IMMUNOREGULATION

Volume 2. Supplement 1. April 2018

ISSN: 2588-5200

# ABSTRACT BOOK

Iranian Society for Immunology and Allergy

14th International Congress of Immunology and Allergy

26-28 April 2018, Tehran, I.R.Iran

ICIA2018

چهاردهمین کنگره بین المللی ایمونولوژی و آلرژی

۶ تا ۸ اردیبهشت ۱۳۹۷ تهران، ایران

www.icia.ir

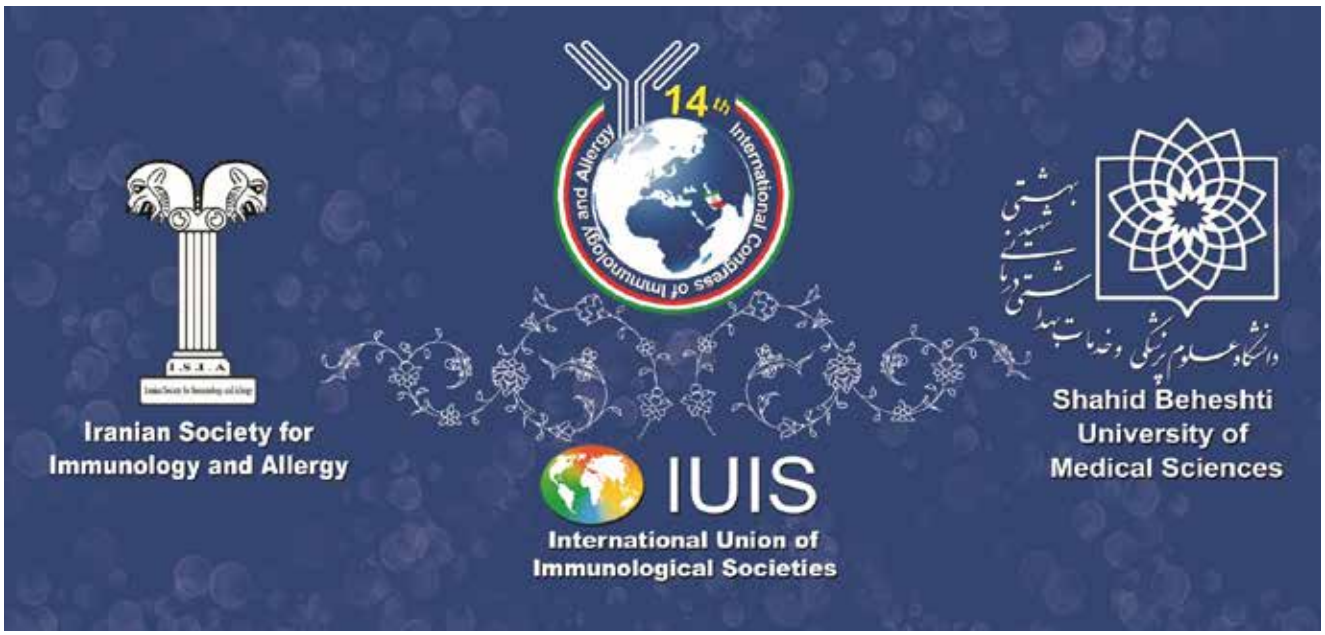
Contents

Allergy and Immunotherapy of Allergic Disease ..... 17
Cancer Immunology ..... 27
Cancer Immunotherapy ..... 38
Computational Immunology and Systems Biology ..... 46
Diagnostic Methods in Immunology ..... 54
Immune Cell Therapy ..... 62
Immunodeficiency ..... 71
Immunogenetics ..... 75
Immunohematology ..... 84
Immunology of Chemical Victims and Environmental Pollution ..... 91
Immunology of Exercise, Aging, and Nutrition ..... 98
Immunology of Infectious Diseases Bacteria and Fungi ..... 104
Immunology of Infectious Diseases Viruses ..... 110
Immunology of Mycobacterium Tuberculosis ..... 118
Immunology of Rheumatic Diseases ..... 124
Immunomodulation and Immunoregulation ..... 132
Immunoparasitology ..... 140
Immunopharmacology and Medicinal Plants ..... 151
Innate Immunity and Inflammation ..... 162
Monoclonal Antibody Diagnostic and Therapeutic ..... 170
Mucosal and Gastrointestinal Immunology ..... 179
Nanoimmunology ..... 191
Oral Immunology ..... 199
Psychoneuroimmunology and Immunoendocrinology ..... 208
Reproductive Immunology ..... 213
Research and Development ..... 225
Stem Cells Based Immunotherapy ..... 236
Tolerance and Autoimmunity ..... 245
Transplantation ..... 255
Vaccine ..... 260
Veterinary Immunology ..... 269
Allergy and Immunotherapy of Allergic Disease ..... 277
Cancer Immunology ..... 287
Cancer Immunotherapy ..... 301
Computational Immunology and Systems Biology ..... 311
Diagnostic Methods in Immunology ..... 317
Immune Cell Therapy ..... 329
Immunodeficiency ..... 335
Immunogenetics ..... 344
Immunohematology ..... 354
Immunology of Chemical Victims and Environmental Pollution ..... 357
Immunology of Exercise, Aging, and Nutrition ..... 360

Immunology of Infectious Diseases Bacteria and Fungi ..... 364
Immunology of Infectious Diseases Viruses ..... 375
Immunology of Mycobacterium Tuberculosis ..... 386
Immunology of Rheumatic Diseases ..... 395
Immunomodulation and Immunoregulation ..... 406
Immunoparasitology ..... 413
Immunopharmacology and Medicinal Plants ..... 424
Innate Immunity and Inflammation ..... 435
Monoclonal Antibody Diagnostic and Therapeutic ..... 446
Mucosal and Gastrointestinal Immunology ..... 457
Nanoimmunology ..... 469
Oral Immunology ..... 477
Psychoneuroimmunology and Immunoendocrinology ..... 487
Reproductive Immunology ..... 491
Stem Cells Based Immunotherapy ..... 506
Tolerance and Autoimmunity ..... 517
Transplantation ..... 527
Vaccine ..... 538
Veterinary Immunology ..... 549
Allergy and Immunotherapy of Allergic Disease ..... 560
Cancer Immunology ..... 602
Cancer Immunotherapy ..... 667
Computational Immunology and Systems Biology ..... 696
Diagnostic Methods in Immunology ..... 711
Immune Cell Therapy ..... 714
Immunogenetics ..... 719
Immunohematology ..... 737
Immunology of Chemical Victims and Environmental Pollution ..... 743
Immunology of Exercise, Aging, and Nutrition ..... 750
Immunology of Infectious Diseases Bacteria & Fungi ..... 760
Immunology of Infectious Diseases Viruses ..... 779
Immunology of Mycobacterium Tuberculosis ..... 813
Immunology of Rheumatic Diseases ..... 815
Immunomodulation and Immunoregulation ..... 832
Immunoparasitology ..... 836
Immunopharmacology and Medicinal Plants ..... 840
Innate Immunity and Inflammation ..... 885
Monoclonal Antibody Diagnostic and Therapeutic ..... 912
Mucosal and Gastrointestinal Immunology ..... 914
Nanoimmunology ..... 923
Psychoneuroimmunology and Immunoendocrinology ..... 933
Reproductive Immunology ..... 940
Index ..... 1063


14th
International Congress of Immunology and Allergy

# ICIA2018 Organizers



# Chairman

# Welcome Message

On behalf of the congress organizers, the ***Iranian Society for Immunology and Allergy (ISIA)*** and ***Shahid Beheshti University of Medical Sciences***, it is my great pleasure to invite you to the "**14th International Congress of Immunology and Allergy" (ICIA2018)**. As the largest meeting in the field of immunology and allergy in Iran, **ICIA2018** is an event which is held biannually.

The theme of the congress is "major breakthroughs and advances in immunology" covering all fields of immunology, ranging from innate and adaptive immunity to emerging diagnostics and therapeutics.

With the participation of invited speakers and chairs from all over the world, the opportunity for discussing science and forming new collaborations is provided. Front-runners in the fields of basic, clinical and diagnostic immunology with keynote lectures will present discoveries in fundamental immunology.

We very much look forward to seeing you in Tehran on 26-28 April 2018, in which we will do our best to make **ICIA2018** the most memorable scientific event for you.

Sincerely,

**Saeid Namaki, Ph.D.**
**Chairman of the Congress**

# President of ISIA

# Welcome Message

As president of the "***Iranian Society for Immunology and Allergy" (ISIA)***, it is my pleasure to extend this invitation to you to join us in Tehran in April 2018 at the "**14th international Congress of Immunology and Allergy" (ICIA2018).** Being held every two years and regularly attracting over 1500 delegates, **ICIA2018** will begin to work on 26th April. ISIA together with ICIA's patrons will work to create a memorable scientific event for participants. The opportunity of exploring the most recent and innovative advances in the field of immunology and allergy from bench to bedside will motivate all attendings with different interests in almost all the fields of modern immunology. Offering a fine balance between basic and applied/translational researchers, we ensure you will totally be satisfied with this scientific session. Like all previous ICIAs, ISIA will follow its missions of fostering young and not so young researchers as scientists. I do hope that you will be able to join us at **ICIA2018** in Tehran and I look forward to seeing you there.
Sincerely,

**Mohammad Vodjgani, Ph.D.**
**President of the Iranian Society for Immunology and Allergy (ISIA)**

# Head of Scientific Committee Welcome Message

We are delighted to invite you to join the ***"14th International Congress of Immunology and Allergy" (ICIA 2018)*** at Tehran, Iran, from 26 to 28 April 2018. Holding such an invaluable academic and scientific meeting provides a good opportunity for sharing ideas and experiences with the most outstanding domestic and foreign scholars to motivate students and graduates of immunology and all other related fields on their scientific path. Moreover, with the participation of scientists and experts of clinical immunology and allergy covering dimensions of diagnosis, prevention and treatment in the field of immunology along with scholars of both cellular and molecular aspects of basic immunology, practical integration of basic and clinical sciences will be provided helping share information in order to strengthen the relationship between basic and clinical sciences.

Keynote speeches of different panels, presentation of abstracts, advanced courses and workshops will form the core of **ICIA 2018**, which will be the center of attention of all scholars, experts, and students attracted by the field of immunology. Thus, **ICIA2018** strives to take advantage of valuable experiences and ideas to make this meeting more effective.

I hope that with the scientific cooperation of nationwide medical universities and research centers, companies related to immunological and laboratory products, pharmaceutical companies and other scientific organizations, **ICIA2018** becomes a memorable meeting.

Sincerely,

**Seyed Mahmoud Masiha Hashemi, Ph.D.**
**Head of Scientific Committee of the Congress**

# Head of Executive Committee Welcome Message

I am delighted to warmly welcome you to attend 14th ICIA, held on April 26-28, 2018, in Tehran-Iran .The congress is organized jointly by ***Iranian Society of Immunology and Allergy (ISIA)*** and ***Shahid Beheshti University of Medical Sciences (SBMU)*** accompanied with other national and international outstanding Medical Universities and Scientific Institutes. As the Executive manager, I would like to express my sincere welcome to all of the distinguished guests and participants to **ICIA2018**.

With the purpose of bringing together leading academic scientists, researchers and scholars to exchange and share their experiences and research results on all aspects of Immunology and promoting the international communication and cooperation, it is our best pleasure to hold the **ICIA2018**, which covers various fields of immunology.

We again invite you to join us at **ICIA2018** to have memorable scientific and cultural experiences. All members of **ICIA2018** organizing committee look forward to meeting you in Tehran-Iran.

**Mahdi Shabani, Ph.D.**
**Head of Executive Committee of the Congress**

# Congress Board

# Strategic Committee of ICIA2018

**Dr. Saeed Namaki**

*Chairman of the Congress*

**Dr. Mohammad Vodjgani**

*President of ISIA*

**Dr. Seyed Mahmoud Masiha Hashemi,**

*Head of Scientific Committee of the Congress*

**Dr. Mahdi Shabani**

*Head of Executive Committee of the Congress*

**Dr. Tooba Ghazanfari**

*Head of Third Festival of Immunology and Allergy*

**Dr. Mehrnaz Mesdaghi**

*Head of International Committee of the Congress*

**Dr. Sara Soudi**

*Secretary of Junior Members of ISIA*

*Coordinator of ICIA2018 Accommodation*

**Dr. Reza Falak**

*Coordinator of ICIA2018 Workshops*

**Dr. Farhad Riazi Rad**

*Coordinator of ICIA2018 Tours*

**Dr. Samira Rajaei**

*Coordinator of ICIA2018 Accomodation*

**Dr. Mohammad Hosein Nicknam**

*Vice President of ISIA*

**Dr. Jamshid Hadjati**

*ISIA Board Member*

**Dr. Abbas Rezaei**

*ISIA Board Member*

**Dr. Mehdi Shekarabi**

*ISIA Board Member*

**Dr. Seyed Mohammad Moazzeni**

*ISIA Board Member*

**Dr. Reza Mansouri**

*ISIA Board Member*

**Dr. Mohammad Mehdi Mohammadi**

*ISIA Board Member*

# Scientific Committee of ICIA2018 (National)

Abbaszadegan, Mohammad Reza
Abd Nikfarjam, Bahareh
Abdolmaleki, Mohsen
Abediankenari, Saeid
Abolhassani, Mohsen
Abolnejadian, Farhad
Aghaie, Afsaneh
Aghamohamadi, Asghar
Aghdami, Nasser
Aghebatimaleki, Leili
Aghili, Babak
Ahanchian, Hamid
Ahmadi, Kazem
Ahmadiafshar, Akefeh
Ajami, Aboulghasem
Ajdary, Soheila
Alijani, Ebrahim
Alimohammadian, Mohammad Hossein
Alsahebfosoul, Fereshteh
Alyasin, Soheyla
Amani, Davar
Amin, Reza
Amini, Abbas Ali
Amirchaghmaghi, Elham
Amirghofran, Zahra
Amiri, Mohammad Mehdi
Amirzargar, Ali Akbar
Anani Sarab, Gholamreza
Andalib, Ali Reza
Anisian, Ali
Arababadi, Mohammad Kazemi
Arandi, Narges
Arasteh, Javad
Ardestani, Soosan
Arefian, Ehsan
Armin, Shahrnaz
Arshi, Saba
Asadi Asl,Sara
Asgarin-Omran, Hossein
Ashoori, Elham
Asnaashari, Ehsan
Assarehzadegan, Mohammad Ali
Ataollahi, Mohammadreza
Atarod, Lida
Athari, Seyyed Shamsadin
Azadmehr, Abbas
Babaie, Delara
Babaloo, Zohreh
Badiee, Ali
Bagheri, Nader
Baharvand, Hosein
Bahrami, Ahmad
Bani Hashemi, Seyd Reza
Baradaran, Behzad
Barzegari, Saeed
Bayanolhagh, Saeed
Bazargan, Nasrin
Behmanesh, Fatemeh
Behniafard, Nasrin
Behzad Poopak
Bemanian, Mohammad Hassan
Bidad, Katayoon
Bozorgmehr, Mahmoud
Chavoshzadeh, Zahra
Cheraghi, Taher
Dabaghian, Mehran
Dabbaghzadeh, Abbas
Darabi, Behzad
Darvishalipor, Shamila
Davoodi, Homa
Delbandi, Ali Akbar
Delirezh, Norouz
Doroudchi, Mehrnoosh
Douraghi, Masoomeh
Ebrahimi, Esmail
Ebrahimi, Marzeih
Ebrahimi, Mohsen
Ebtekar, Massoumeh
Eftekharian, Mohammad Mahdi
Ehsani Arkani, Mohammad Javad
Emam, Mahdi
Entezami, Kobra
Erfani, Nasrollah
Eskandari, Nahid
Eslami, Mohammad Bagher
Eslamian, Mohammad-Hossein
Esmaeil, Nafiseh
Esmaeilnejad, Atefe
Esmaeilzadeh, Abdolreza
Esmaeilzadeh, Hossein
Falak, Reza
Fallahpour, Morteza
Faridhosseini, Reza
Farjadian, Shirin
Farrokhi, Shokrollah
Farsad, Faraneh
Farzaneh, Parvaneh
Fatemeh, Beyzaei
Fathi, Seyed Mohammad
Fattahi, Javad
Fayezi, Abbas
Fazeli, Bahare
Fazlollahi, Mohammad Reza
Fereidouni, Mohammad
Feyzollahzade, Sadegh
Foroughi Mehrnoush
Foroughi, Farshad
Forouzan, Karimi
Fotohi
Ganjalikhani Hakemi, Mazdak
Ganji, Ali
Ghaderi, Abbas
Ghadiri, Ata Allah

Ghaemi, Amir
Ghafari, Javad
Ghafourian Borujerdnia, Mehri
Ghalam Farsa, Ghasem
Ghaneie, Mostafa
Gharagozloo, Marjan
Gharagozlou, Mohammed
Gharebaghian, Ahmad
Gharesifard, Behrouz
Ghatrehsamani, Mahdi
Ghazanfari, Tooba
Ghazavi, Ali
Gholamin, Mehran
Gholamzad, Mehrdad
Gholijani, Nasser
Ghorban Khodayar
Givtaj, Nozar
Golsazshirazi, Forough
Gorgin, Ali
Hadinedoushan, Hossein
Hadjati, Jamshid
Haghmorad, Dariush
Haji Molla Hoseini, Mostafa
Hajibehzad, Mehdi
Hajighasemi, Fatemeh
Hajilooi, Mehrdad
Hamedifar, Haleh
Hamidieh, Amir Ali
Harandi, Ali
Hashemi, Seyed Mahmoud
Hassan, Zuhair Mohammad
Heidarzadeh Arani, Marzieh
Hekmatdoost, Azita
Hojjat-Farsangi, Mohammad
Hosseinali Izad, Maryam
Hosseinzadeh, Morteza
Houshmand, Hamidreza
Idali, Farah
Isanezhad, Amin
Jabbari Azad, Farahzad
Jadidiniaragh, Farhad
Jafarzadeh, Abdollah
Jalali, Amir
Jalali, Seyed Amir
Jalili, Ali
Jamshidian, Azam
Javad Ahmadian, Haris
Jazayeri, Mirhadi
Jeddi Tehrani, Mahmoud
Kafami, Laya
Kafshdozan, Khatere
Kalantar, Kurosh
Kalantari, Arash
Kalantari, Tahereh
Kamali-Sarvestani, Eskandar
Kardor, Gholamali
Karimi, Mohammad Hossein
Kavosi, Kaveh
Kawosi, Hoda
Kazemi, Bahram
Kazemi, Tohid
Keramatipour, Mohammad
Keshavarz, Maryam
Keyhani, Abdolhossein
Khaghanzadeh, Narges
Khaki, Pejvak
Khalili, Abbas
Khansari, Nematollat
Khazaei, Hossein Ali
Kheirandish, Maryam
Khodadadi, Ali
Khorrami, Samaneh
Khosravi, Afra
Khosravi, Alireza
Kiani,Jafar
Kokhaei, Parviz
Langroudi, Ladan
Mahboudi, Fereidoun
Mahdaviani, Seyed Alireza
Mahmoodzadeh-Niknam, Hamid
Mahmoudi, Mahdi
Mahmoudi, Mahmoud
Majidi, Jafar
Mansouri, Davoud
Mansouri, Mahboubeh
Mansouri, Reza
Marashi, Mahdi
Marjani, Majid
Masjedi, Mohsen
Masoudinejad, Ali
Massoud, Amir Hossein
Mehdipour, Fereshteh
Mehrzad, Jalil
Memarian, Ali
Mesdaghi, Mehrnaz
Mirahmadian, Mahroo
Mirshafiey, Abbass
Moaidi Esfahani, Behjat Sadat
Moazzeni, Seyed Mohammad
Modabber, Farrokh
Modarressi, Mohammad Hossein
Mofazzal Jahromi, Mirza Ali
Moghbeli, Meysam
Mohammadi, Marjan
Mohammadi, Mohammad Mehdi
Mohammadi, Mojgan
Mohammadi-Shahrokhi, Vahid
Mohammadnia, Mousa
Mohammadreza, Rahmani
Mohammadzadeh , Adel
Mohammadzadeh, Iraj
Moin, Mostafa
Mojtabavi, Nazanin
Mojtahedi, Zahra
Mokhtari Azad, Talaat
Mollanouri Shamsi, Mahdieh
Momen, Tooba
Moniri, Afshin
Moravej, Ali
Mortaz, Esameil
Mortazavi, Seyed Hamidreza
Mosaffa, Nariman
Mosayebi, Ghasem
Mostafaie, Ali
Mostafazadeh, Amrollah
Motedayen, Mohammad Hassan
Mousavi Shabestari, Tahereh
Mousavi, Seyed Fazlullah
Movahedi, Masoud
Nabavi, Mohammad
Naderi, Nadereh
Nadji, Seyed Alireza
Namaki, Saeed
Namdar Ahmadabad, Hassan
Nasiri Kalmarzi, Rasoul
Namaki, Saeid
Negar, Seyed
Nejatollahi, Foroogh

Nicknam, Mohammad Hosein
Nikbakht Brujeni, Gholamreza
Nikbin, Behrouz
Nikoo, Shohreh
Nikoueinejad, Hassan
Noorbakhsh, Farshid
Noorbala, Ahmad-Ali
Nosratabadi, Reza
Nouri, Hamid Reza
Nourizadeh, Maryam
Ostadali, Mohammadreza Reza
Pakravan, Nafiseh
Pakzad, Parviz
Parvaneh, Nima
Pezeshki, Mohammad
Pishdadian, Abbas
Pourfathollah, Ali Akbar
Pourpak, Zahra
Poyan Mehr, Mehrdad
Rabbani, Mohammad
Rafati, Sima
Rafatpanah, Hoshang
Rafiei Tehrani, Shahanaz
Rafiei, Alireza Reza
Rahbarizadeh, Fatemeh
Rahimi, Roghayeh
Rajaei, Samira
Rasaie, Mohammad Javad
Rashno, Mohammad
Razavi, Seyed Alireza
Razmkhah, Mahboobeh
Rekabi, Mahsa
Rezaei, Abbas
Rezaei, Nima
Riazi Rad, Farhad
Rostamian, Abdolrahman
Rostamian, Abdolrahman
Rostaminejad, Mohammad
Saadat, Farshid
Saboor Yaraghi, Ali Akbar
Sabzehei, Mohammad Kazem
Sadeghi, Rokhsareh
Sadeghi-Shabestari, Mahnaz
Sadighimoghaddam, Bizhan
Sadri, Homa
Saeidi, Mohsen
Saeidi, Reza
Safa, Omid
Safari, Elahe
Safari, Mojgan
Saghafi, Shiva
Sahebi, Leyla
Sahraeian, Mohammad Ali
Salari, Farhad
Salehi, Eisa
Salekmoghadam, Alireza
Salimi, Vahid
Salimian, Jafar
Samadi, Morteza
Samiei, Afshin
Samira Mohammadi-Yeganeh
Sandoghchian Shotorbani, Siamak
Sankian, Mojtaba
Sardari, Soroush
Sarrafnejad, Abdolfattah
Sattari, Mandana
Sedghy, Farnaz
Seyedi, Shahram
Shabani, Mahdi
Shafi Mojadadi, Mohammad
Shahabi, Shahram
Shahbazi, Mehdi
Shahidi, Minoo
ShahMohammadi, Shohreh
Shahrokhi, Somayeh
Shahsavar, Farhad
Shaiegan, Mojgon
Shakib Jafari, Reza
Shakiba, Yadollah
Shams, Ali
Shamsdin, Seyedeh Azra
Shamsi, Ali
Shariat, Mansoureh
Sharifi, Nourieh
Sharifpaghaleh, Ehsan
Sharifzadeh, Sedigheh
Sheikhi, Abdolkarim
Sheikhian, Ali
Shekarabi, Mehdi
Shirzad, Hedayatollah
Shokohi, Shervin
Shokrgozar, Mohammad Ali
Shokri, Fazel
Mohammad Ali Shokrgozar
Soleiman jahi, Horie
Soleimanifar, Narges
Solgi, Ghasem
Soroush, Mohammadreza
Soudi, Sara
Tabaresi, Payam
Taghadosi, Mahdi
Taghvaei, Behrang
Taheri, Tahereh
Tahoori, Mohammad Taher
Tajbakhsh, Hassan
Tajik, Nader
Taleghani, Feryal
Tavakol Afshari, Jalil
Tavakol, Marziyeh
Tavakoli, Maryam
Tebyanian, Majid
Tehrani, Mohsen
Vaezzadeh, Farhad
Varahram, Mohammad
Varasteh, Abdolreza Reza
Varzi, Ali Mohammad
Vaziri, Behrouz
Vodjgani, Mohammad
Yadegarynia, Davood
Yaraee, Roya
Yari, Fatemeh
Yazdani, Reza
Yazdani, Yaghoub
Yeganeh, Farshid
Yosefi, Bahman
Younesi, Vahid
Yousefi, Mehdi
Zahed, Ghazal
Zahra Faghih
Zamani, Alireza
Zamani, Mohammad Ali
Zamani, Zahra
Zandieh, Fariborz
Zarnani, Amir-Hassan
Zavaranhosseini, Ahmad
Zavvar, Mahdi

# Scientific Committee of ICIA2018 (International)

Ciacci, Carolina

Harandi, Ali M

Jalili, Ahmad

Kabelitz, Dietrich

Kheradmand, Farrah

Kropf, Pascale

Manjili, Masoud H

Modabber, Farrokh

Nademi, Zohreh

Rajalingam, Raja

Sadegh-Nasseri, Scheherazade

Schmidt, Reinhold E.

Simon, Hans-Uwe

Stear, Michael J

Wahn, Ulrich

# Scientific Committee of ICIA2018

## Workshops

Dr. Ahad Zare
Dr. Ali Mostafaie
Dr. Ali Norozi Aghideh
Dr. Ali Pormohammad
Dr. Amir Jalali
Dr. Babak Arjmand
Dr. Ebrahim Faghihloo
Dr. Ehsan Jan Zamin
Dr. Elham Ashouri
Dr. Farhad Jadidi-Niaragh
Dr. Fatemeh Faraji
Dr. Flora Forouzesh
Dr. Forouzan Karimi
Dr. Hossein Asgarian-Omran
Dr. Jafar Salimian
Dr. Kamran Mansouri
Dr. Kaveh Baghaei
Dr. Koroush Kalantar
Dr. Mahmood Bozorgmehr
Dr. Majid Khoshmirsafa
Dr. Maryam Khairandish
Dr. Marzieh Ebrahimi
Dr. Mirhadi Jazayeri
Dr. Maryam Kheirandish
Dr. Mitra Nourbakhsh
Dr. Mohamad Keramatipour
Dr. Mohamad Reza Haghshenas
Dr.Mohammad Davoodzadeh Gholami
Dr. Mohammad Javad Nasiri
Dr. Mohammad Mehdi Amiri
Dr. Mohammad Rostami Nejad
Dr. Nariman Mosaffa
Dr. Parvane Farzane
Dr. Raheleh Shokouhi Shoormasti
Dr. Reza Falak
Dr. Reza Zolfaghari Emameh
Dr. Rozita Khanafari
Dr. Saeid Abediankenari
Dr. Sattar Gorgani-Firuzjaee
Dr. Siavash Ahmadi Nourbakhsh
Dr. Vahid Jajarmi
Dr. Vahid Salimi
Dr. Zahra Alizadeh
Dr. Zohreh Mohamadi
Dr. Mohammad Davoodzadeh Gholami

# Executive Committee

Abassi, Ardashir
Abbasi-kenarsari, Hajar
Abdolmohammadi, Kamal
Abedini, Fateme
Afzali, Shima
Aghamajidi, Azin
Ahmadi Fesharaki, Raoufe
Ahmadifard, Reza
Alimohammadi, Masoumeh
Alimohammadi,Reza
Alinezhad, Maede
Alirezaee, Atefe
Asadirad, ALI
Asgari, Marzieh
Asghari, Mohadeseh
Askarzadeh, Monireh
Atashzar, Mohammad reza
Azempour, Elham
Azizi, Mahdieh
Babaei, Erfan
Bagheralmoosavi, Sarvin
Bagheri, Salman
Baghernezhad, Zainab
Baradaran, Behnoosh
Bazargan, Mahsa
Beheshti, Elahe
Changaei, Mostafa
Dalil Roofchayee, Neda
Davoudzade Gholami, Mohammad
Delsuz,Rezaee
Doroudian, Fateme
Eshghi, Fateme
Eskandarian, Maryam
Esmaeili, Seyed Alireza
Ezzatifar, Fatemeh
Faghih, Manizhe
Fakhari, Yaser
Fallah Mehrjardi, Keyvan
Fathi, Farshid
Fathollahi, Anwar
Fattahi, Pooya
Gaeini, Amirhossein
Ghahremani, Maryam
Ghasemi, Nazanin
Ghasiyari, Haniye
Ghazanfari, Hadi
Gholami, Parastou
Golmohammad pour, Ladan
Habibzadeh, Sima
Haghdoost, Sepideh
Hajatbeigi, Baran
Haji Khodaverdi Khani, Hedieh
Hajivalili, Mahsa
Hajmahmoodi, Somayeh
Hasanzadeh, Mohammad Ali
Hashemi, Zahra
Hassan Pour, Zahra
Heidari, Maryam
Heidari, Neda
Heidari, Sahel
Hesseini, Vahedeh
Hosseini, Maryam
Iranshahi, Nasrin
Jafari, Davood
Jafarpour, Roghaye
Jafarzadeh, Fatemeh
Javidan, moslem
Johari nad, Fatemeh
Kakadezfuli, Neda
Karimi, Mozhde
Katebi, Asal
Kazemi Sefat, Nazanin Atieh
Kazemi, Mohammad Hossein
Keshavarzian, Nafise
Khabareh, Maryam
Khakpoor Koosheh, Mohammad
Khan Nazer, Nikoo
Khani chamani, Fateme
Kheiri, Farzaneh
Khoshmirsafa, Majid
Khosroopour, Zahra
Khubraftar, Saeedeh
Kiaa, Vahid
Lotfinejad, Sedighe
Mahmoudi, Mohammad
Mami, Fatemeh
Mami, Somaye
Mansourabadi, Amirhossein
Mardani, Fatemeh
Mashhadi, Fatemeh
Masoudnia, Mina
Masoumi, Farima
Masoumikarimi, Masoumeh
Mazarzaei, Abdulbaset
Mehdinejadiani, Kobra
Mehdinejadiani, Shayesteh


Mohammadi kordkheyli, Marziyeh

Mohammadi, Maryam

Mojtahedi, Haniye

Moloudi Zargari, Milad

Momeni, Zahra

Moradi, Maryam

Mortezagholi, Sahar

Moulazade, Alireza

Mousavi, Seyed Mohammad Javad

Mousavinasab, Fatemehsadat

Namazi, Shideh

Nazari, Farzad

Nejatbakhsh Samimi, Leila

Niknam, Bahare

Nikoonezhad, Maryam

Norouzbeygi, Mina

Omidi, Zahra

Paydarfar, Shadi

Pormohammad, Ali

Pouya, Sedigheh

Rahimi, Arezou

Rahmani, Hoda

Ramezani, Mahya

Rammezani, Khadijeh

Rashidi, Nesa

Rasouli, Shima

Rastegari-Pouyani, Mohsen

Rezaei Lotfi, Alaleh

Rezaei, Ramazan

Rostami, Elham

Rostamian, Hosein

Rostamzadeh, Zainab

Sadeghi, Atefe

Sadeghi, Somaye

Sadegholvad, Mona

Sadri, Maryam

Saeidi Brojeni, Ali

Safaei, Elmira

Safarzadeh, Mehrnoush

Salehi, Saeedeh

Seyyednajib, Mirali

Shahidi, Maryam

Shahir, Mehri

Shams, Forough

Sheikhi, Shima

Shirazi

Shojaei, Samaneh

Shojaei, Zeinab

Shokri, Mohammad Reza

Shokrollahi, Mahdieh

Taene, Abdulghader

Taghavi, Mahsa

Tavakkoli, Sajjad

Touzandehjani, Danial

Vafa, Ashraf

Vaziri Nezam Dousت, Fereshte

Yaftiyan, Atefeh

Yousefi, Roya

Zadian, Seyed Sajjad

Zand, Nafiseh

# Invited Speakers

# Allergy and Immunotherapy of Allergic Disease

## Oral Presentation

ICIA2018\Allergy and immunotherapy\Oral\5314
**5314**

# Status of IL-10 gene expression in patients with allergic rhinitis who received immunotherapy and symbiotic

Sajad Dehnavi[1], Mojgan Mohammadi[2]* Farahzad Jabbari Azad[2], Reza Farid Hosseini[2], Jalil Tavakol Afshari[3], Maryam Khoshkhooi [2], Hamid Ahanchian[2], Nasrin Moazzen[1], Maryam Davarpanah[1], Amirabbas Salmani[1]

1. *Department of Immunology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
2. *Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
3. *Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** Allergic Rhinitis (AR) as the most common allergic disease has an increasing incidence in all over the world. Annually, billion dollars are paid for various types of allergic diseases. There are several lines of evidence showing the effects of symbiotic in the treatment of allergic diseases. We aimed in this study to evaluate the effects of symbiotic on the gene expression of IL-10 as one of regulatory T cell (Treg) cytokines in patients with AR who received immunotherapy.

**Methods:** Twenty patients with AR who underwent cluster immunotherapy were randomized to receive symbiotic and placebo for two months. Before and after the procedure, clinical symptoms were evaluated and whole blood samples were collected to extract RNA from peripheral blood mononuclear cells (PBMCs), then cDNA was synthesized and finally gene expression was evaluated by SYBR Green real-time PCR technique.

**Results:** Along with improvement of clinical symptoms and quality of life, gene expression of IL-10 increased in both symbiotic and placebo groups, this increase was more evident in symbiotic group but the difference was not significant (p=0.72).

**Conclusion:** Our study showed that administration of symbiotic may have a positive effect on Treg cells leading to improved clinical symptoms and quality of life in AR patients who underwent immunotherapy. However, increasing the sample size might help us get a better conclusion.

**Keywords:** Allergic Rhinitis, Immunotherapy, Symbiotic, IL-10

ICIA2018\Allergy and immunotherapy\Oral\7586
**7586**

# PD-1 and Tim-3 immune checkpoint receptors are more expressed on T-CD4+ lymphocytes of patients with asthma

Ali Mosayebian[1], Zohreh Koohini[1], Hadi Hossein-Nataj[1], Saeid Abediankenari[1,2], Siavash Abedi[3*], Hossein Asgarian-Omran[1,2*]

*1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
*2. Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
*3. Department of Internal Medicine, Pulmonary and Critical Care Division, Mazandaran University of Medical Sciences, Sari, Iran*

**Background:** Asthma is a chronic disorder of the airways characterized by reversible airway obstruction, inflammation and bronchial hyperresponsiveness. Different immune cells and molecules have been shown to be involved in the pathogenesis mechanisms of asthma. T cell immunoglobulin and mucin domain-containing molecule-3 (Tim-3) and programmed death-1 (PD-1) are two surface immune checkpoint receptors expressed mainly on Th1 and Th2 cells, respectively which play an important role in regulating the activity of these cells. Due to the limited studies on the roles of Tim-3 and PD-1 in the pathogenesis and development of asthma, in the present study, we studied the expression of Tim-3 and PD-1 on $CD4^+$ T cells of asthmatic patients.

**Methods:** A total of 37 asthmatic patients and 32 healthy controls were enrolled in this study. Fresh peripheral blood mononuclear cells (PBMC) were collected from all subjects and were used for subsequent experiments. The frequency of $Tim\text{-}3^+$/ $PD\text{-}1^+$/ $CD4^+$ T cells was determined by three-color flowcytometry method. To evaluate the Th1/Th2 ratio, PBMCs were stimulated with PMA/ionomycin for 18 h. IFN-γ and IL-4 were measured in culture supernatants by ELISA. Serum total IgE was also measured in all samples.

**Results:** Significant increase in the percentage and absolute count of $Tim\text{-}3^+$/ $PD\text{-}1^+$/ $CD4^+$, $Tim\text{-}3^+/CD4^+$ and $PD\text{-}1^+/CD4^+$ T cells was found in asthmatic patients compared to healthy controls. The IFN-γ/IL-4 ratio (Th1/Th2 ratio) was significantly higher in healthy controls than that of asthmatic patients.

**Conclusion:** Our data showing the increased expression of PD-1 and Tim-3 on $CD4^+$ T cells of patients with asthma suggest the potential roles of these immune checkpoint receptors on the pathogenesis of asthma and promotion of Th2-mediated immune responses.

**Keywords:** Asthma, Tim-3, PD-1, Th1/Th2 ratio

ICIA2018\Allergy and immunotherapy\Oral\7636
**7636**

**Identification and Characterization of major allergens from elm (Ulmus glabra) pollen grains**

Elham Mahlooji [1], Mohammad Reza Mozdianfard[1], Marzieh Heidarzadeh Arani[2], Mostafa Moin[3], Maryam Sharifshoushtari[3,4] , Morteza Djamali[5], Reza Mansouri[6], Zahra pourpak[3], Hossein Batooli[7], Gholam Ali Kardar[3]

1. *Department of Chemical Engineering, Faculty of Engineering, University of Kashan, IR Iran*
2. *Pediatric Department, Faculty of Medicine, Kashan University of Medical Sciences, Kashan*
3. *Immunology, Asthma and Allergy Research Institute, Children's Medical Center, Tehran University of Medical Sciences, Tehran, IR Iran*
4. *Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
5. *Institut Méditerranéen d'Ecologie et de Paléoécologie (IMEP UMR CNRS 6116), Europôle Méditerranéen de l'Arbois, Bâtiment Villemin BP80, 13545 Aix-en-Provence, Cedex 04, France*
6. *Department of Physics, Sharif University of Technology, Tehran, 14588-89694, Iran and Saeedi Institute for Advanced Studies, University of Kashan, Kashan, 87317-51167, Iran*
7. *Isfahan Research Center of Natural Sources and Agriculture, Kashan Station, Kashan, Iran*

**Background:** Elm (Ulmus) tree pollen is a known aeroallergen, found commonly in the semi-arid climate of Kashan-Iran, planted primarily for its shade and aesthetic appeal. It pollinates from early February to mid March and is distributed widely throughout the world's temperate and tropical regions. Four species have been identified in Iran, namely: U. glabra, U. minor, U. boissieri and U. umbraculifera with the former being an important species in the northern forests of Iran. In this research, we identified and characterized major allergens in elm pollen.

**Methods:** Physical appearance specifications of U.glabra species were examined using SEM. Pollen protein was analyzed using SDS PAGE while, total protein content was measured by Bradford assay. Total IgE was examined by taking venous blood from 18 patients (8 male and 10 female), sensitive to elm with positive skin prick test (SPT). Major allergens were identified by immunoblot technique on pooled patients' sera (using 5% pollen extract taken from locally collected pollen). Allergenic proteins were later purified using gel filtration chromatography.

Results: Pollen grains were oblate-spheroidal to angular and square in form and contained 4-6 elliptical pores with thick and rugulate exine, sized 16-50 X 18-50 µm. Four components showed IgE-binding activity and were therefore identified as the elm pollen allergens, having molecular weight ranged between 39-70 kDa. Average total IgE was 162.98 IU/mL which reacted with a distinct major band at nearly 55 kDa. Immunoblot analysis identified a novel allergen protein, named Ulm g1.

**Conclusion:** The 55 kDa allergen was the major allergen of U. glabra. Moreover, combination of total IgE and immunoblot techniques, could be effectively used in the diagnosis of elm pollen allergy.

**Key words:** Pollen grains, Elm tree, allergen, seasonal allergy, Ulm g1

ICIA2018\Allergy and immunotherapy\Oral\7879
**7879**

# Loratadine counter-regulates YKL-40 release from human monocytic cell line (THP-1) but not human epithelial cells (A549)

Zeynab Baghernejadan [1], Mostafa Haji Molla Hoseini [2]

*1. Department of Medical Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
*2. Department of Medical Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

**Background:** Recent research has found the evidence of chitinase and chitinase like proteins contributions to the mechanisms of allergic response and their association with atopy. The chitinase like protein, YKL-40, plays an important role in the pathogenesis of allergic diseases. Allergens induce its production from macrophages and epithelial cells.
Some investigations indicate that loratadine has anti-inflammatory effects apart from its H1 receptors anatagonistic activity. We sought to know whether human epithelial cells and macrophages stimulated for YKL-40 synthesis by host dust mite (HDM) extract, might be influenced by loratadin. We used the human pulmonary type II epithelial cell line A549 and the human monocytic cell line (THP-1) for our in vitro study.
**Method:** After dose finding studies, using neutral red cytotoxicity assay, the cells were pre-incubated with loratadin (0.5 µg/mL) and then activated by HDM (Greer Lab). Also in post-treatment conditions; A549 and THP-1 cells were stimulated with HDM extract followed by incubation with loratadine. Then, the amounts of IL-8 and YKL-40 released into the cell supernatants were determined by a specific ELISA assay. The classic chemotaxis assay was performed, using modified Boyden chambers as well.
**Results:** Loratadine has no effect on IL-8 production and neutrophil chemotaxis under these conditions. Unlike A549, THP-1 cells stimulated with HDM significantly increased the release of YKL-40. Pre-incubation with loratadin diminished the YKL-40 release from THP-1 cells in a significant manner.
**Conclusions:** Loratadin might exert anti-inflammatory effects beyond its H1-receptor antagonistic activity in the context of inflammatory disorders of the respiratory tract such as bronchial asthma and allergic rhinitis.

**Keywords:** Loratadine, YKL-40, A549, THP-1

ICIA2018\Allergy and immunotherapy\Oral\7996
**7996**

# T cell-specific transcription factors gene are differently expressed in Allergic Rhinitis

Soraya Bilvaieh[1], Hamid reza Mortazavi[1], Farhad Salari[1], Ali Grgin karaji[1].

1. Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences

**Background:** Allergic rhinitis (AR) is the most common form of IgE-mediated disease. T cells subsets have a main role in induction and control of AR. The aim of this study was to evaluate the level of GATA-3, RORγt and FoxP3 (the hallmark of Th2, Th17, and T regulatory cells, respectively)gene expression in peripheral blood cells of AR patients.

**Methods:**RNA was extracted from blood cells of 32 AR patients and 20 healthy controls and then transcribed to cDNA. The cDNA was used to measure the level of GATA-3, RORγt and FoxP3 gene expression by means of Real Time-PCR and specific primers. In addition, AR patients and healthy controls Total serum IgE was measured by ELISA.

**Results:** Our results showed that while the level of FoxP3 expression in AR patients was higher than that of control group (P<0/05), thelevel of GATA-3 expression in AR patient was lower than controls (P<0/05). On the other hand, the level of RORγt expression in patient group was higher than that of control group, although it was not statistically significant. Total serum IgE in AR patients was higher than control group (P<0/05).

**Conclusion:** Taken together these results showed that the level of FoxP3 expression in AR patients was higher but the level of GATA-3 expression was lower than that of healthy controls. These unexpected results may be related to chronic state of the disease and consumption of anti-inflammatory drugs such as Glucocorticoids by AR patients.

**Key words:** Allergic Rhinitis, GATA-3, RORγt, FoxP3, Th2, Th17, T regulatory

ICIA2018\Allergy and immunotherapy\Oral\8089
**8089**

# Epidemiology of Chronic Rhinosinusitis in Southwestern Part of Iran: A GA²LEN Study

Shokrollah Farrokhi[1], Afshin Ostovar[2], Wytske J. Fokkens[3]

*1. Department of Immunology, Asthma and Allergy, The Persian Gulf Tropical Medicine Research Center, The Persian Gulf Biomedical Research Institute, Bushehr University of Medical Sciences, Bushehr, Iran*
*2. Department of Otorhinolaryngology, Academic Medical Center, Amsterdam, the Netherlands*
*3. Department of Epidemiology, The Persian Gulf Tropical Medicine Research Center, The Persian Gulf Biomedical Research Institute, Bushehr University of Medical Sciences, Bushehr, Iran*

**Background:** Population based studies by the European Position Paper on Rhinosinusitis (EPOS) criteria for assessment of the prevalence of chronic rhinosinusitis (CRS) play important roles in the development and promotion of public health policies.
**Methods:** A multistage, stratified cluster random sampling method was used to select participants randomly from people living in Bushehr, southwestern part of Iran. Standardized questionnaire of GA²LEN were completed by 5201 participants after personal interviewing. Logistic regression analyses were used to examine the association between variables and risk factors of CRS.
**Results:** The overall prevalence of CRS was 28.4%. It was slightly higher among females (28.3%) than males (28.2%) (P = 0.4). CRS was more prevalent in smokers, 25-34 age range group, individuals with a history of asthma, allergic rhinitis or eczema, non-educated persons and health care workers (P < 0.05).
**Conclusions:** Bushehr had the highest prevalence of CRS in the world. Therefore, CRS can pose high economic burden on the population and health system. Our study also provides important information for the development and promotion of public health policies associated with CRS particularly in developing countries.

**Key words:** chronic rhinosinusitis, EPOS criteria, GA²LEN study, prevalence

ICIA2018\Allergy and immunotherapy\Oral\8244
**8244**

# Evaluation of the efficacy of nicotine in the treatment of allergic asthma in BALB/c mice

Ebrahim Mazloomi[1], Behrooz Ilkhanizadeh[2], Ahad Zare[3], Adel Mohammadzadeh[4], Nowruz Delirezh[5] Shahram Shahabi[1*]

1. *Cellular and Molecular Research Center, Urmia University of Medical Sciences, Urmia, Iran*
2. *Department of Pathology, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran*
3. *Immunology, Asthma and Allergy Research Institute, Tehran University of Medical sciences, Tehran, Iran*
4. *Departments of Genetics and Immunology, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran*
5. *Department of Cellular and Molecular Biotechnology, Institute of Biotechnology, Urmia University, Urmia, Iran*

**Background:** Nicotine, an nAChR agonist, shows prominent anti-inflammatory properties, and some studies have shown its suppressive effects on inflammation. Here, we examined whether nicotine as a medicine has beneficial effects on asthma treatment in a mouse model of allergic asthma

**Methods:** BALB/c mice were sensitized with OVA and alum. Two weeks later, the mice received nicotine in concentrations of 1 and 10 mg/kg three times. After 10 days the mice were challenged with OVA (5 percent) using an ultrasonic nebulizer and sacrificed the next day. To evaluate Treg cells proliferation against the allergen, splenocytes were cultured with OVA; the cells were stained with anti-mouse CD4 and FOXP3 antibodies 72 hours later.

**Results:** Our results showed that the administration of nicotine reduced lung-tissue inflammation, number of eosinophils in bronchoalveolar fluid, allergen-specific IgE and IL-4 production, while it increased the TGF-β/IL-4 ratio and Treg cells proliferation against the allergen. Our results showed that nicotine exerts its suppressive effects in a dose-dependent manner: administration of 10 mg/kg of nicotine showed more suppressive effects than 1 mg/kg

**Conclusion:** These data suggest that nicotine, by decreasing allergic inflammation severity and potentiating Treg cells proliferation against the allergen, could be used as a medicine in the treatment of allergic asthma by.

**Keywords:** Allergic asthma, anti-inflammatory cholinergic pathway, nicotine,nAChRs

ICIA2018\Allergy and immunotherapy\Oral\8594
**8594**

# Ash (Fraxinus excelsior) pollen grains Proteomic approach of most common allergens sensitization pattern in Iranian allergic patients

Maryam Sharifshoushtari[1,2], Ahmad Majd[3], Mostafa Moin[2,4] , Zahra pourpak[2,4], Taher Nejadsattari[1], Shaghayegh Tajik[2], Mohsen Badalzadeh[2], Mohamad Reza Fazlollahi[2], Raheleh Shokohi Shoormasti[2], Behnoosh Tayebi[5], Zakiyeh Azadi[2], Ahad Zare[2],Gholam Ali Kardar[3]

1. *Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
2. *Immunology, Asthma and Allergy Research Institute, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran*
3. *Department of Plant Biology, faculty of biology science, Islamic Azad University, North Tehran Branch, Tehran, Iran*
4. *Department of Immunology and Allergy, Children Medical Center, Tehran University of Medical Sciences, Tehran, Iran*
5. *Royan institute_ institute for Stem Cell Biology and Technology (RI-SCBT), Tehran, IRAN*

**Background:** Ash (Fraxinus excelsior) is a popular tree, climate change patterns and as a cause of winter-spring pollinosis in some regions worldwide. The aim of this research we investigated to determine the profile of main allergen components diagnosis for patients with sensitivity to ash pollen implicated in the common ash pollen allergy.

**Methods:** Ash pollen count analysis and data were documented from 2010 through 2017 in Tehran. Pollen extracts were resolved by one and two-dimensional electrophoresis and assayed with sera of allergic subjects. Sera from three groups, group one: (n=19), patients polysensitized with ash positive specific IgE, group two: (n=10), patients were ash negative, but positive to other pollens and group three: (n=9), sera control. All serum samples did immunoblot analysis with 5% pollen extract.

**Results:** Total pollen counts varied extensively between 87 to 2037 grains/$m^3$/24 h. The allergens with apparent molecular masses ranging from nine to 70 kDa were detected. Generally, twelve spot allergens have been identified in ash pollen while just one allergen has been novel allergen in hsp70 KDa. The interesting result is; all patients of group two sera have shown positive reaction with ash extract in western blot.

**Conclusion:** According to results, it seems the local F.excelsior extract is difference with source extract used for developed RIDA strip and also prick test solution and/or this is cause of different result of commercial RIDA and this research western blot on group two samples. Maybe we should be overview in our diagnosis methods for avoiding to false negative and also false positive results.

**Keywords:** Proteomic approach, Ash pollen, Mass Spectrophotometry, Allergen

ICIA2018\Allergy and immunotherapy\Oral\9822
**9822**

# An asthma-associated IL4R variant exacerbates airway inflammation by promoting conversion of regulatory T cells to TH17-like cells

Massoud AH[1,2], Charbonnier LM[1,2], Phipatanakul W[1,2], Chatila TA[1,2].

*1. Division of Immunology, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts, USA.*
*2. Department of Pediatrics, Harvard Medical School, Boston, Massachusetts, USA.*

**Background:** Mechanisms by which regulatory T ($T_{reg}$) cells fail to control inflammation in severe asthma remain poorly understood. A glutamine- (Q) to arginine (R) substitution at position 576 of IL-4Rα (IL-4Rα-Q576R) has been linked to asthma exacerbation and severity.

**Methods:** By utilizing a mouse model of airway inflammation in IL-4Rα-Q576R mice we demonstrated an aberrant increase of IL-17 and IL-6 expression in mutated Treg cells (by flow cytometry), associated with skewing of Treg cell to the T helper ($T_H$) 17 cell fate. $T_{reg}$ cell lineage-specific deletion of RAR related orphan receptor C (Rorc) reverses the aggravated airway inflammation in $Il4ra^{R576}$ mice.

**Results:** We here show the IL-4Rα-R576 mutation enables recruitment of the adaptor GRB2 to promote airway inflammation by a novel mechanism involving IL-4-directed $T_{reg}$ cell differentiation towards the T helper ($T_H$)17 cell lineage.

**Conclusion:** This study elucidates a novel mechanism for the development of mixed $T_H2$ and $T_H17$ cell responses relevant to asthma and other allergic inflammatory diseases. By activating an additional branch of IL-4R signaling initiated by the adaptor protein GRB2, an IL-4Rα polymorphism associated with asthma exacerbations and severity potentiates $T_H17$ and to a lesser extent $T_H2$ responses.

**Keywords:** Asthma, Regulatory T cells, $T_H17$

# Cancer Immunology

## Oral Presentation

ICIA2018\Cancer Immunology\Oral\7634
**7634**

# The significance of CD45RO+ tumor-infiltrating lymphocytes in breast cancer

Simin Ahmadvand[1, 2], Zahra Faghih[2], Akbar Safaei[3], Mehdi Montazer[3], Abbas Ghaderi[1, 2*]

1. *Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
3. *Department of Pathology, School of Medicine, Shiraz University of Medical Sciences, Iran*

**Background:** Breast cancer (BC) is the most common and the second leading cause of cancer related deaths among women worldwide. The presence of lymphocytic infiltrates in breast tumors have long been proved and seems to have prognostic impact on patients' outcome.

**Methods:** The study population included 94 females with invasive ductal carcinoma of the breast who received surgical resection between 2009 and 2011 in Shahid Faghihi Hospital of Shiraz University of Medical Sciences, Iran. Four µm sections were prepared from their FFPE tissue blocks and immunohistochemically stained for CD3, CD8, CD45RO and Foxp3 molecules. Digital photos were taken from both, center (CT) and invasive margins (IM) of tumors by an expert pathologist and number of the positive cells were counted by FIJI image analyzer. Differences in cells' frequencies among different clinicopathological features of the disease as well as recurrence were analyzed by Mann-Whitney U test.

**Results:** We observed that higher frequencies of CD45RO+ TILs as well as other subpopulations were associated with lymph node involvement, high grade, advanced stages, ER negativity and HER2 positivity. The frequency of CD45RO+ TILs was significantly higher in relapse free patients ($P<0.05$).

**Conclusion:** more TILs infiltration was associated with more aggressive features of BC, additionally our results implied that increase in frequency of CD45RO+ TILs which probably enriched for effector/memory cells could reduce the risk of post-operative risk of relapse.

**Keywords:** Breast cancer, CD45RO+tumor-infiltrating lymphocytes

ICIA2018\Cancer Immunology\Oral\7746
**7746**

# Functional study of $CD25^{-}FoxP3^{+}CD127^{-}CD4^{+}$ cells in tumor draining lymph nodes of breast cancer patients

Zahra Faghih[1], Andisheh Niakan[1, 2], Abbas Ghaderi[1, 2]

*1. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
*2. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Tumor-derived $CD4^{+}CD25^{+}FoxP3^{+}$ Tregs have been extensively studied in different types of cancer including breast cancer (BC) and in most cases, they are associated with tumor progression mostly through suppressing immune responses at tumor sites. Recently, a $FoxP^{+}$ subtype with $CD4^{+}CD25^{-}FoxP3^{+}$ phenotype has been observed in some diseases especially autoimmune diseases. As little is known about their function in cancer patients, in this study, their frequency as well as their ability to produce cytokines were investigated in TDLNs of BC patients.

**Methods:** Mononuclear cells were isolated from auxiliary lymph nodes of 20 BC patients. Following activation with PMA/Ionomycin in the presence of Golgi inhibitors, the cells were stained for CD25, CD4, CD127, FoxP3, IFN-γ, IL-2, IL-22, IL-17 and IL-10 and acquired on four-color flow cytometer. Data were analyzed by CellQuest Pro software.

**Results:** Our analysis showed that less than 1% of $CD4^{+}CD25^{-}FoxP3^{+}$ cells produced cytokines which were significantly lower than $CD4^{+}CD25^{+}FoxP3^{+}$ regulatory and $CD4^{+}CD25^{+}FoxP3^{-}$ effector subpopulations. Nevertheless, the MFI of IFN-γ and IL-2 in $CD4^{+}CD25^{-}FoxP3^{+}$ subset was significantly higher than the MFI of these cytokines in Tregs; but lower than effector subset. On the other hand, the MFI of IL-22 in Tregs was significantly higher than in $CD4^{+}CD25^{-}FoxP3^{+}$ population. The MFI of IL-10 in $CD4^{+}CD25^{-}FoxP3^{+}$ subset was significantly higher than in effector cells.

**Conclusion:** Our results showed that $CD4^{+}CD25^{-}FoxP3^{+}$ cells in TDLNs of BC patients have an intermediate phenotype between effector and regulatory cells, since they produce more IL-2 and IFN-γ than Tregs and more IL-10 than effector cells. These cells are probably a heterogeneous population of effector and regulatory T cells and/or a subset of dysfunctional Tregs which have lost their CD25 expression because of chronic stimulation, high suppressive function and repeatedly exposure to tumor antigens in tumor microenvironment.

**Key words:** Breast cancer, TDLNs, Treg, $CD4^{+}CD25^{-}FoxP3^{+}$ T cells.

ICIA2018\Cancer Immunology\Oral\7845
**7845**

# MicroRNA-330 inhibit colorectal cancer proliferation and migration

Behzad mansoori[1, 2], Ali Mohammadi[1], Solmaz Shirjang[1], Behzad Baradaran[1]

1. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** miR-330 has been reported in several cancers, the cellular and molecular mechanism of miR-330 in colorectal cancer remains unclear. A recent study suggested that miR-330 could be one of the key regulators of CRC. The aim of this study is to investigate the role of miR-152 in CRC and its mechanisms.

**Methods:** The pCMV-GFP-miR-330 construct was transfected into SW-480 cells. The miR-330 positive cells were selected by Geneticin antibiotic (G418) for two-weeks. To evaluate the effect of miR-330 on the colorectal cancer cell proliferation and migration, MTT and scratch wound healing assays were performed, respectively. Then, the expression level of miR-330 and the inhibitory effect of miR330 on caspase3 expression was evaluated by the qRT-PCR.

**Results:** The induction of miR-330 in SW-480 cells was confirmed by GFP channel imaging system and the 10 fold increase in miR-330 expression in stable cells. MTT and wound healing assays showed that miR-330 have anti-proliferation and cell migration effects in stable miR-330 colorectal cancer cell line compared to control group. The result of qRT-PCR on caspase3 expression showed miR-330 could decrease the expression level of miR-330 in stable miR-330 cells compared to control cells.

**Conclusions:** As the results displayed miRNA-330 could regulate the colorectal cancer proliferation and migration by targeting the caspase3 mRNA. Also the result suggested that miR-330 can be a therapeutic molecule in target therapy of colorectal cancer.

**Keywords:** miR-330, Colorectal cancer, Proliferation, Migration, Caspase3

ICIA2018\Cancer Immunology\Oral\7911
**7911**

# Increased frequency of CD4$^+$ and CD8$^+$ T-cell expressing CD11c and PD-1 in lymphoid organs of mice with colorectal cancer

Davood Rostamzadeh[1,2], Ahmad Hosseini[2], Zohreh Babaloo[3], Alireza Rezvani[4], Zahra Mojtahedi[2]

*1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
*2. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Science, Shiraz, Iran*
*3. Tabriz, Iran. Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
*4. Department of Hematology, Medical Oncology and Stem Cell Transplantation, Hematology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** CD11c is an alpha integrin classically employed to define myeloid dendritic cells. Recent reports identified CD11c-expressing CD8$^+$ T-cells as a new subset of CD$^{\wedge+}$ regulatory T-cells. There is some evidence that CD11c$^+$CD8$^+$ T-cells may exert their effector functions or regulatory functions in different conditions. Until now, there have no studies published in which the frequency of CD11c$^+$ T-cells in cancer. There is also limit evidence on expression of immune-checkpoint receptor programmed cell death-1 (PD-1) in mouse lymphoid organs, especially in bone marrow. Therefore, we analyzed the frequency of CD11c$^+$CD8$^+$ and CD11c$^+$CD4$^+$ T-cells as well as PD-1 expressing CD4$^+$ and CD8$^+$ T-cells in different tissues of young, mature and colorectal cancer-bearing (CRC) mice.

**Methods:** C57BL/6 mice divided into three groups, including young mice (3-4 weeks), mature mice (13-14 weeks old) and CRC mice. Mice were sacrificed and frequency of immune cells in bone marrow, lymph nodes and spleen were analyzed by flow cytometry.

**Results:** In contrast to young and mature mice, CRC mice displayed higher percentage of CD11c$^+$CD8$^+$ and CD11c$^+$CD4$^+$ T-cells in bone marrow than those compared to lymph nodes and spleen. Interestingly, higher percentage of CD11c$^+$CD8$^+$ T-cells, but not CD11c$^+$CD4$^+$ T-cells was observed in bone marrow of CRC mice in compared to lymph nodes and spleen. We also higher frequency of CD8$^+$PD-1$^+$ T-cells in BM, spleen and LN of CRC mice compared with young and mature mice.

**Conclusion:** As T-cell exhaustion is associated with inhibitory receptor PD-1 and according to regulatory roles of CD11c expression CD8$^+$ T-cells, elevated percentage of CD11c and PD-1 expressing T-cells are related with dampens antitumor immunity.

**Keywords:** CD11c$^+$CD8$^+$ T-cells, CD11c$^+$CD4$^+$ T-cells, PD-1, Colorectal cancer

ICIA2018\Cancer Immunology\Oral\8329
**8329**

## CD4$^+$Treg cells in tumor draining lymph nodes of bladder cancer patients

Zahra Faghih[1], Yasmin Vahidi[1], Maryam Fakhimi[1], Ardalan Asadollah pour[2], Shahriar Zeighami[2], Ali Ariafar[2,3]

*1. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
*2. Department of Urology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
*3. Urology-Oncology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Regulatory T cells (Tregs) are a suppressive subset of CD4$^+$ T cells which play an important role in the regulation of immune responses. It has been also demonstrated that Tregs at tumor sites could significantly suppress immune responses, leading to immune tolerance of tumor cells. In the present study, we investigated the frequency of CD4$^+$ Tregs in tumor draining lymph nodes of patients with bladder cancer (BLC).

**Methods:** Mononuclear cells were isolated from lymph nodes of 35 untreated BLC patients subjected to radical cystectomy and stained for CD4, FoxP3, CD25, and CD127. The cells were then acquired on four-color flow cytometer and data were analyzed by CellQuest Pro software. The frequency of differentcell subtypes i.e. CD25$^+$FoxP3$^+$CD127$^{low/-}$ and CD25$^-$FoxP3$^+$CD127$^{low/-}$ were assessed in CD4$^+$ cells.

**Results:** The frequency of Tregs with CD4$^+$CD25$^+$CD127$^{low/-}$Foxp3$^+$ phenotype is 57.40±2.92 in draining lymph nodes of BLC patients though no significant difference was found in their prevalence in patients with different clinicopathological characteristics. However, statistical analysis showed that the frequency of CD4$^+$CD25$^-$CD127$^{Low/-}$Foxp3$^+$ cells significantly increased in involved lymph nodes (p=0.027). Additionally, CD4$^+$Foxp3$^+$ cells (p=0.021) and CD4$^+$CD25$^-$CD127$^{Low/-}$Foxp3$^+$ cells (p=0.017) increased in patients with positive perivesical fat invasion.

**Conclusion:** Collectively, our results indicated that regulatory cells in draining lymph nodes of bladder cancers are probably a heterogeneous population with various phenotypes. Previous studies suggested that CD4$^+$CD25$^-$CD127$^{Low/-}$Foxp3$^+$ cellscan be a subset of dysfunctional Tregs which have lost their CD25 expression because of chronic stimulation, high suppressive function and repeatedly exposure to tumor antigens in tumor microenvironment. Increasing in the frequency of this subsets in patients with involved nodes suggested an inhibitory role for these cells in BLC.

**Key words:** Bladder cancer, lymph node, regulatory T cells

ICIA2018\Cancer Immunology\Oral \8414
**8414**

# Comparison of the immunomodulatory effects of isolated stage 2 and stage 4 carcinoma associated fibroblasts in mouse breast cancer model

Ladan Langroudi[1], Zuhair MohammadHassan[2], MasoudSoleimani[3], Seyed Mahmoud Hashemi [4]

1. *Department of Immunology, School of Medicine, Kerman University of Medical Sciences, kerman, Iran*
2. *Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
3. *Department of Hematology, Faculty of Medical Sciences Tarbiat Modares University, Tehran, Iran*
4. *Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** studies have shown that the microenvironment of solid tumors affect the development and progression of cancer. Additionally they are the cells responsible for immune evasion and drug resistance of cancer cells. However the crosstalk between CAFs and the immune system is still unknown.

**Methods:** in this study, we characterized and compared the phenotype and the immunomodulatory properties of stage 2 and 4 CAFs isolated from mammary tumor of Balb/C mouse. **Results:** Flow cytometry of the surface marker panel showed that stage 4 CAFs express significantly higher levels of HLA-DR. Also Higher levels of IL10, COX-2 and MMP9 enzyme were also observed in stage 4 CAFs. Whereas in stage 2 CAFs higher amounts of TFG-β and IDO expression was observed.

**Conclusion:** CAFs represent the supporting stroma of cancer. They are known responsible for immune evading and growth of cancer. Functional differences showed by their surface markers, cytokine and enzyme production indicates to induction of different microenvironments in their presence. The discrepancy observed in cancer therapies may be attributed to the. Therefore, further studies are required to fully elucidate the role of CAFs in various stages of cancer.

**Keywords:** Carcinoma Associated Fibroblasts, Breast Cancer, Immunomodulatory Properties

ICIA2018\Cancer Immunology\Oral\8176
**8176**

# Silencing of Tim-3 Expression by miR-326 Affects Apoptosis and Proliferation of Human HL-60 Leukemia Cell Line

Maryam Mohammad-Ganji[1], Mazdak Ganjali khani-Hakemi[1*], Vida Homayouni[2], Abbas Rezaei[1], Hossein Khan-Ahmad[3]

1. *Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
2. *Acquired Immunodeficiency Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*
3. *Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background:** Leukemia Stem Cells (LSCs) are the main reason for drug-resistance and disease relapse in Acute Myeloid Leukemia (AML). Current drugs destroy normal Hematopoietic Stem Cells (HSCs) rather than LSCs. T cell immunoglobulin mucin-3 (TIM-3), an immune regulatory molecule, is a CD34+CD38- LSCs specific surface markerwith high expression on these cells compared to HSCs. The interaction between TIM-3 and its ligand, Galectin-9 (Gal-9), mediates signaling pathways involved in apoptosis and proliferation. We hypothesized that miR-326 could have a suppressive activity on TIM-3 expression and hence, affects the proliferation and apoptosis processes in AML cells.

**Methods:** Bioinformatics predictions were done using Mirwalk and Target Scan softwares. TIM-3 expression was induced on HL-60 cells by PMA. After miR-326 transfection, MTT, q-RT-PCR, flowcytometery were performed to evaluate the cells survival and TIM-3 expression level. Then, after adding recombinant Galectin-9, apoptosis and proliferation rates were measured with Annexin-V and CFSE assays, respectively.

**Results:** Flow cytometry assay confirmed our bioinformatics prediction of suppressive effect of miR-326 on TIM-3 expression (66.4% silencing) in HL-60 cell line (p=0.002). The qRT-PCR results were also confirmatory. Annexin-V and MTT assays showed increased cell apoptosis and decreased cell survival, while data from CFSE assay indicated a severe reduction in HL-60 cells proliferation.

**Conclusion:** Our results demonstrated that, miR-326 can silence TIM-3 expression in AML HL-60 cells. Moreover, it is shown that miR-326 can enhance AML cells apoptosis and reduce their proliferation and survival and hence, might be considered as a novel target for therapeutic approaches against AML.

**Keywords**: Acute myeloid leukemia; T cell immunoglobulin mucin-3; microRNA-326; proliferation; apoptosis; .Galectin-9

ICIA2018\Cancer Immunology\Oral\8320
**8320**

## Immunomodulation Effect of Gastro-spheres Through Disturbance of Th17/Treg Balance

Alaleh Rezalotfi[1,2], Ghasem Solgi[*1], Marzieh Ebrahimi[*2]

*1. Immunology Department, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
*2. Dept. Stem Cells and developmental Biology, Cell Sciences Research Center, Royan Institute for Stem Cell Biology and Technology, Tehran, Iran*

**Background:** Gastro-spheres with capacity to form tumor, believed to be responsible forescape from immune-mediated destruction. Adaptive immune system plays a major role in gastric cancer including Th17/Treg cells. Th17 producing IL-17 cells considered as crucial cells in pathogenesis of autoimmune diseases. In opposite, Tregs are immune-suppressive cells, prominent with tumor progression. Although it is indicated the prevalence of Treg/Th17 in the most cancers, their interaction with gastro-spheres have been elusive. The object of this study is to find the effect of gastro-spheres on Th17/Treg balance.

**Methods:** Isolated PBMCs from two healthy donors were cultured with MKN-45 cells and its derived gastro-spheres, as the model for enriched cancer stem cells conditioned medium (CM) through mixed lymphocyte reaction for 5 days at 37˚C with 3 times stimulation. Responder groups expansion were evaluated by CFSE and the percentages of $CD4^+CD25^+FOXP3^+$Treg cells and $CD4^+IL\text{-}17^+$ Th17 cells were analyzed by flowcytometry. ELISA measurement also performed for cytokines IL-17, TGF-β and IL-10.

**Results:** Flowcytometry analysis revealed an increase in percentages of Tregs and Th17 cells in PBMCs treated with gastro-spheres compared to parental cells CM. It was also demonstrated that T cell expansion was insignificantly decreased in gastro-spheres CM compared to parental and control group. ELISA measurement also revealed an increase in concentration of IL-10 and IL-17 but a decrease in TGF-β in gastro-spheres CM.

**Conclusion:** We concluded that gastro-spheres CM may increase the percentage of Tregs and Th17 cells more than their parental counterpart. This fact exhibited the possible existence of differentiation factors among gastro-spheres secretion, which contribute to T cells differentiation. Furthermore, it has been shown that gastro-spheres secretion can be possibly more immunomodulator than its parental and leads to limited T cell expansion.

**Key words**: Regulatory T cells, Th17 cells, Gastro-Spheres

ICIA2018\Cancer Immunology\Oral\8485
**8485**

# Snail-1 silencing inhibits mir-34a and Let-7 expression in esophageal squamous cell carcinoma cell line

Maryam Hemmat zadeh[1,2], Hamed Mohammadi[1,2], Behzad Baradaran[1,2]

1. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** In cancer cells, the transcription factor Snail-1 promotes epithelial-to-mesenchymal transition (EMT), a process related to the emergence of invasion, and cancer progression. The purpose of this study was to evaluate the effects of the Snail-1 specific small interference RNA (siRNA) on the expression of miR-34a and Let-7 in TE-8 human esophageal squamous cell carcinoma cell line, in vitro.

**Methods:** TE-8 cells were transfected with the Snail-1 specific siRNA. The relative expression levels of Snail-1 mRNA, miR-34a, and let-7a were investigated by Quantitative Reverse Transcription PCR. Western blotting was carried out to evaluate the Snail-1 protein level. In vitro migration assay of TE-8 was also performed following the treatment with or without the Snail-1 specific siRNA.

**Results:** It was found that treatment of cancer cells with the Snail-specific siRNA effectively downregulated the expression of Snail-1 in mRNA and protein level. However, it had elevated the expression of miR-34a and let-7a expressions. Furthermore, suppression of Snail-1 led to diminished cell migration.

**Conclusion:** Our findings imply that increased level of miR-34a and let-7a might be associated with downregulation of Snail-1 and, thereby, subsided cell migration.

**Keywords:** Snail-1, miR-34a, Let-7a, Esophageal squamous cell carcinoma

ICIA2018\Cancer Immunology\Oral\9772
**9772**

# Safety of intravenously applied mistletoe extract: results from a phase I dose escalation study in patients with advanced cancer

Seyed Ehsan Asadi[1]*, Ahmad Rahimi[2] ,Matin Aghalar[2] ,Akram Jamali[2]

1. *Phd OF Nursing, Sina Hospital , Isfahan, Iran*
2. *Nursing Student OF Dehaghan University, Isfahan, Iran*

**Background:** Mistletoe extracts have anti-tumor properties and are approved for subcutaneous use in cancer patients. Data on Intravenous application are limited.

**Methods:** An aqueous extract from pine-mistletoe was used to investigate maximum tolerable dose (MTD) and safety of intravenous application. It was infused once weekly for 3 weeks in patients with advanced cancer. Any type of cancer was included; relevant exclusion criteria were concurrent chemo- or radiation therapy. The classical phase I 3 + 3 dose escalation scheme was followed. Predefined dose groups were 200, 400, 700, 1200 and 2000 mg. Maximum planned dose was 2000 mg. With the MTD three more patients should be treated for 9 weeks in order to evaluate intermediate term tolerability. Weekly during the treatment and 1 week later tolerability, clinical status, safety laboratory parameters and adverse events were documented.

**Results:** Twenty-four patients (3 in the dose groups 200, 400, 700 and 1200 mg, respectively, 10 in the dose group 2000 mg) were included. MTD was not reached. Because one dose-limiting toxicity (DLT), an allergic reaction, occurred during infusion of 2000 mg, three more patients had to be included in this dose group and tolerated it, as well as the three patients who received 2000 mg for 9 weeks. Occasionally in the dose group 2000 mg mild to moderate fever occurred.

**Conclusion:** Weekly infusions of 2000 mg of the pine-mistletoe extract were tolerated and can be used in further studies but had a risk for allergic reactions and fever.

**Keywords:** Cancer, Clinical trial, Intravenous, MTD, Side effects, Viscum album

# Cancer Immunotherapy

## Oral Presentation

ICIA2018\Cancer Immunotherapy\Oral\5244 cancer immuno
**5244**

# Polyclonal antibody against different extracellular subdomains of HER2 induces tumor growth inhibition in vitro

Reza Hosseini-Ghatar[1], Tahereh Soltantoyeh[1], Motahareh Bahadori[2], Jalal Khoshnoodi[1], Forough Golsaz-Shirazi[1], Mahmood Jeddi-Tehrani[2], Mohammad Mehdi Amiri[1*], Fazel Shokri[1,2*]

1. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
2. *Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*

**Background:** Human epidermal growth factor receptor 2 (HER2) has a crucial role in several malignancies and is overexpressed in 20-30% of patients with breast cancer. The extracellular domain of HER2 (HER2-ECD) has been extensively employed as an important target in passive and active immunotherapy. Isolated recombinant prokaryotic HER2-ECD subdomains were previously found to be ineffective to induce anti-tumor antibody response. In this study, we employed recombinant eukaryotic HER2-ECD subdomains to raise anti-HER2 antibodies and to determine their anti-tumor activity in vitro.

**Methods:** Two paired subdomains of HER2-ECD (DI+II and DIII+IV), which represent Pertuzumab and Trastuzumab binding domains, respectively, along with the full extracellular domain of HER2 were generated in eukaryotic CHO cells. Polyclonal antibodies were raised against these subdomains and characterized using ELISA, flow cytometry, immunoblot and their anti-tumor activity was assessed by XTT assay. The cross-reactivity of these antibodies with other members of the human HER family was also determined.

**Results:** Anti-DI+II and DIII+IV polyclonal antibodies react with recombinant HER2-ECD and native HER2 expressed on tumor cells similar to Trastuzumab and anti-HER2-ECD antibody. These two polyclonal antibodies were able to inhibit binding of Pertuzumab and Trastuzumab to HER2, respectively, and did not cross-react with other members of HER family. Assessment of the functional activity of these antibodies shows that they inhibit tumor cells growth in vitro, similar to Trastuzumab.

**Conclusion:**High immunogenicity of human HER2 DI+II and DIII+IV subdomains in rabbits and the tumor inhibitory activity of the purified specific antibodies imply that they might be suitable for active immunotherapy in formulation with appropriate adjuvants and in combination with other HER2 specific therapeutics.

**Keywords:** Breast cancer, subdomains of HER2, polyclonal antibody, immunotherapy

ICIA2018\Cancer Immunotherapy\Oral\7402 cancer immuno
**7402**

# Vaccination with Human Amniotic Epithelial Cells Confer Effective Protection in a Murine Model of Colon Adenocarcinoma

Meraj Tabatabaei[1], Nariman Mosaffa[1], Roya Ghods[2,3], [4], Somaieh Kazemnejad [5], Manijeh Khanmohammadi[5], Ebrahim Mirzadeghan[5], Ahmad-Reza Mahmoudi[4], Mohammad-Reza Bolouri[4], Reza Falak[4], Bahareh Keshavarzi[1], Mehrnoosh Ramezani[6], Amir-Hassan Zarnani[7,4]

*1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
*2. Oncopathology Research Center, Iran University of Medical Sciences, Tehran, Iran.*
*3. Department of Molecular Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medicine Sciences, IUMS, Tehran, Iran.*
*4. Immunology Research Center (IRC), Iran University of Medical Sciences, Tehran, Iran.*
*5. Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.*
*6. Department of Biochemistry, school of medicine, Ardabil University of Medical Science, Ardabil, Iran.*
*7. Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.*

**Background:** The immunologic similarities between cancer development and placentation have helped researchers to unravel molecular mechanisms responsible for carcinogenesis and to take advantage of stem cells from reproductive organs to elicit robust anti-cancer immune responses.

**Methods:** Human amniotic epithelial cells (hAECs) were isolated and characterized by assessment of their multi-lineage differentiation potential and expression of mesenchymal, hematopoietic and embryonic markers. Their effectiveness as a preventive and therapeutic vaccine in a mouse model of colon cancer was then evaluated by profiling of tumor growth parameters, cross-protective specific CTL and antibody responses, frequency of T cell subsets and cytokine profile.

**Results:** hAECs expressed markers of mesenchymal and embryonic origin, namely cytokeratin, CD9, CD10, CD29, CD73, CD105, HLA-I, HLA-G, STRO-1, SSEA-4 and OCT-4 with considerable capacity to differentiate toward chondrocytes and osteocytes. Vaccination with both CT26 and hAECs resulted in complete inhibition of tumor development in all vaccinated mice. Similarly, mice vaccinated with hAECs were significantly protected against melanoma, B16F10, challenge. Vaccination with hAECs led to expansion of systemic and splenic cytotoxic T cell population and induced tumor specific cross-protective cellular immunity against CT26 cells with increased splenic IFN-γ production in response to CT26 challenge. Interestingly, cross-reactive antibodies were generated against CT26 cells after vaccination with hAECs. As therapeutic vaccine, hAECs immunization reduced tumor burden leading to expansion of peripheral blood and splenic CD8+ T cells and induction of Th1 cytokine profile.

**Conclusion:** The results of our study clearly demonstrated that hAECs possess the potential for being used as an effective prophylactic vaccine for immune prevention of colon cancer most probably due to the shared antigenic determinants.

**Key words:** Human amniotic epithelial cells, Cancer vaccine, Colon cancer, Immunotherapy, Anti-tumor immunity

ICIA2018\Cancer Immunotherapy\Oral\7820 cancer immunotherapy
**7820**

## Metabolic reprogramming in T cells reduces Treg cells and improves the PD-1 blockade cancer immunotherapy

Maryam Akrami Somea Bozorg, Kenji Chamoto, Tasuku Honjo

*Department of Immunology and Genomic Medicine graduate School of Medicine*

**Background:** Tumor microenvironment is profoundly immunosuppressive. FoxP3$^{+}$ regulatory (Treg) T cells represent a major barrier to effective antitumor immunity and immunotherapy. Consequently, there has been considerable interest to target Foxp3$^{+}$Tregcells in tumor. There are various signaling and metabolic pathways to induce instability of FoxP3 expression and to convert Foxp3$^{+}$Treg cells into Foxp3-lost T cells (ex-FoxP3 T cell). Unlike Foxp3$^{+}$Treg cells, the ex-FoxP3 cells express an activated-memory T cell phenotype, and produced inflammatory cytokines.Therefore targeting Foxp3$^{+}$ instability has been potential therapeutic strategy for antitumor immunotherapy. Some reports showed that instability of Foxp3 expression might be associated with PD-1 signaling and metabolic pathways. However, from these aspects, exact connecting pathways between Foxp3 stability and PD-1 blockade is unknown.

**Methods:** Here we investigated the effect of metabolic modulators in Foxp3 stability during the PD-1 blockade therapy using mice carrying the Foxp3-GFPcre knock-in (KI) locus and Rosa26-tdRFP transgene, which can present ex-Foxp3 cells as a GFP$^{-}$RFP$^{+}$ cells.

**Result:** We followed the history of Foxp3 expression during the combination therapy of PD-1 blockade and mTOR inhibitor, AMPK inhibitor, HIF-1a inhibitor, and Foxo1 inhibitors. Among them, only Rapamycin reduced the Foxp3$^{+}$ T cells, but not others tested. Interestingly, when Foxp3 was reduced, ex-Foxp3 increased, indicating that Foxp3 cells were converted to ex-Foxp3 by the inhibition of mTOR signaling.

**Conclusion:** Therefore combination of PD-1 blockade and metabolic modulator targeting Treg is a novel strategy, and our results shed light on improving PD-1 blockade cancer immunotherapy.

**Keywords:** ex-Foxp3, PD-1, T regulatory

ICIA2018\Cancer Immunotherapy\Oral\7988 cancer immuno
**7988**

# Myeloid-Derived Suppressor Cells suppress antitumor immune responses through STAT3 expression in patient with breast cancer

Elham Safarzadeh[1, 2], Shahryar Hashemzadeh [3], Behzad Mansoori[1], Ali Mohammadi[1], VahidKhazeShahgoli[1], Milad Asadi[1], Elham Bagbani[1], Behzad Baradaran[1,2]*

1. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Faculty of Medicine, Department of Immunology, Tabriz University of Medical Sciences, Tabriz, Iran*
3. *General and Vascular Surgery Department of Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** The capacity of tumors to evade immune-mediated destruction is recently included in the list of hallmarks of tumor development and progression. Given the role of expansion and proliferation of immunosuppressive cells within the tumor microenvironment, these cells remain under investigation in cancers. Myeloid-derived suppressor cells (MDSCs), a heterogeneous population of myeloid cells, are thought to be a major subset of cells that induce an immunosuppressive tumor microenvironment. The purpose of this study was to characterize the different subsets of MDSCs and determine their level and function in the circulation of breast cancer patients.

**Methods:** We analyzed the frequency of MDSCs in the freshly-isolated peripheral blood mononuclear cell (PBMC) of patients with breast cancer or healthy donor by flow cytometry. These MDSCs were further characterized for phenotype using anti-human monoclonal fluorescently-labeled antibodies. Then effects of MDSCs on the CD3+T cell response were evaluated. STAT3 is considered as an important transcription factor for MDSC expansion and function, so we assessed the effect of inhibition of STAT3 signaling on MDSC suppressive activity.

**Results:** Our results showed significant increases in circulating HLA-DR – CD33+ MDCSs in breast cancer patients when compared with healthy donors. Moreover, co-culture experiments of magnetically purified HLA-DR – CD33+ MDCSs and CD3+ T cells indicated that accumulation of MDSCs was associated with defective T cell function. Of note, STAT3-targeted siRNA abolished MDSC's suppressive activity.

**Conclusion:** This finding suggest that MDSCs play an important role in breast cancer and future extensive validation studies and progress in this field may help identify new immunotherapeutic strategies to inhibit MDSC function.

**Keywords:** Breast cancer, Myeloid-derived suppressor cells, Immunosuppressive cells, tumor microenvironment

ICIA2018\Cancer Immunotherapy\Oral\8076 cancer immuno
**8076**

## Inhibition of tumor growth by mouse ROR1 specific antibody in a syngeneic mouse tumor model

HadiHassannia[1], Mohammad Mehdi Amiri[1*], FarhadJadidi-Niaragh[2], Reza Hosseini-Ghatar[1], Jalal Khoshnoodi[1], Ramezan-Ali Sharifian[3], Forough Golsaz-Shirazi[1], Mahmood Jeddi-Tehrani[4], Fazel Shokri[1,4*]

1. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.*
2. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*
3. *Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran.*
4. *Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.*

**Background:** Immunotherapy with tumor-associated antigens (TAAs) is a potentially powerful approach to eradicate tumor cells. The receptor tyrosine kinase-like orphan receptor 1 (ROR1) plays a crucial role for survival of tumor cells and is overexpressed in various malignancies. In the present study, we developed a syngeneic mouse tumor model to assess anti-tumor effect of mouse ROR1 specific polyclonal antibody (pAb) in vivo.

**Methods:** Mouse ROR1 specific antibody was produced in rabbit using recombinant ROR1 protein. Tow mouse tumor cell lines, (4T1 and CT26), were transfected with full length mouse ROR1 construct and stable clones were selected and characterized by immunocytochemistry, Western blot and flow cytometry. In vitro and in vivo anti-tumor activities of anti-ROR1 antibody were assessed by XTT and syngeneic BALB/c mouse model, respectively.

**Results:** We successfully established two mouse ROR1-overexpressing tumor cell lines. The in vitro results indicate that the ROR1p Ab did not significantly inhibit growth of ROR1+ cell lines. One of these cell lines (CT26-ROR1) was implanted in syngeneic BALB/c mice to assess anti-ROR1 tumor inhibitory activity in vivo. The tumor size was significantly reduced in mice treated with ROR1p specific Ab.

**Conclusion:** Our results demonstrated for the first time tumor inhibitory effect of mouse ROR1 specific antibody in a syngeneic mouse tumor model. This model is a promising tool for preclinical assessment of ROR1 therapeutics and investigation of the underlying molecular mechanisms.

**Keywords:** ROR1, syngeneic tumor model, anti-ROR1 antibody, tumor-associated antigen

ICIA2018\Cancer Immunotherapy\Oral\8385 cancer immuno
**8385**

# Evaluation of anti-tumor responses by combination therapy with oxaliplatin and anti-PD-L1 antibody in a mouse model of colorectal carcinoma

Soheila Golchin[1], Seyed Amir Jalali[2]

1. *Department of Immunology, School of Medicine Shahid Beheshti University of Medical Sciences*
2. *Department of Immunology, School of Medicine Shahid Beheshti University of Medical Sciences*

**Background:** Chemotherapy has been widely used in cancer treatment. Chemotherapy drugs remain the backbone of current treatment, but are limited by a narrow therapeutic index, significant toxicities, and frequently-acquired resistance. Oxaliplatin (OXP), a platinum-based chemotherapeutic agent, can promote a favourable immune microenvironment and stimulate anti-cancer immune responses. Exposure to OXP markedly increased expression of the T-cell inhibitory molecule programmed death receptor-ligand 1 (PD-L1). Overexpression of immune checkpoint molecules affects tumor-specific T-cell immunity in the tumor microenvironment, and can reshape tumor progression and metastasis. Many tumors can upregulate PD-L1 expression, thus avoiding immune surveillance and elimination. Many molecularly-targeted agents have not shown curative properties when administered as monotherapies. With that in mind, the current opinion is that combining molecularly-targeted anticancer therapy with conventional cytotoxic chemotherapy will improve treatment outcomes. Important considerations regarding optimizing dosage, sequence, and timing of targeted therapies will be required when designing future clinical treatments.

**Methods:** To determine whether combined treatment affects the local immune cell populations we evaluated the anti-tumor activity of anti-PD-L1 combined with OXP against a murine colon carcinoma in vivo and examined the tumor immune microenvironment by flowcytometric analysis of immune cells from the tumor site, lymph nodes, and spleen.

**Results:** Our study showed that the in-vivo administration of OXP combined with anti-PD-L1 markedly inhibited the growth of murine colorectal carcinoma tumors in mice. The anti-tumor effect of anti-PD-L1 plus OXP correlated with a marked increase in the number of tumor-infiltrating activated CD8+ T cells and a marked decrease in the number of regulatory T cells in drainage lymph nodes and spleen.

**Conclusion:** Mice treated with anti-PD-L1 combined with OXP had smaller tumors and greater survival rates than mice receiving either anti-PD-L1 or OXP alone. We also found that the timing of drug administrations affected tumor responses.

**Keywords:** Anti PD-L1, combination therapy, cancer, immunotherapy, chemotherapy, timing

ICIA2018\Cancer Immunotherapy\Oral\8533 cancer immuno
**8533**

# Combined blockade of PD-1and Tim-3immune checkpoint receptors to restore the function of exhausted CD8+ T cells in chronic lymphocytic leukemia

Hadiseh Rezazadeh[1], Hadi Hossein-Nataj[1], Ramin Shekarriz[2], Mohsen Tehrani[1], Hossein Asgarian-Omran[1,3]*

*1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
*2. Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran*
*3. Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

**Background:** Although, common treatment strategies have improved overall survival in chronic lymphocytic leukemia (CLL), majority of patients continue to relapse and therefore finding a new strategy seems to be necessary. Blocking antibodies against programmed death-1 (PD-1) immune checkpoint receptor have been approved by FDA for treatment of many malignancies. But, in recent studies it was demonstrated that PD-1 blockade alone cannot completely restore the function of exhausted T cells. In the current in vitro survey, combinatorial blockade of PD-1 and T cell immunoglobulin- and mucin-domain-containing molecule-3 (Tim-3) regulatory pathways were done to restore the function of exhausted $CD8^+$ T cells in CLL patients.

**Methods:** Peripheral blood mononuclear cells were separated from fifteen patients with CLL and $CD8^+$ T cells were positively isolated using magnetic beads separation method. Purified $CD8^+$ T cells were treated with either blocking antibodies against PD-1 and Tim-3 and isotype matched control antibodies. Treated $CD8^+$ T cells were stimulated with anti-CD3/CD28 antibodies and PMA/Ionomycin to assess their proliferation capacity and cytokines production by MTT assay and ELISA, respectively. Concentrations of IL-2, IL-10, IFN-γ, and TNF-α were measured in culture supernatants. Degranulation properties of $CD8^+$ T cells were also assessed by CD107a degranulation assay.

**Results:** Our results showed that combined blockade of PD-1 and Tim-3 by inhibitory antibodies reversed the effector functions of exhausted $CD8^+$ T cells and improved their proliferation, cytotoxicity and production of pro-inflammatory cytokines.

**Conclusion:** Considering potential roles of PD-1 and Tim-3 molecules in T cells exhaustion, obtained results from this study in restoring the function of exhausted $CD8^+$ T cells could be a potential support in formation of a new treatment option for patients with CLL.

**Keywords:** Chronic lymphocytic leukemia, exhausted $CD8^+$ T cells, Tim-3, PD-1, immunotherapy

# Computational Immunology and Systems Biology

## Oral Presentation

ICIA2018\Computational Immunology and Systems Biology\Oral\5311
**5311**

# Modeling the tumor growth and production of anti-tumor cytotoxic T lymphocyte after deletion of Myeloid-Derived Suppressor Cells using Differential equation of system

Zahra Mirsanei[1], Nassim Kheshtchin[1], Samaneh Arab[1], Nazanin mojtabavi[2], Jamshid Hadjati[1,*]

1. *Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
2. *Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*

**Background:** The tumor microenvironment is a complex tissue that is participated in anti-tumor activity or suppress antitumor immune responses. The ability of MDSCs (Myeloid-Derived Suppressor Cells) to inhibit the cytotoxic immune responses mediated by NK cells and CTL led to the elimination of these cells as a target for immunotherapy that increases the efficiency of these methods. For eliminate MDSC's inhibitory mechanisms, the use of drugs is effective.

**Method:** 4T1 cells were implanted subcutaneously in right flank of BALB/c mice in two groups: The control group contains 15 mice without any treat and the test group contain 15 mice, which treated by 5-fluorouracil (5-FU) from 5th day (when the tumor was tangible for the first time) by 3 days interval. Tumor growth in the course of treatment and survival of mice after that was followed by measuring tumor size and Immunohistochemistry (IHC) assay was used for counting tumor infiltrating CD8+ cells as a result of MDSCs elimination.

**Result and conclusion:** A class of mathematical models called Ordinary Differential Equation (ODE) model was developed using our aquired empirical data to analyze and simulation the behavior of the immune system in the tumor microenvironment. We show that our model is capable of capturing the observed experimental results, and hence can be potentiallyused in designing future experiments involving this approach to immunotherapy.

**Key Word:** Tumor Modeling, MDSC, ODE

ICIA2018\Computational Immunology and Systems Biology\Oral\7950
**7950**

# Game Theory Modeling as a Novel Methodology to Describing the Host-Microbe Interactions: Mycobacterium Tuberculosis (Mtb) and Host Immune Defense as an Example

Hiva Sharebiani[1], Shadi Abbasnia[1], Sara Hajimiri[1], Rezvan Pilpa[1], Arezo Firoozeh[1], SamanSoleimanpour[2], Kiarash Ghazvini[2], S.A Rahim Rezaee[1]

*1. Inflammation and Inflammatory Diseases Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
*2. Microbiology & Virology Research Center, Bu-Ali Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran.*

**Background:** The interactions between host and microbe determine the outcome of infections. To understand the underlying pathogenesis of infectious diseases, epigenetic study and then the game theory models which describes the outcome (pay-off) from specific interactions (game) between two individuals (players) were considered. In this study, Mtb and host interactions in tuberculosis manifestation were evaluated as a model.
**Methods:** The virulence factors (Ag85A, CFP-10 and ESAT-6) and main host defense strategies (CCR1, CCR2, T-bet, iNOS, IDO, TGF-β, MMP3 and 9) were evaluated by real-time-PCR, TaqMan method. Then using these assessing interactions modeled as a dynamic game with complete and perfect information. The pay-off matrix is designed based on gene expression of host and microbe using the Naive Bayes classifier (NBC) by Rapid minerV5.3 software. Extensive form of game modeled via backward induction and detection of SPE strategies by Min-Max method to determination of Nash equilibrium.
**Results:** Host and Mtb strategies evaluated by Pareto and PSPE efficiency indices as appropriate response markers and classified to dominant, dominated and optimal strategies. Cooperative and grim trigger strategies was defined. Therefore, the mechanisms of leaving the latency or remaining in the same situation, were determined. The optimum levels of gene expression as the best strategies of each player were: Mtb high expression of Ag85 and high level of CCR1 and medium level of CCR2, T-bet, iNOS, IDO and TGF-β that confirmed by MMPs level and patient's outcome as the host protective immune response. The role of each gene in this interaction was investigated. Gene expression changes of TGF-β/IDO, T-bet/iNOS and CCR1/CCR2 were responsible for reactivation or remaining in latency, response to CFP-10: ESAT-6 or Ag85A and patient's outcome, respectively.
**Conclusion:** A rational way for assessing interaction outcomes for vaccine design, therapeutic strategy or prognosis of an infection is epigenetics studies of the players in a time course and then evaluation of different outcomes by game theory model.

**Keywords:** Game theory, Nash equilibrium, Interaction, Epigenetic.

ICIA2018\Computational Immunology and Systems Biology\Oral\7971
**7971**

# Bioinformatics approach to identifying molecular biomarkers in multiple sclerosis

Mina Rezaei[1], Shirin Farivar[2*]

1. *Department of cellular and molecular biology, shahid Beheshti University, Tehran, Iran*
2. *Department of cellular and molecular biology, shahid Beheshti University, Tehran, Iran*

**Background:** Multiple sclerosis (MS) is a chronic autoimmune, inflammatory neurological disease of the central nervous system (CNS). Considering the complexity of its etiopathogenesis, early diagnosis of MS and individualized management are challenging in clinical practice. It is also difficult to obtain CNS samples for diagnostic purposes. Therefore, studies on biomarkers in MS are useful for early prediction and diagnosis, monitoring of disease activity and predicting treatment response.

The present study aimed to analyze the molecular mechanism of MS using microarray analysis combined with bioinformatics techniques.

**Methods:** We downloaded the gene expression profile of MS from NCBI Geo datasets, Gene Expression Omnibus (GEO) and analyzed the microarray data. Genes are compared with logfc for their expression in normal controls and MS patients and sorted by their p-value.

**Result:** Finally gene with most modification in multiple sclerosis is selected for further analysis. Also its most related pathways and gene function are identified by DAVID database and Gene Card database and its protein structure such as secondary and domains are identified through ExPASy database.

**Conclusion:** The result suggested some genes may play central regulatory roles in controlling gene expression in the pathogenesis of MS. Our findings confirm the presence of multiple molecular alterations in MS and indicate the possibility for identifying prognostic factors associated with MS pathogenesis.

**Keywords:** multiple sclerosis, microarray analysis, RNA and protein structure, Gene expression

ICIA2018\Computational Immunology and Systems Biology\Oral\7980
**7980**

# Dissection of neutrophils responses to sepsis via network analysis: From weighted co-expression networks to protein-protein and gene regulatory networks

Rasoul Godini[1], FatemehHadi[1], Hossein Fallahi[1]

*1. Department of Biology, Faculty of Science, Razi University*

**Background:** Infection of blood leads to a clinical condition known as sepsis. Sepsis comes with systemic dysregulation of inflammation. Neutrophils are important immune cells in blood that move to the infection site, catch and digest infectious agents. The importance of neutrophils in sepsis has been shown. Herein, we analysed neutrophil responses to sepsis condition through network based bioinformatics.

**Methods:** Free available dataset GSE49758 was obtained from NCBI. After preprocessing of the genes for adequate variable ones, the matrix analyzed through Weighted Gene Co-expression Network Analysis (WGCNA) method using R program. Septic samples were compared against normal ones to determine differentially expressed genes (DEGs). Using DEGs, the Protein-Protein Interaction (PPI) and Gene Regulatory Network (GRN) were constructed by STRING and ChEA databases, respectively. Visualizing and analyzing of the networks were performed by Cytoscape and Gephi programs software.

**Results:** WGCNA showed that there are 3 module eigengenes which are related to sepsis with a correlation of 50 percent (p-value less than 0.01). Module membership and gene significance correlation in the detected modules was at least 0.45 (p-value = 3.3e-10). The members of modules were related to inflammatory responses, protein transport and signal transduction. For other types of networks, we obtained a list containing 633 DEGs that resulted in a PPI network constructed of 260 nodes and 524 edges. We detected 9 sub-networks by overlap neighborhood expansion methods in PPI. Different hubs were detected in this method such as SMAD3 and IL1B. GRN was constructed by two differentially expressed transcription factors including BCOR and SMAD3 which regulates 79 and 95 target genes, respectively.

**Conclusion:** In this study, we comprehensively analyzed responses of neutrophils to sepsis by various network analysis. The results determined genes and molecular processes which could be focused on in further studies.

**Keywords:** Differentially expressed genes, Protein-protein interaction, Sepsis, WGCNA

ICIA2018\Computational Immunology and Systems Biology\Oral\8463
**8463**

# The power of whole exome sequencing in detection of causative mutation in a lost primary immunodeficient baby based on the whole exome data of its healthy parents

Ali Aghebati Maleki[1], Majid Changi-Ashtiani[2], Taravat Talebi[1], Neda Mohsenpour[1], Mohammad Shahrooei[3-4], Alireza Biglari[1], Tina Shahani[1], Hassan Rokni-Zadeh[5]

*1. Department of Genetics and Molecular Medicine, School of Medicine, Zanjan University of Medical Sciences (ZUMS), Zanjan, Iran*
*2. School of Mathematics, Institute for Research in Fundamental Sciences (IPM), Tehran, Iran.*
*3. Experimental Laboratory Immunology, Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium*
*4. Specialised Immunology Laboratory of Dr. Shahrooei, Ahvaz, Iran*
*5. Department of Medical Biotechnology and Nanotechnology, School of Medicine, Zanjan University of Medical Sciences (ZUMS), Zanjan, Iran*

**Background:** Whole Exome sequencing (WES) is used to efficiently detect common single-nucleotide variants (SNVs) across a broad spectrum of applications such as identification of rare genetic variants that are presumably responsible for complex genetic diseases. Primary immunodeficiency diseases (PIDD) are a group of more than 300 rare, genetic and chronic disorders that the immune system functions improperly. In this study we aimed to find the causative mutation(s) by WES, performed on healthy parent samples of a lost PIDD baby; among the dozens of Iranian families whose children had died due to PIDD and routine diagnostic procedurehave not been able to determine the cause of the illness.

**Methods:** After selecting the appropriate pedigree, extracting gDNA was performed from the parents' blood. Samples were sent to WES and then data were analyzed to find common heterozygous mutations associated with patient phenotype. In order to confirm the results, the candidate mutation segregation in the family was examined by Sanger sequencing method.

**Results:** Variant calling presented about sixty thousand variants in parent data which differed from the human reference genome. Of these variants, we reached two hundred heterozygouscommon variants in parent. Eventually, only one strong candidate variant associated with the disease phenotype was considered according to WES pipeline as the only probable cause of the disease that confirmed by sanger sequencing too. Finally, homozygous transversion of G>T at exon 33 of Dock8 gene was identified as causative mutation at lost baby.

**Conclusion:** Here, we present reliability of WES to infer genomic variants in disorders with genetic heterogeneity, particularly, for detection of causative mutation from healthy parent genomic data. This approach will improve management of PID families when the proband sample is not available. Due to life instability of such patients, this approach is of high value.

**Keywords:** Primary immunodeficiency, Whole Exome Sequencing, Carrier detection

ICIA2018\Computational Immunology and Systems Biology\Oral\9920
**9920**

# Determination and Ranking of Involved Signaling Pathways in Systemic Lupus Erythematosus Using PBMC Transcriptome Profile by Bayesian Network Approach

Samaneh Maleknia[1], Dr.Ali Shirifi Zarchi[2], Dr.Kaveh Kavousi[1]

1. *Department of Bioinformatics Institute of Biochemistry and Biophysics (IBB); Tehran University (UT)*
2. *Department of computer engineering, Sharif University of Technology*

**Background:** In recent years, studying immune system related diseases has a great importance in medicinal research. According to these studies, several specific biological pathways are influenced by different types of disordered immune systems. Many of these studies focus on autoimmune diseases. Computational methods have pivotal roles in generating results and making new discoveries. The aim of this study is to nominate signaling pathways that are specially related to “systemic lupus erythematosus” (SLE) with Bayesian network approach.

**Methods:** The first phase of this study involves performing enrichment analysis on 5 datasets of “PBMC” cells gene expression, with both case and control groups for each dataset. All datasets were downloaded from NCBI. Enrichment analysis was performed based on best-curated signaling pathway databases like KEGG, Reactome, and wiki pathway. More than 20 effective signaling pathways were identified with a significance level of less than 0.05. In the next step, to identify the most affected genes and network modules, Bayesian networks were inferred from involved pathways. These networks were trained using gene expression data.

**Results:** According to the results, most of these pathways are related directly to the immune system. Sufficient data must be provided to permit evaluation by the reviewers and public reading of the abstracts. Statements such as “additional information to be presented at the meeting” are not acceptable.

**Conclusion:** Finally, in the current state, the most influenced subnetworks of pathways are nominated, the most influenced genes (by this approach) were ranked and a validation process was used to select the most related ones.

**Keywords:** Systems biology, Autoimmunity, Bayesian network, Systemic lupus erythematosus

ICIA2018\Computational Immunology and Systems Biology\Oral\8471
**8471**

**Immunology of Glioblastoma**

FatemeShaabanpour Aghamaleki[1], Shirin Farivar[2]

*1. Department of molecular and cellular Biology School of Biotechnology and Life Science University of Shahid Beheshti*
*2. Department of molecular and cellular Biology School of Biotechnology and Life Science University of Shahid Beheshti*

**Background:** glioblastoma is a malignant, high grade and a progressive brain tumor that affects both children and adults. It has a multifactorial pathogenesis such as genetics, epigenetics and environmental factor. But it has genetics originally in 10 percent of pediatric patients. Among this genetics factor, immune system genes may play an important role in glioblastoma pathogenesis, progression, and metastasis. Therefore, identification of these genes can help select the best therapy for glioblastoma.
**Methods:** Gene expression profiling by array of 12 glioblastoma patients and 3 healthy individuals were extracted from Geo datasets. Genes are compared with logFC for their expression between two groups and sorted by their p-value. Also, the most related pathway, location, function, and protein networks of this gene were identified with STRING, GeneCard and DAVID databases.
**Results:** several important immune genes associated with glioblastoma tumors are identified such as CXCL2, NFATC1, TGFB2, PTPN9, FCGR1, XCL1, CCL4 and etc. These genes have hyperexpression in glioblastoma patients. According to DAVID database, all of the immune genes that have hyperexpression in glioblastoma play important roles in the inflammatory response, cytokine and growth factor. Also, their related pathways are chemokine, TNF signaling pathways, and G-protein coupled receptor (GPCR) signaling pathway.
**Conclusion:** Although, there are a wide variety of immune pathways and genes that play important roles in many diseases, some of these pathways and genes are important and specific in some diseases such as glioblastoma. Therefore, identification of these critical and specific immune genes in glioblastoma can lead to appropriate targeting in immunotherapy and chemotherapy.

**Keywords:** glioblastoma, immune system, glioblastoma immunology, glioblastoma therapy

# Diagnostic Methods in Immunology

## Oral Presentation

ICIA2018\Diagnostic Methods in Immunology\Oral\7399
**7399**

# IL-10 as a potential predictor of grave prognosis in premature infant

Farshid Saadat[1], Seyedeh Zohreh Jalali[2]

1. *Department of of Immunology, School of Medicine, Guilan University of medical sciences, Rasht, Iran*
2. *Department of Neonatology, School of Medicine, Guilan University of medical sciences, Rasht, Iran*

**Background:** Prematurity is the direct cause of neonatal death worldwide. To increase survival rates, mechanical ventilation with highly inspired oxygen, corticosteroids and exogenous surfactant therapy are used. The present study was designed to explore the outcome in premature neonate based on the anti inflammatory cytokine levels.

**Methods:** This was a prospective, randomized investigation of infants who were less than 37 weeks of gestational age. Blood samples were collected on day 1 and plasma cytokine was assayed for interleukin (IL)-10.

**Results:** This study showed that the plasma IL-10 levels stand as a promising biomarker for predicting prognosis. Our findings show that the higher IL-10 levels statistically associated to poor outcome after excluded other confounders. ($P<0.001$).

**Conclusion:** Although, further studies are required to elucidate the mechanistic role of IL-10, it might be considered as a valuable marker for outcome assessment.

**Keywords:** Prematurity, interleukin-10, prognosis

ICIA2018\Diagnostic Methods in Immunology\Oral\7689
**7689**

# Developing a specific anti-TAZ (WWTR1) Polyclonal Antibody

Mahsa Mohammadzadeh[1], Fatemeh Ghaemimanesh[2], Mehrdad Hashemi[1], Babak Negahdari[2,3], Ali Ahmad Bayat[2], Reza Hadavi[2], Saeideh Milani[2], Hodjatallah Rabbani[2]

*1. Department of Genetics, Islamic Azad University, Tehran Medical Sciences Branch, Tehran, Iran*
*2. Antibody-Antigen Engineering Department, Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*
*3. Department of Medical Biotechnology, School of Advanced Science in Medicine, Tehran University of Medical Science, Tehran, Iran*

**Background:** TAZ (WWTR1) is a transcriptional co-activator protein in Hippo signaling pathway which plays a pivotal role in organ size control during embryogenesis. TAZ has been shown to be overexpressed in various human carcinomas such as breast, hepatocellular and lung cancers. The aim of this study was to produce a specific polyclonal antibody (pAb) against TAZ to be used as a research tool for further investigation of TAZ characteristics in human carcinomas.
**Methods:** A white New Zealand rabbit was immunized using a Keyhole limpet hemocyanin conjugated synthetic peptide of TAZ. Following the raise of antibody titration, the developed polyclonal anti-TAZ antibody (pAb anti-TAZ) was purified using affinity chromatography. After determining the specificity of pAb in ELISA, its reactivity with TAZ molecule was assessed in different cancer cell lines by various immunoassays.
**Results:** The produced antibody could recognize the immunizing peptide in ELISA. PAb anti-TAZ was able to specifically recognize TAZ in human carcinoma cell lines containing A431, MCF7 and Raji in Western blot, flow cytometry and ICC. Immunohistochemistry results showed that the developed pAb detected TAZ in prostate and bladder carcinoma tissues.
**Conclusion:** The developed anti-TAZ pAb might be used as a research tool in expression profiling of many cancer cells with application of Western blot, flow cytometry, ICC and IHC.

**Keywords:** Polyclonal Antibody, TAZ, cancer, detection

ICIA2018\Diagnostic Methods in Immunology\Oral\8069
**8069**

# Evaluation of Phagocytosis in Human Neutrophils using Enhanced Green Fluorescent Protein (EGFP) expressing E. coli

Hosain AqaHosaini, Sayyed Hamid Zarkesh Esfahani

*1. Department of Biology, Faculty of Sciences, University of Isfahan, Iran*

**Backgrounds:** Phagocytosis plays a very important role in innate immunity and helps body against bacterial infections. Patients who have defect in phagocytosis suffer from recurrent bacterial infections that may be life threatening. It is important to detect the defect in phagocytosis as early as possible in life. Patients who receive immunosuppressive drug may also have suppressed phagocytosis. There are different tests for evaluation of phagocytosis such as NBT and DHR which use chemical compounds not real bacteria to test the phagocytosis. Bacteria such as E. coli can be transformed to express Enhanced Green fluorescent protein (EGFP).

To introduce and evaluate a method for testing Neutrophil's phagocytosis using real bacteria.

**Methods:** EGFP sequence was cloned in an expression vector called pColdI using the enzymes NdeI and XhoI. BL21 strain of E. coliwas transformed by pColdI containing EGFP. Presence of the EGFP sequence was confirmed by PCR and sequencing. The expression of EGFP in bacteria was detected by fluorescent microscope and Flow cytometry. EGFP expressing bacteria were added to heparinized whole blood of either healthy volunteers or patients receiving immunosuppressive medicines. The engulfment and digestion of fluorescent bacteria was detected by flow cytometry at different time points.

**Results:** The E. coli transformed with vector containing EGFP expressed high levels of green fluorescence as detected by both fluorescence microscope and flow cytometry. Neutrophils that engulfed fluorescent bacteria became highly fluorescent as could be detected by flow cytometry. Digestion of bacteria during the time resulted in decrease in Neutrophil's fluorescence.

**Conclusion:** EGFP expressing bacteria and flow cytometry technique can be used to evaluate the phagocytosis capability of neutrophils.

**Key words:** phagocytosis, pColdI, EGFP, E. coli, fluorescent, flow cytometry.

ICIA2018\Diagnostic Methods in Immunology\Oral\8088
**8088**

# Severe Decline in Mir-20a and Mir-92a in the Context of the Mir-17-92 Cluster: Ideal Biomarkers of Various COPD Subtypes

Akbar Ghorbani, Alvanegh Mahmood Tavallaei, Houri Edalat[1]*

1. *Human Genetics Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*

**Background:** chronic obstructive pulmonary disease (COPD) is going to be the third leading cause of death by 2020. Spirometry is laborious that results in a great under-diagnosis of COPD. On the other hand, circulating miRNAs are among the most beneficial feasible non-aggressive biomarkers for the diagnosis and treatment of many diseases. Among members of the significant miR-17-92 cluster, miR-20a and miR-92a are greatly involved in both inflammation and hypoxia (that are known as the main reasons for COPD comorbidities).

**Methods:** Thus, the expression of these miRNAs was evaluated in 26 COPD patients and 19 normal individuals by a highly sensitive approach called stem-loop RT-qPCR. An explanation of the study design and experimental method.

**Results:** The expressions of the studied miRNAs were significantly reduced in the serum of the COPD patients relative to that of the controls ($p<0.001$).

**Conclusion:** This finding may be attributed to the susceptible genetic background of the COPD group and/or the epigenetic changes caused by smoking. Altogether, our findings indicated that the clusteral expression pattern of miR-17-92 reflects the cluster's ideal position as a biomarker of the pathogenesis of COPD subtypes. Decreased expression of miR-20a in a patient might reflect a progressive stage of the disease. MiR-92a might be used as an early-detection biomarker of COPD. A higher reduction level for miR-92a compared with miR-20a might reveal the chronic nature of the disease. The miRNAs can be used as therapeutic targets specifically in the context of the miR-17-92 cluster to address the various clinic pathological aspects of the disease.

**Keywords:** COPD, biomarker, treatment, serum, inflammation, stem-loop real-time quantitative PCR (stem-loop RT-qPCR)

ICIA2018\Diagnostic Methods in Immunology\Oral\8233
**8233**

**Total PSA tumor marker detection based on nano-bio-optical sensor**

Zahra akbari jonoush[1], Reza Mansouri [1], FatemehYazdiyan [2]

1. *Department of Immunology, Shahid Sadoughi University of Medical Sciences & Health Services, Yazd,*
2. *Faculty of New Science and Technology, University of Tehran, Iran*

**Background:** Nowadays, early cancer detection is crucial for improved prognosis and cancer management. Therefore, identification of sensitive and specific methods for early cancer detection is required. One of the methods for early cancer detection are biosensor and gold nanoparticles.

**Methods:** Herein, antibody-coated gold nanoparticles were employed in optical immunoassay for detecting total PSA biomarker. To achieve this goal, antibody was covalently conjugated to gold Nano spheres using 11-Mercaptoundecanoic acid (MUA). Finally, serum sample was investigated by localized surface Plasmon resonances. The results obtained by LSPR and Chemiluminescence assay were compared with each other.

**Results:** The results suggested that Nano bio-optical sensor had good potential and good sensitivity in detecting of total PSA. The proposed Nano bio-optical sensor delivered a good sensitivity with average sensitivity 1.1 nm/ng $ml^{-1}$, and a low detection limit of 0.9 ng/ml

**Conclusion:** These results indicated that the Nano bio-optical sensor has a great potential in diagnosis of cancer biomarkers.

**Keywords:** Nano bio-optical sensor, total PSA biomarker, total PSA monoclonal antibody

ICIA2018\Diagnostic Methods in Immunology\Oral\8656
**8656**

# Determining laboratory reference values of TREC and KREC in different age groups of Iranian healthy individuals

Leila Shakerian[1], Zahra Pourpak[1], Somayeh Shamlou[1], Erna Domsgen[2], Anoshirvan Kazemnejad[3], Hossein Dalili[4], Lennart Hammarstrom[2], Maryam Nourizadeh[1]

1. *Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran*
2. *Department of Laboratory Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden*
3. *Department of Biostatistics, Tarbiat Modares University, Tehran, Iran*
4. *Breastfeeding Research Center, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Assessment of T-cell receptor excision circles (TREC) and kappa-deleting recombination excision circles (KREC) copies has been recently described as biomarkers of thymus and bone marrow's functions, respectively. In this study, first we aimed to explore the effects of demographic variables including age, sex, race as well as weight and height at birth on these two episomal molecules. Secondly, for the first time in our country, we determined the reference values of TREC and KREC copy numbers in different age groups of Iranian healthy individuals as a thresholds for identifying the T cell and B cell lymphopenia.

**Methods:** TREC and KREC copy numbers were evaluated in 251 dried blood spot (DBS) samples from healthy volunteers (age range: 0-60 years), 11 premature newborns (age range: 30-35 weeks) and also six primary immunodeficiency (PID) patients as disease controls using multiplex quantitative real time PCR.

**Results:** TREC and KREC copies were notably reduced with increasing the age. We could not find any significant differences between male and female as well as among various ethnicities in Iran.

**Conclusion:** These finding suggest that demographic variables including age as important interfering factor, should be considered for interpretation of TREC/KREC results.

**Keywords:** T-cell receptor excision circles, kappa-deleting recombination excision circles,primary immunodeficiency disorders.

ICIA2018\Diagnostic Methods in Immunology\Oral\9813
**9813**

# Development and evaluation of specific scFv antibody against human dentin sialophosphoprotein

Seyed Nooreddin Faraji[1,3], Mozafar Mohammadi[3,4], Foroogh Nejatollahi[2,3]

1. *School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Shiraz HIV/AIDS Research Center, Institute of Health, Shiraz University of Medical Sciences, Shiraz, Iran.*
3. *Recombinant Antibody Laboratory, Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran*
4. *Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*

**Background:** Prostate cancer (PCa) is the second leading cause of cancer related death in men. Diagnosis of PCa faces many challenges especially its biomarker-based detection methods. Dentin sialophosphoprotein (DSPP) has been shown with higher specificity and sensitivity for PCa diagnosis compared to current biomarkers.

**Methods:** A single chain antibody (scFv) against DSPP was isolated using the panning procedure. PCR and DNA fingerprint were done following 4th round of panning. The reactivity of the isolated scFv to DSPP was evaluated by phage ELISA on PCa urines (number: 10) and compared to healthy individuals (number: 10). Following plasmid extraction and DNA sequencing, the 3D structure of isolated scFv was simulated base on homology modeling. Docking of the isolated scFv on DSPP was also simulated to investigate the antigen-antibody interaction.

**Results:** The frequency of isolated scFv against DSPP was 40%. Phage-ELISA results showed significant difference between PCa patients (OD: 0.92) and healthy individuals (OD: 0.34). Sequence analysis of the isolated scFv showed several amino acid substitutions in CDRs and FRs of both VH and VL. Docking results showed that the scFvcan tightly bind to the DSPP by several interaction especially by hydrogen bonds between antigen and antibody interface.

**Conclusion:** The results of the new isolated anti DSPP-scFv antibody evaluated by experimental and in silico techniques, introduce a specific and sensitive antibody for designing DSPP-based PCa detection methods.

**Keywords:** Dentin sialophsphoprotein (DSPP), single chain antibody (scFv), prostate cancer (PCa), panning, docking

# Immune Cell Therapy

## Oral Presentation

ICIA2018\Immune Cell Therapy\Oral\8000
**8000**

# Interlukine-27, a Key Player in Rejecting 4T1 Breast Carcinoma, an in vitro and in vivo Experiment

Abdolreza Esmaeilzadeh[1,2], Masoumeh Ebtekar[1], Alireza Biglari[3], Zuhair M. Hassan Saraf[1], Takayoki Yoshimoto[4]

*1. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University Tehran, Iran.*
*2. Cancer Gene Therapy Research Center, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran*
*3. Department of Genetic and Molecular Medicine, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran*
*4. Department of Immunoregulation, Institute of Medical Science, Tokyo Medical University, Tokyo, Japan*

**Background:** Cytokines are the chief participant in regulating immune responses, and cytokine-based cancer therapy have been proved in treating malignancies. Interleukin (IL)-27 is a cytokine from IL-12 family and promotes antitumor immunity by enhancing NK cells, CTLs and through exerting antiangiogenic potential. IL-27 has played an anti-tumoral role in numerous animal tumor models and suppressed tumor development. Here we have assessed whether IL-27 might be a feasible treatment for breast cancer.

**Methods:** In this study, murine IL-27 gene was cloned into expression vector P3XFLAG-CMV-9, and then recombinant plasmid was cloned into 4T1 cell line. We injected 4T1 cells to BALB/c mice to establish the antitumor activity of IL-27 in a syngeneic mouse model of breast cancer. The immunologic effects, anti proliferative function of IL-27 and tumor size have been evaluated. The injected tumoral mice and 4T1 treated cells with P3XFLAG-CMV-9 + IL-27 exhibited a significant reduction in tumor size and 4T1 cell numbers respectively.

**Result and conclusion:** Our results for the first time, suggest that IL-27 efficiently displays capability to suppress breast tumor, through upregulating IFN-γ and granzyme B as well as down regulating IL-4. We interestingly demonstrated that IL-27 can hold notable promise for breast cancer immunotherapy.

**Keywords:** Interleukin (IL)-27, Breast Cancer, immune gene therapy, Anti-tumor

ICIA2018\Immune Cell Therapy\Oral\9758
**9758**

**A new way for miRNA delivery and priming dendritic cells: electroporated exosome with miRNA**

Ali asadirad, Seyed Mahmoud Hashemi, Kaveh Baghai , Hosein Ghambarian , Esmaiil Mortaz, Davar Amani

*Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Science*

**Background:** Exosomes, membranous Nanovesicles, naturally carry bio-macromolecules and play pivotal roles in both physiological intercellular crosstalk and disease pathogenesis. Tumor-derived exosomes are proposed as a new type of cancer vaccine.

**Methods:** In this study we purpose to show that tumor cell-derived exosomes can function as vehicles to deliver exogenous miRNA-155 mimic in to dendritic cells (DC) for simultaneous miRNA delivery and Antigen priming of DC.

For this purpose, miRNA -155 was electroporated into tumor cell-derived exosomes then DCs were pulsed with electroporated exosomes.

**Results:** DCs pulsed miRNA-155 loaded exosomes can significantly increase the level of maturation and uniformity of DCs. In addition, these DCs in comparison with DCs in control group can proliferate lymphocytes insignificantly.

**Conclusion:** Furthermore, simultaneous miRNA delivery and antigen priming of DC can improve maturity and uniformity compared to control groups. Similar approaches could be useful in modification of target biomolecules in vitro and in vivo.

ICIA2018\Immune Cell Therapy\Oral\8470
**8470**

# Exosomes from Neuroblastoma Cell-Exposed Natural Killer Cells can educate Naïve NKs to eradicate neuroblastoma tumors in vivo

AlirezaShoae-Hassani, Maryam Behfar, Seyed Abdolreza Mortazavi-Tabatabaei, Rashin Mohseni, Amir Ali Hamidieh

*1. Applied Cell Sciences & Tissue Engineering Department, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Immune cell-derived exosomes can increase immunity against tumors. On the other hand, tumor-derived exosomes can reduce the immunity and change the tumor microenvironment to further develope and initiate metastasis. These effects take place via alterations in the functions of innate and adoptive immune cells. In this experiment, we have studied the NK cell (NKs) effectiveness on tumor cells after expansion and incubation with exosomes.

**Methods:** The exosomes were derived from two populations of NKs: 1) Naïve NKs and, 2) neuroblastoma cell-exposed NKs. Also, we studied the effects of neuroblastoma derived exosomes (NB-Ex) on NK function. The molecular load of the characterized exosomes (by means of nanoparticle tracking analysis, flow cytometry, scanning electron microscopy and western blot) from NKs exposed to the neuroblastoma cell showed the expression of NCRs in addition to CD56, NKG2D, and KIR2DL2 receptors. These exosomes were used to treat NKs and then administered to NB tumor cells both in vitro and in vivo.

**Results:** Our results showed that some educations from exosomes derived from neuroblastoma cell-exposed NK cells activate NK cells to exert more efficient cytotoxicity against NB tumors, while NB-Ex act as tumor promoters by providing a tumor supporting niche.

**Conclusion:** This method of preparing the exosomes dramatically increases NK-mediated anti tumor effects against neuroblastoma cells.

**Keywords:** Cancer Therapy, Exosome, Immune Cell Therapy, Natural Killer Cell, Neuroblastoma

ICIA2018\Immune Cell Therapy\Oral\8294
**8294**

# Construction of Chimeric Antigen Receptor (CAR) bearing Anti-Prostate Specific Membrane Antigen (PSMA) nanobody (VHH)

Mahmoud Hassani[1,2], FatemehHajari Taheri[2], Mohsen Abolhassani[2], Mohammad Hossein Modaresi[1], Zahra Sharifzadeh[2], Arash Arashkia[3]

*1. Department of Molecular Medicine, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran*
*2. Hybridoma Lab, Department of Immunology, Pasteur Institute of Iran, Tehran, Iran*
*3. Department of Molecular Virology, Pasteur Institute of Iran, Tehran, Iran*

**Background:** Adoptive transfer of T cells expressing chimeric antigen receptors (CARs) is a promising anti-cancer therapy. T cells isolated from the body and equipped with CARs; when given back to the patient, these "CAR T cells" recognize and attack cancer cells. In 2017, two CAR T-cells were approved by the FDA, one for the treatment of acute lymphoblastic leukemia and the other for the advanced lymphomas. This technique is being investigated for use in other solid tumors and blood cancers.

**Methods:** For the first time, we used camelid nanobody (VHH) against prostate specific membrane antigen (PSMA) to construct the CAR T cells. T cells were transfected with construct composed of co-stimulatory molecules (CD28 and CD3ζ) joined with spacer (CH2-CH3 fragment of IgG1) to nanobody. After confirmation of surface expression, the functions were evaluated when co-cultured with prostate cancer cells.

**Results:** Our data show that co-culture of CAR-T cells with $PSMA^+$ prostate cancer cell (LNCap) not only increased the expression of IL-2 cytokine (about 400 ng/ml), but also expressed T cell activation marker (CD-69) almost 29%. In addition, CAR-T cells proliferated nearly 60% as compared with PSMA negative prostate cancer cell (DU145). This result indicates the specificity of CAR-T cells against PSMA.

**Conclusion:** The engineered VHH-CAR T cells may be developed to target virtually any tumor associated antigen in the future for the adoptive T-cell immunotherapy of solid tumors.

**Keywords:** CAR, PSMA, Prostate Cancer, Nanobody, Immunotherapy

ICIA2018\Immune Cell Therapy\Oral\8186
**8186**

# Immunoregulatory function of Treg cells in experimental animal model of inflammatory bowel disease

Kaveh Baghaei[1], Seyed Mahmoud Hashemi[2], Sedigheh Pouya[2], Maryam Heidari[2], Hamid Asadzadeh Aghdaei[1], Mohammad reza Zali[3]

*1. Basic and Molecular Epidemiology of Gastrointestinal Disorder Research Center, Research Institute for Gastroenterology and Liver Disease, Shahid Beheshti University of Medical Science, Tehran, Iran*
*2. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences Tehran, Iran*
*3. Gastroenterology and Liver Disease Research Center, Research Institute for Gastroenterology and Liver Disease, Shahid Beheshti University of Medical Science, Tehran, Iran*

**Background:** T regulatory (Treg) cells have a basic role in maintain stability of immune homeostasis and reduce autoimmune responses by modulating cells of innate and adaptive immune system. The immunoregulatory functions of Treg cells are mediated through different mechanisms including create tolerance against external factors such as bacteria, antigens and allergens. Therefore, the immunological function of these cells in the intestine is very important as a place that is constantly associated with foreign antigens and commensal bacteria. As Treg therapy has the potential to be a clinical treatment in the field of transplantation, its set up in the treatment of the IBD diseases can be much more effective than broad-spectrum immunosuppressive therapies. In current study, beneficial of Treg therapy in acute crohn's disease model in mice is considered.

**Methods:** Acute colitis was induced in female C57BL/6 mice using DSS in drinking water. Naïve T cell were isolated from spleen by Macs cell separation columns. By using CD3, CD28 and TGF-B, naïve T cells were differentiated to Treg cells. Tregs, $10^6$ cells per mouse, was injected intraperitoneally, on the last day of DSS. Clinical, serological and pathological tests were applied to analyze clinical outcomes.

**Results:** After receiving T regulatory cells in treated group, the clinical sign of colitis were moderated recovered. Clinical signs include body weight changes, bleeding and stool consistency were significantly changed and histopathology results also showed restriction of inflammation.

**Conclusion:** Regarding to immunoregulatory effects of Treg in inflammatory reactions, using of Treg could be presented as a new potential for therapeutic approach in IBD diseases. In following, clinical trial and comparative studies could be carrying out to identify effective strategy in order to help patients treatment.

**Keywords:** Treg, IBD, Acute colitis, Animal model

ICIA2018\Immune Cell Therapy\Oral\7964
**7964**

# Bone marrow dendritic cells derived from CD40 and p19 knockout mice have in-vitro tolerogenic effect

Tahereh Kalantari[1, 2],Bogoljub Ciric[4], Eskandar Kamali-Sarvestani[3], And Abdol mohamad Rostami[4]

*1. Diagnostic Laboratory Sciences and Technology Research Center, School of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran.*
*2. Department of Laboratory Sciences, School of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran.*
*3. Immunology Department, Shiraz University of Medical Sciences, Shiraz 7144155, Iran*
*4. Department of Neurology, Thomas Jefferson University, Philadelphia, PA 19107, USA*

**Background:** Multiple sclerosis (MS) as an auto immune disease are characterized by perivascular inflammatory lesions, demyelination and axonal damage. The role of dendritic cells (DCs) as antigen presenting cells (APCs) in MS or its animal model EAE (experimental autoimmune encephalomyelitis) development has been shown in different studies. In this study, Potency of Knockout mice (KO) derived BMDCs (Bone Marrow Dendritic Cells) (CD40KO-DCs and p19KO-DCs) were compared to C57BL/6 mice (Cont-DCs) for their tolerogenic/ immunogenic efficacy.

**Methods:** Flow cytometry was used to analyze the expression of maturation markers MHCII and co-stimulatory molecules CD40, CD80 and CD86. Expression of CD40 and p19 was measured by RT-PCR. The ability of DCs to stimulate $CD4^+$ T cells of 2D2 mice was evaluated by $^3$H-thymidine uptake and the endocytic capacity was evaluated in KO derived DCs compared to Cont-DCs using fluorescent OVA.

**Results:** CD40 expression of DCs obtained from CD40KO was significantly different ($\rho<0.05$) to Cont-DCs. IL-6 and IL-12 expressions of KO derived BMDCs were significantly lower when compared with Cont-DCs ($p<0.05$). The gene expression of CD40 and/or IL-23p19 was measured by quantative RT-PCR in KO derived DCs compared to control group. As compared to Cont-DCs groups, CD40KO-DCs and P19KO-DCs showed a significant difference in mRNA expression of CD40 and p19 respectively ($p<0.05$). CD40KO-DCs in stimulation of MOG-specific$CD4^+$ T cell proliferation were decreased significantly compared to Cont-DCs group($p<0.05$). The percentage of endocytosed FITC-OVA was not statistically different in KO derived DCs when compared to Cont-DCs group (($p>0.05$).

**Conclusion:** This study shows that BMDCs derived from CD40KO and p19KO mice has a tolerogenic potency in-vitro. We suggest a further in-vivo study using these KO derived DCs to check their tolerogenic effect in therapy of auto immune diseases such as MS.

**Keywords:** Knockout mice, CD40KO, p19KO, tolerogenic DC.

ICIA2018\Immune Cell Therapy\Oral\7821
**7821**

# Mesenchymal Stem Cells pulsed with 17-β estradiollead to a more favorable outcome in collagen induced model of Rheumatoid Arthritis

Monireh Jahantigh, Seyyed Meysam Abtahi Froushani, Nahideh Afzale Ahangaran

*Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Insufficient of Mesenchymal stem cells (MSCs) reaching to inflamed tissues has limited their potential therapeutic benefits. Some documents indicated that 17-β estradiol augments homing of stem cells. Here, we evaluate the effect of 17-β estradiol treatment of Mesenchymal stem cells (MSC) on their potential for modulation of the animal model of Rheumatoid arthritis (RA).

**Methods:** Bone marrow-derived MSCs were pulsed with 100µM of 17-β estradiol for 24 h. RA was induced by collagen peptide and complete Freund's adjuvant in Wistar rats. Animals were allocated in 3 groups, one week after induction: treated with MSCs alone, treated with MSCs pulsed with estradiol and untreated group. The change in the diameter of wrists and ankles of each rat were recorded every 5 days until 33 days after induction. Then, splenocytes were tested to assess proliferation rate and cytokines production.

**Results:** The swelling and edema of ankles of RA rats received 17-β estradiol pulsed MSCs were significantly regressed compared to RA rats received MSCs alone. Aside from reducing lymphocyte proliferation, 17-β estradiol pulsed MSCs significantly reduced the production of proinflammatory IL-17 as well as TNF-α through antigen-specific re-stimulation more profound than RA rats treated with MSCs alone. Moreover, the level of anti-inflammatory IL-10 was significantly increased in RA rats treated with17-β estradiol pulsed MSCs compared to RA rats received MSCs alone. Nevertheless, the levels of IFN-γ didn't show any significant changes between MSCs treated group.

**Conclusion:** MSCs pulsed with 17-β estradiol lead to a more favorable outcome in RA model compared to un-treated MSCs.

**Keywords:** 17-β estradiol, Mesenchymal stem cell, Rheumatoid arthritis.

ICIA2018\Immune Cell Therapy\Oral\11082
**11082**

# Mesenchymal Stem Cell-Derived Exosomes Ameliorate Inflammation in Mouse Model of Acute Colitis

Neda Heidari[1], HajarAbbasi[1] , Seyed Mahmoud Hashemi[1] , Saeed Namaki[1] , Mohammad Reza Zali[1]

*1. Department of Immunology, School of medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** inflammatory bowel disease (IBD) is called a group of diseases which causes chronic and recurrent inflammation of the large intestine and narrow intestine that the imbalanced immune responses have an important role in development and progression of the disease. Studies on mesenchymal stem cells (MSCs) have shown that these cells have immunomodulatory properties that can exert their effect through exosomes secretion. Therefore, this study was designed to investigate the effects of the mesenchymal stem cell-derived Exosome injection in mouse model of acute colitis.

**Methods:** Acute colitis was induced by 3 % dextran sulfate sodium (DSS) in C57Bl/6. The disease was induced in 1 cycle (4 days use of water containing DSS, followed by 6 days of water). After induction of disease in mice, injection of Exosome (100µl) for 4 times was done intraperitoneally. During the study, changes in body weight, bleeding, stool consistency, disease activity index (DAI), and mortality rate were recorded. After euthanizing the mice, weight, and length of the colon, the percentage of Treg cells were measured. The pathology examination of the colon was also performed.

**Results:** After Exosomes injection body weight decrease, bleeding, DAI was improved and mortality rates decreased. The stool consistency was improved too. The percentage of Treg cells and the TGF-β production was increased while the level of IL-17 decreased. Pathologic findings showed after injection of Exosomes, infiltration of inflammatory cells and epithelial destruction decreased.

**Conclusion:** Given that exosome has immunomodulatory effects, Exosomes can be used as a cell-free therapy in the treatment of inflammatory bowel disease.

**Keywords:** Mesenchymal stem cell, Inflammatory bowel diseases, Dextran sulfate sodium, Exosome

# Immunodeficiency

## Oral Presentation

ICIA2018\Immunodeficiency\Oral\8548
**8548**

# Clinical course, laboratory data and cell-surface expression of IL12Rβ1 in MSMD suspected patients with disseminated or atypical local BCGosis

Leila Moradi[1], Leila Parvaneh L[1,4], Hamid Eshaghi[3], Reza yazdani[2], Nima Parvaneh[1,2], Asghar AghaMohammadi[1,2]

*1. Department of Immunology, Asthma and Allergy, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran*
*2. Research center for immunodeficiency Diseases (RCID), Tehran University of Medical Sciences, Tehran, Iran*
*3. Department of Infectious Diseases, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran*
*4. Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran*

**Background:** Autosomal recessive IL12Rβ1 deficiency is known as the most common cause of Mendelian Susceptibility to Mycobacterial Diseases (MSMD). This rare congenital disorder caused by mutations in genes encoding cytokines or receptors of IL12/23-INF-γ immunity axis, characterized by increased susceptibility of these patients to infections due to weakly virulent mycobacteria such as BCG vaccine or environmental mycobacteria (EM) and salmonella.

**Methods:** Clinical course and immunological laboratory data of Patients with atypical-local or disseminated BCGosis referred to Children's medical center in Tehran were studied to investigate the suspected MSMD patients, after rulling out more common PIDs presented with BCGosis, such as SCID or CGD. Blood samples of healthy controls and 19 patients were analyzed. Lymphocytes from both group were activated and stimulated in vitro with PHA and IL2 and evaluated for flowcytometric IL12Rβ1 expression.

**Results:** Reduced IL12Rβ1 expression was noticed in 4 patients' blood sample. All of them had consanguineous parents and history of BCG vaccination at birth. Whereas all of them had recurrent episodes of diarrhea, just one had documented history of disseminated salmonellosis. Two patients had recurrent oral candidiasis. There was no evidence of viral infection or malignancies.

**Conclusion:** Regarding to high frequency of consanguinity in Iran and BCG vaccination program at birth, it seems advisable to investigate IL12Rβ1 deficiency as the most prevalent cause in patients with suspicious MSMD. It may be beneficial postponing BCG vaccination in neonates with family history of documented or suspected diagnosis of a PID or an unusual consequence of BCG vaccination.

**Keywords:** BCGosis, IL12Rβ1 deficiency, MSMD, primary immunodeficiency, Salmonellosis

ICIA2018\Immunodeficiency\Oral\9878
**9878**

## Evaluation of early and late apoptosis in patients with CVID

Reza Yazdani[1,2*], Mazdak Ganjalikhani-Hakemi[2], Nima Rezaei[1], Fateme Babaha[3], Asghar Aghamohammadi[1]

*1. Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Science, Tehran, Iran*
*2. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
*3. Department of Immunology, School of Medicine, Tarbiat Modares University, Tehran, Iran*

**Background:** Common variable immunodeficiency (CVID) is a primary immunodeficiency with heterogeneous complications. One of the most important issues with regard to pathogenesis of CVID is defect in survival and differentiation of B-cells. Accordingly, we evaluated apoptosis (early and late apoptosis) with and/or without stimulation in B-cells, as well as measurement of B-cell subsets in CVID patients and controls.

**Methods:** B-cells were purified from PBMCs by negative selection and were stimulated by anti-IgM and anti-CD40 antibody for 24 hours. We measured spontaneous and induced apoptosis of B cells by annexin V/PI apoptosis detection kit. Four-color flow cytometric immunophenotyping determination of B-cell subsets (transitional, $CD21^{low}$, naive, marginal zone and memory B cells as well as plasmablasts) were performed using FACS. Moreover, BCL-2 gene expression was measured using real-time PCR.

**Results:** The expression of annexin V and PI in both unstimulated and stimulated B cells from patients were significantly higher than controls. Moreover, early apoptosis ($P = 0.04$), late apoptosis ($P = 0.04$) and necrosis ($P = 0.03$) in both unstimulated and stimulated B-cells were strongly further in the patients than controls. Furthermore, transcript levels of Bcl-2 were lower in patients than controls. On the other hand, our patients presented a significant reduction in absolute counts and percentage of B-cell subsets in patients than controls.

**Conclusions:** Our data suggest that increased apoptosis leads to abnormality in B-cell subset numbers, as B-cells could be unable to complete their maturation and differentiation. Thus, apoptosis could be one of the mechanisms involved in the significant reduction of B-cell subsets in these patients.

**Keywords:** apoptosis, immunophenotyping, common variable immunodeficiency

ICIA2018\Immunodeficiency\Oral\9923
**9923**

# Finding Two Autosomal Dominant Gain-Of-Function Mutations in STAT1 Gene of Three Chronic Mucocutaneous Candidiasis Patients

Vajiheh Ostadi[1,2], Roya Sherkat[1], MazdakGhanjalikhan Hakemi[2], Seyed Mehran Modaressadegi[4] , Anne Puel[3]

1. *Acquired Immune Deficiency Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*
2. *Department of Immunology, School of Medical University of Isfahan, Iran*
3. *Laboratory of Human Genetics of Infectious Diseases, Necker Branch, Paris, France*
4. *Isfahan University of Medical Sciences, Department of Genetics, School of Medical University of Isfahan, Iran*

**Background:** Chronic mucocutaneous candidiasis disease (CMCD) is characterized by susceptibility to recurrent or resistant candida infections, typically Candida albicans, on cutaneous and mucosal surfaces. In this survey, function and genetics of Th17 cells were studied in susceptible patients to mucocutaneous candidiasis.

**Methods:** for suspected patients who suffered from chronic candidiasis, CFSE proliferation test with candida antigen, detection of Th17 cells and pSTAT1 were done by flow cytometry method. Also, expression of IL-17A, IL-17F and IL-22 genes were measured with real-time PCR on all suspected patients. At the same time, whole exome sequencing (WES) was done for all patients.

**Results:** we detected three patients with gain-of-function (GOF) mutation in STAT1 molecule. The frequency of Th17 cells and their proliferation against candida antigen were reduced significantly in these patients compared with healthy controls. IFN-γ stimulation resulted in higher generation of pSTAT1 in the T lymphocyte cultures from patients compared to healthy controls. Gene expression of IL-17A, IL-17F and IL-22 from PBMCs was significantly reduced in these patients.

**Conclusion:** In summary, our study showed importance of STAT1 molecule in IL-17A T cells differenciation against Candida. Since, previous studies are reported STAT1 GOF mutations to be causative in more than half of CMC patients, for finding an effective treatment, more studies are required for displaying of relevance of this molecule with autoimmunity and other clinical symptoms.

# Immunogenetics

## Oral Presentation

ICIA2018\Immunogenetics\Oral\7355
**7355**

# KIR2DS1, 2DS5, 3DS1 and KIR2DL5 are associated with the risk of head and neck squamous cell carcinoma in Iranian patients

Shaghik Barani[1,3], Bijan Khademi[2], Abbas Ghaderi[1, 3 *]

1. *Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz Iran*
2. *Otolaryngology Research Center, Department of Otolaryngology, Shiraz University of Medical Sciences, Iran*
3. *Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Activating and inhibitory KIR receptors (aKIR, iKIR) control the development and function of NK cells whose function alterations adjust the tumor microenvironment immunity. This research was conducted to determine the KIRs gene impact on genetic predisposition to Head and Neck Squamous Cell Carcinoma (HNSCC) as the sixth most prevalent cancer worldwide and its clinicopathological features in Iranians.

**Methods:** KIR genotyping using sequence-specific primers-polymerase chain reaction (SSP-PCR) method was performed to identify the presence of all 16 KIR genes in 285 Head and Neck Squamous Cell Carcinoma (HNSCC) patients, including laryngeal, oral cavity and pharyngeal SCC (L.SCC, O.SCC, P.SCC) and 273 controls (CNs).

**Results:** Comparison of KIRs gene frequency between HNSCC and CN groups revealed a highly significant increase in KIR2DL5, 2DS1, 2DS5, 3DS1 and CxT4 genotype and a decrease in KIR2DS4 deleted variant and AA genotype carriers. A significant increase was noted in individuals with more inhibitory KIRs than activating KIRs in HNSCC compared with CNs. Individuals with ≥ 4 inhibitory KIR and those with ≥ 5 activating KIRs were significantly more common in HNSCC than CNs. The carrier frequency of KIR2DS1 and C4T4 genotype is lower in young-onset HNSCC compared with late-onset ones. 68 distinct KIR genotypes were identified in 558 individuals. A higher frequency of lymph node metastasis (LNM) was observed in patients with KIR2DL2 gene than those who lacked it, as well as in patients with 5 inhibitory KIR than those with less than it.The frequency of KIR2DL2 and KIR2DS4 deleted variant carriers was increased in patients with advanced and early clinical stages respectively.

**Conclusion:** Our findings discovered the detrimental impact of KIR2DS1, 2DS5, 3DS1, 2DL5 and CxT4 genotype as well as the positive impact of KIR2DS4del and AA genotype on genetic predisposition to HNSCC and KIRs associations with clinicopathological characteristics.

**Keywords:** killer immunoglobulin like receptors (KIRs), SSP-PCR, Head and neck squamous cell carcinoma (HNSCC), NK cells

ICIA2018\Immunogenetics\Oral\7394
**7394**

# Association between TRA3IP2 (rs33980500)and left main coronary artery stenosis in Acute Myocardial Infarction

Pordel, S.[1], SajediKhanian, M.[2], Karimi, M.H[3], Nikoo H[4], Doroudchi M.[1]

1. *Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Department of Cardiology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
3. *Fars Blood Transfusion Center, Shiraz, Iran*
4. *Cardiovascular Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** There is compelling evidence showing connections between inflammation, immune system and cardiac pathology. IL-17A and its adaptor Act1 protein, which is encoded by TRAF3IP2 gene, may have a role in this process. Due to the association of TRAF3IP2- rs33980500 with inflammation, we assessed this single nucleotide polymorphism (SNP) in acute myocardial infarction (AMI).

**Methods:** AMI patients (n=201) referring to the affiliated hospitals of Shiraz University of Medical Sciences in a one year period were included in this study. DNA was extracted from venous blood and the genetic variation in theTRA3IP2-rs33980500was identified using PCR-RFLP method. Control individuals (n=201) were recruited from among healthy blood donors of the same age range and gender.

**Results:** No significant difference was observed inallelic and genotype distributions of TRA3IP2-rs33980500 between patients and controls. However, there was an association between this SNP and left main coronary artery stenosis. MI patients with left main coronary artery stenosis had a significantly higher frequency of T allele (p=0.03) and TT genotype (p=0.01) compared to patients without this complication.

**Conclusion:** The association of TT genotype and T allele of TRA3IP2-rs33980500 SNP with left main coronary artery stenosis is in accordance with its link with inflammation. T allele of TRA3IP2-rs33980500 SNP is reported to be associated with increased inflammation which is a key factor in atherosclerotic plaque formation, atherosclerosis progression and narrowing of the arteries.

**Keywords:** Myocardial infarction, Single nucleotide polymorphism, TRA3IP2-rs33980500

ICIA2018\Immunogenetics\Oral\7876
**7876**

## Assessment of protein prenylation pathway in multiple sclerosis patients

*Mohammad Taheri[1,2], Mir Davood Omrani[1,2*]*

1. *Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
2. *Urogenital Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Multiple sclerosis (MS) is a chronic inflammatory disorder with several genetic and environmental factors being implicated in its pathogenesis. Protein prenylation as one of the important post-translational modifications of proteins has crucial role in immune system regulation.

**Methods:** In the current case–control study we compared expression of five genes coding for the different subunits of proteins implicated in protein prenylation in 50 Iranian MS patients with those of healthy subjects.

**Result:** No significant difference has been found in FNTA and PGGT1B expressions between cases and controls. Spearman Correlation analysis between FNTA relative expression and disease duration showed significant correlation in male patients (r=-0.671, P=0.024) but not female patients (r=0.253, P=0.12). FNTB expression was significantly higher in MS patients compared with healthy subjects. Spearman Correlation analysis between FNTB relative expression and disease duration showed significant correlation in male patients (r=-0.876, P=0.004) but not female patients (r=0.296, P=0.06). RABGGTA was significantly up-regulated in total MS patients, total male patients, female patients aged between 30 and 40 and male patients aged >40 compared with corresponding control groups. RABGGTB was significantly down-regulated in total MS patients, total female patients and female patients aged >40 compared with corresponding control groups.

**Conclusion:** Totally, we demonstrated dysregulation of protein prenylation pathway in MS patients compared with healthy subjects. Future studies are needed to find the clinical implication of this pathway in MS patients.

ICIA2018\Immunogenetics\Oral\7894
**7894**

# Negative regulation of Semaphorin 3A expression in peripheral blood mononuclear cells using microRNA-497-5p

Shima Shapoori[1], Mazdak Ganjalikhani-hakemi[1], Mahsa Rezaeepoor[1], Fereshteh Alsahebfosoul[1], Sharifeh Khosravi[2], Masoud Etemadifar[3], Marjan Mansourian[4]

*1. Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
*2. Department of Genetics, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
*3. Multiple Sclerosis and Neuroimmunology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*
*4. Department of Biostatistics and Epidemiology, Faculty of Health, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background:** Semaphorin3A (Sema3A) as a secreted semaphorin, is generated by a wide spectrum of cells in the human body including most of the immune cells. Thus, this protein as an immune modulator could participate in the pathogenesis of autoimmune diseases like Multiple sclerosis (MS). MicroRNAs (miRNAs) which are non-coding and single-stranded RNAs with ~21-25 nucleotides length, modulate target gene expression at the post-transcriptional level. MiRNAs play significant roles in many physiologic processes as well as semaphorins. Accordingly, it proposes that miRNAs may be crucial to modulate the semaphorins functions. Previous findings have proved that miR-497-5p is up-regulated and Sema3A is down-regulated in some autoimmune disorders. Thus, this paper aimed to increase our understanding of correlation between the Sema3A and Mir-497-5p in Peripheral Blood Mononuclear Cells (PBMCs).

**Methods:** In this study, we cultured PBMCs in optimum condition and transfected miR-497-5p mimic into them using X-treme gene Reagent. There were 4 groups: 1- Positive control, 2- Mock-transfection control, 3- Scrambled control, 4- Mimic transfection which in all groups PBMCs were treated with PHA. After 48 hours, we assessed supernatant and cells by ELISA assay and qRT-PCR, respectively. Also,we evaluated cell viability using MTT assay. All experiments were done in triplicates and P values equal to or less than 0.05 were considered significant. All the statistical analysis were performed through the SPSS 20 and P values equal to or less than 0.05 were considered significant.

**Results:** We observed down-regulation of Sema3A gene (p value = 0.0001) and its secretion (p value = 0.021) in PBMCs through miR-497-5p transfection. Moreover, transfection with miR-497-5p mimic and down-regulation of Sema3A did not affect viability of PBMCs (p value = 0.156).

**Conclusion:** Based on the obtained results, we suggest that miR-497-5p has a high suppressive effect on Sema3A expression and both Sema3A and miR-497-5p could be considered as critical targets for further studies on future therapeutic attempts for autoimmune diseases such as MS and rheumatoid arthritis (RA).

**Keywords:** Autoimmune disease, miRNA, miR-497-5p, Sema3A

ICIA2018\Immunogenetics\Oral\8116
**8116**

# IRF7 gene expression profile and methylation of its promoter region in patients with systemic sclerosis

Ramazan Rezaei[1,3], Mahdi Mahmoudi[1], Farhad Gharibdoost[1], Mohammad Vodjgani[2]

1. *Rheumatology Research Center, Tehran University of Medical Sciences,*
2. *Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
3. *Department of Immunology, Medical School, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** The aim of the current study was to evaluate if methylation status of CpG sites of interferon regulatory factor 7 (IRF7) promoter in peripheral blood mononuclear cells (PBMCs) of systemic sclerosis (SSc) patients is involved in pathogenesis of the disease.

**Methods:** PBMCs were isolated from whole blood of 50 SSc patients and 30 controls. After the extraction of total RNA and DNA contents from PBMCs, complementary DNA (cDNA) was synthesized. Afterwards, quantitative analysis of IRF7 messenger RNA (mRNA) was conducted by real-time polymerase chain reaction (PCR). To evaluate the methylation status of the promoter region of IRF7 gene, PCR products of bisulfite-treated DNA from SSc patients and controls were sequenced.

**Results:** The mRNA expression of IRF7 in PBMCs from patients compared with controls was significantly upregulated. While limited cutaneous SSc patients expressed the mRNA of IRF7 higher than controls, the diffuse cutaneous SSc group did not demonstrate significantly increased expression in comparison to controls. Insignificant promoter hypomethylation of IRF7 was observed in SSc patients compared with the control group. However, CpG2 hypomethylation was significantly associated with increased SSc risk. Furthermore, overall promoter methylation and mRNA level of IRF7 were significantly correlated with each other. Nonetheless, none of them correlated with Rodnan score of SSc patients. There was significant difference in IRF7 mRNA expression between CpG8 methylated and unmethylated SSc patients. Moreover, the difference of methylation and expression was not significant between anti-nuclear antibody (ANA)-positive and ANA-negative SSc patients.

**Conclusions:** It is suggested that hypomethylation of the IRF7 promoter might play a role in SSc pathogenesis, probably through promoting the IRF7 expression in PBMCs of patients with SSc.

**Keyword:** IRF7, systemic sclerosis

ICIA2018\Immunogenetics\Oral\8297
**8297**

# Association between variants of FOXP3 gene and the occurrence of pre-eclampsia in Iranian women

Milad Gholami[1,2], Sahar Bayat[3], RezaMirfakhraie[4], Reihaneh Pirjani[5], Robabeh Taheripanah[2],Sayyed Mohamad Hossein Ghaderian[6]

*1. Department of Biochemistry and Genetics, faculty of Medicine, Arak University of Medical Sciences, Arak, Iran*
*2. Infertility & Reproductive Health Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Department of Medical Genetics, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
*4. Department of Medical Genetics, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*5. Associate professor, Obstetrics and Gynecology Department, Arash hospital, Tehran University of Medical Sciences, Tehran, Iran*
*6. Urogenital Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Pre-eclampsia (PE), is a pregnancy-specific disorder, with serious consequences for both the mother and the fetus that affecting 4–8% of pregnant women around the world. Despite intense studies, the pathophysiology of PE remains enigmatic. Previous studies suggested that regulatory T cells (Treg) dysfunction is involved in the pathogenesis of PE. We hypothesized that functional variants of the Forkhead Box Protein 3 (FOXP3) gene might be associated with PE via dysregulation of Treg cells.

**Methods:** We genotyped three variants of FOXP3 by PCR-RFLP and Tetra ARMS-PCR methods in 133 PE patients and 143 healthy age-matched controls.

**Results:**The genotypic frequencies of rs2232365 were found to be protective from the development of PE under codominant [odds ratio (OR) 0.49, 95 percent confidence interval (CI) 0.28 – 0.87, P-value = 0.043], dominant [odds ratio (OR) 0.54, 95 percent confidence interval (CI) 0.32 – 0.94, P-value = 0.027] and over dominant [odds ratio (OR) 0.57, 95 percent confidence interval (CI) 0.35 – 0.92, P-value = 0.02] models. Moreover, the rs3761548 conferred a risk of PE in recessive model [odds ratio (OR) 2.05, 95 percent confidence interval (CI) 1.08 - 3.88, P-value = 0.025]. However, no mutation was detected in FOXP3 exon2 in any of the studied samples.

**Conclusion:** Based on our results, thought that FOXP3 variants may be an important contributor for development of PE in Iranian women, probably by altering FOXP3 function and/or its expression.

**Keywords:** Association, Variants, Pre-eclampsia, FOXP3

ICIA2018\Immunogenetics\Oral\8719
**8719**

# The Relationship PDCD1 polymorphisms with Systemic Lupus Erythematosus in an Iranian Population

Farshid Fathi[1], Hossein Motedayyen[2], NoushinLotfi[1], Erfan Sadeghi[3], Mehdi Raie[4], Hossein Hafezi[5]

1. *Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
2. *Department of Laboratory Medicine, Kashan University of Medical Sciences, Kashan, Iran*
3. *Department of Biostatistics and Epidemiology, School of Public Health, Isfahan University of Medical Sciences, Isfahan, Iran*
4. *Health Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*
5. *Department of Dermatology, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background:** Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease that characterized by the breakdown of tolerance and the production of autoantibodies against self-antigens. PD-1 is one of the most important inhibitory receptors for regulating the immune system and preventing the development of autoimmune disorders. Therefore, we evaluated whether PDCD1 (PD-1) gene polymorphisms are associated with the incidence of SLE disease in the Iranian population.

**Methods:** Blood samples (5ml) were collected from 120 SLE patients and 120 healthy volunteers'. Genomic DNA extracted by salting out method and genotype determination of PD1.1, PD1.3, PD1.5 and PD1.9 was done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

**Results:** Our results showed a significant difference between PD1.5 genotypes in SLE patients and control group (P = 0.02). The frequency of genotypes CC, CT and TT were, respectively, 60%, 31.7% and, 8.3% in SLE patients, while those of control group were 42.5%, 46.7% and 10.8% for CC, CT and TT, respectively. In addition, the allelic analysis indicated that there was a significant difference in PD1.5C allele between SLE patients and control group. The frequency of C allele of PD1.5 in SLE patients was significantly higher than the control group (p = 0.01). However, there was no difference between PD1.1, PD1.3 and PD1.9 genotypes and alleles in SLE and control group.

**Conclusion:** Our study suggested that PD-1.5 polymorphisms in PDCD1 gene may increase SLE susceptibility in our Iranian population. However, the PD1.1, PD1.3 and PD1.9 were not correlated to genetic predisposition of SLE in our Iranian population.

**Keywords:** PDCD1, Polymorphism, Systemic lupus erythematosus, autoimmune disease.

ICIA2018\Immunogenetics\Oral\9950
**9950**

# Variation in the Interleukin 4–Receptor α Gene (I50V) Confers Susceptibility to Asthma

Masouma Mowahedi[1], Morteza Samadi[2]

*1. Department of Immunology, International Campus, Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran*
*2. Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran*

**Background:** Th17 cells apart from Th2 cells have been found to participate in the development of asthma. Recent studies have implicated polymorphisms in the signaling pathway of interleukin 4 (IL-4) receptor in the progress of inflammation. The aim of the present study was to investigate how to associate the single nucleotide polymorphism (SNP) in the interleukin 4 receptor α chain (IL-4Rα) gene rs1805010 (I50V) to total immunoglobulin E (IgE) and IL-17A in the etiology of asthma in an Iranian population.
**Methods:** To evaluate how associate SNP I50V and asthma, in a case-control study, I50V variant were screened using PCR-RFLP followed by Fisher exact test. To investigate how associate SNP I50V and serum levels of IL-17A and total IgE, first ELISA was performed. To determine how to communicate SNP I50V, IL-17A and total IgE with the severity of the disease, patients were classified into three groups according to GINA criteria: mild, moderate and severe. Next student's t-test or one-way ANOVA were performed.
**Results:** We found a significant association between SNP I50V with asthma ($p = 0.001$). Despite IL-17A and total IgE in asthmatic patients were significantly higher than the control group ($p = 0.026$ and $p < 0.01$ respectively), neither total IgE nor IL-17A associated to SNP I50V.
**Conclusion:** I allele is more common in patients and more likely to reflect the importance of Th2 cells in asthma. Despite higher levels of total IgE and IL-17A were in accordance with the disease subgroups, increase of total IgE in tune with the severity of disease while enhancement of IL-17A in serum against the total IgE and according to decrease of disease severity.

**Keywords:** Asthma, I50V, IL-17A, IgE

# Immunohematology

## Oral Presentation

ICIA2018\Immunohematology\Oral\7712
**7712**

# Preliminary investigation of unexpected antibodies against blood group antigens in major β-thalassemia patients in Lorestan province in 2017-2018

Alimohammad Varzi[1], Mona Hemmati Chegeni[2], Mohammad Ziaee[3], Babak Abdolkarrimi[4]

1. *Department of Immunology, Lorestan University of Medical Sciences, Khorramabad, Iran*
2. *Department of Immunology, Lorestan University of Medical Sciences, Khorramabad, Iran*
3. *Medical School, Lorestan University of Medical Sciences, Khorramabad, Iran*
4. *Assistant professor of children hematologist and oncologist, Lorestan University of Medical Sciences*

**Background:** Major β-thalassemia is a congenital hemolytic disorder that is classified as a hemoglobinopathy which is caused by defect in β-globin chain synthesis and considered as the most common genetic disorder world-wide. Regular blood transfusion plays a key role in treatment of the patients with this disorder. However, the development of hemolytic alloantibodies and autoantibodies against red blood cells (RBCs) can be culminated to development of different complications in these patients. Therefore, our study is focused on determination of the frequency of RBC alloimmunization and the most common alloantibodies involved among Lorestan patients who have received transfusion regularly.

**Methods:** We will use and test the information of 91 major β-thalassemia patients of Lorestan who have transfused RBC components. Our clinical and laboratory information including age at the start of transfusions, total number of transfusions, splenectomy status and other variants have been obtained through questionnaires. In this preliminary study alloantibody screening and identification of alloantibodies have been carried out on 15 cases out of 91 thalassemia patients using three cell and 11 cell panel, provided by Iranian blood transfusion organization, respectively.

**Results:** Out of 15 analyzed samples, two (13.34%) were positive for alloantibodies against $C^w$ and $Lu^a$. Moreover anti-lewis, anti-kell and anti-$Jk^a$ were detected in one of these two samples.

**Conclusion:** In this preliminary work, alloantibodies against $C^w$, kell, lutheran, lewis and kidd were detected in 13.34% of patients. Alloimmunization in transfused major β-thalassemia patients is very common which is an important issue for the blood bank department to find a fully matched blood components easily. However, working on new technology with high-throughput blood group typing that are developing in different research centers could be a good answer for reducing these problems in next future.

**Keywords:** alloimmunization, β-thalassemia major, regular blood transfusion, antibody identification

ICIA2018\Immunohematology\Oral\8340
**8340**

# Relationship between HPA-1 and HPA5 Gene Polymorphisms and Refractory to Platelet Therapy and Recombinant Factor VII in Glanzmann Thrombasthenia Patients in Southeast of Iran

Shaban Alizadeh[1], Manizheh Habibpour[1] , Zahra Kashani Khatib[2] , Akbar Dorgalaleh[3] , Majid Naderi[4]

*1. Department of Hematology, School of Allied Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran*
*2 Hematology Department, High Institute for Research and Education in Transfusion Medicine, Iranian Blood Transfusion Organization, Tehran, Iran*
*3. Department of Hematology, School of Allied Medical Sciences, Iran University of Medical Sciences, Tehran, Iran*
*4. Genetic Researcher Center in Non-Communicable Disease, Zahedan University of Medical Sciences, Zahedan, Iran*

**Background:** Glanzmann Thrombasthenia (GT) is a rare autosomal disease. HPA (Human Platelet Alloantigen) is a surface polymorphic alloantigen of platelets. This study was intended to investigate and compare the polymorphism of HPA-1 and HPA-5 genes in two groups of GT patients, with and without resistance to platelet and recombinant factor VII therapy.

**Methods:** This case control study was performed on GT patients (n=16) with resistance to platelet therapy and recombinant factor VII and control group of GT patients (n=16) without resistance to platelet therapy and recombinant factor VII. The consent form was completed by each patient. Gene polymorphisms of HPA-1 and HPA-5 were investigated using SSP-PCR, and the obtained data were analyzed using statistical software SPSS16.0.

**Results:** The results indicated no significant relationship between the studied genes and their resistance to platelet therapy and recombinant factor VII. The frequencies of HPA-1 genotype a/a were 98% and 94% in patient and control groups, respectively. The frequency of allele b was found to be less than allele a. The value of this allele was 4% in patient group and 1% in control group. In addition, the HPA-5a/a (98%) was the most frequent alloantigen in both groups. Seven percent (7%) of the patients had the HPA-5a/b genotype, and the HPA-5b/b was found to be absent in these individuals.

**Conclusion:** According to the results obtained, it could be concluded that these genes play no role in resistance to platelet therapy.

**Keywords:** Human platelet Ag-1, Human platelet Ag-5, Platelet therapy, Glanzmann thrombastenia

ICIA2018\Immunohematology\Oral\8405
**8405**

# Alloimmunization in transfusion dependent thalassemia major patients in South Khorasan

Leila Mobasheri[1], Masoomeh Asadifard[2], Gholamreza Anani Sarab[1]

1. *Department of immunology, Birjand University of Medical Science, Birjand-Iran*
2. *Blood transfusion center, Birjand-Iran*

**Background:** Patients with major beta thalassemia require regular blood transfusion in order to overcome deficient hemoglobin production. Multiple transfusion associated with some complications especially erythrocyte alloimmunization. In this study we aimed to perform antibody identification tests for transfusion dependent patients with major thalassemia in South Khorasan during 1395 to 1396.

**Methods:** This cross-sectional study was carried out on transfusion dependent beta thalassemia major patients registered at the thalassemia clinic in Birjand University of Medical Sciences. The demographic and clinical information of the patients for age, sex and history of transfusion were recorded. Alloantibody screening was done and then positive cases were examined to identify antibodies, using panel cells provided by Iranian Blood Transfusion Organization.

**Results:** Out of 68 patients recruited in this study, 36(53%) were male and 32 (47%) were female with the mean age of 15 years. Alloimmunization was detected in 3 (4.4%) of patients with male gender. Coincidence of Anti-C and Anti-E was detected in one case and Anti-K developed in the second patient. A cold agglutinin was found in the third case through antibody screening.

**Conclusion:** Based on our findings, Anti-Rh and Anti-Kell were the most prevalent alloantibodies. Despite multiple transfusions, favorable condition in terms of alloimmunization prevention was achieved. However, the results re-emphasized the importance of red cell antigens typing from the beginning of transfusion and antigen matching, especially for Rh and Kell antigens.

**Keywords:** Major beta thalassemia, Alloimmunization, Alloantibody screening

ICIA2018\Immunohematology\Oral\8412
**8412**

# Global Plasma Industry: A Narrative on Outlines with Glance at the Endeavors in EMR Members

Afsaneh Aghaie[1], Seyed Mehrdad Jalali[2], Ali Akbar Pourfathollah[1,3]

*1. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran*
*2. Iranian Blood Research and Fractionation Company*
*3. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

**Background:** Well solidified in developed countries, plasma industry is going to penetrate into several emerging economies in any aspects. Plasma supply could be described as the promotor of such industry and nowadays, there are plenty of intentions in order to manufacture products from locally produced plasma in such countries. On the other hand, traditional fractionation method has been modified in order to shortcut the process, enhance the yield or lessen the risk of Transfusion Transmitted Infections (TTI) and it has a great impact on the final product price. In addition, plasma derivatives have mostly had a remarkable share in the global biopharmaceuticals' market. Eastern Mediterranean Region (EMR) consists of various range of developing countries, some of which has magnificent foundation in national plasma supply and years of worthwhile experience in local fractionation and toll manufacturing programs. Great efforts have been made across the region in order to progress the plasma industry in terms of the quality of plasma domestically produced as well the programs align with self-sufficiency in plasma derivatives.

It is to overally review but in brief on outlines and trends in global plasma industry such as plasma supply, industrial process and a short market study followed by a narrative on the endeavors made in EMR members with this regard.

**Keywords:** EMR, plasma industry, plasma supply, plasma derivatives

ICIA2018\Immunohematology\Oral\8596
**8596**

# Developing hemovigilance in University Teaching hospitals of Birjand – South khorasan

Gholamreza Anani Sarab[1], Hediyeh Gholampour Sardashti[2]

1. *Department of Immunology. Birjand University of Medical Sciences*
2. *Department of Laboratory Sciences. Birjand University of Medical Sciences*

**Background:** Blood transfusion monitoring and supervision (hemovigilance) has been established as one of the relatively new branches of transfusion medicine in many countries of the world. After conducting initial studies in the blood transfusion organization and emphasizing the importance of the blood and blood products and surveillance system, the implementation of this important system in health centers was recommended and approved by the Supreme Council of Iranian Blood Transfusion Organization in the winter of 2007. The implementation of this system in Iran since 2009 was started in 50 medical centers all over the country (19 hospitals in Tehran and 31 hospitals in 12 provinces of the country). The aim of this study was to evaluate the monitoring status and the level of completion of hemovigilance forms in teaching hospitals in Brigand.

**Methods:** The information regarding incomplete/inappropriate blood products requisition forms and blood transfusion monitoring forms were recorded in different wards of the teaching hospitals between the years. The results of the survey in 2014 were compared with the same research results in 2017 and the Summarized data were analyzed.

**Results:** The most common deficiency in vigilant forms was the neglect in record of vital signs of the patient over time after starting the transfusion. The well documented parameters were pulse pressure, Blood pressure and respiratory rate. Maximum numbers of incomplete forms were received from Intensive Care Units followed by Cardiac Care Unit. Overall, 17% improvement for completing blood request forms, administering blood, monitoring transfusions and being vigilant for the signs and symptoms of adverse reactions was detected in 3 years period from 2015 to 2017.

**Conclusion:** According to the results of the study, there is a significant improvement in the completion of blood transfusion monitoring form in year 2017 compared to 2015. This is a significant contribution to reducing the error in the process, and allows for safe and effective blood transfusion, system evaluation and collecting data on all types of transfusion-related adverse reactions. However, the study found that different wards do not have the same focus in completing the form, which can be due to the size of the work force or the lack of supervision in some sectors. It is suggested that, in order to improve the quality of supervision, nurses should be offered further courses to increase their awareness of the human and legal responsibilities in blood transfusion safety.

**Keywords:** hemovigilance, blood transfusion, Birjand

ICIA2018\Immunohematology\Oral\8659
**8659**

# An association study between Lewis Blood group phenotype and IL-6 and Il-8 production by Staphylococcus aureus stimulated PBMC in blood group A humans

Sedigheh Younessi Amirabad[1], Kambiz Bagheri[2]

1. *Department Of Microbiology, Kazerun Branch, Islamic Azad University, Kazerun, Iran*
2. *Department Of Immunology, Faculty Of Medicine, Kazerun Branch, Islamic Azad University, Kazerun, Iran*

**Background:** Blood antigens are a major category of human antigens which are formed at the level of red blood cells and different epithelial cells. Adults with a Le gene have Le $^{a}$ or Le $^{b}$. If the people are secretor ABHs, their red blood cells are Le $^{b}$ and if they are non-secretor they will be Le $^{a}$.

**Methods:** In this study, volunteers with a blood type A were classified in to Le$^{a}$ and Le $^{b}$ using blood group test. Mononuclear cells of each individual were separated and subdivided into two subtypes: the control (cultured without bacterial stimulation) and test group (cultured in the presence of Staphylococcus aureus extract). The suspension containing bacterial extract was prepared, and added to the mononuclear cell culture. Following the optimization and finding the best dose, duration, and appropriate exciting pattern, the levels of IL-8 and IL-6 in the cultured groups were measured by the ELISA assay.

**Findings:** The results show that after examining dose and time intervals for incubation and stimulation of PBMCs by bacterial extract, the best dose and time was 50 microliters which fractinated into 2 dose and 24 hours, respectively. In fact, by increasing the dose, the cytotoxic effect of the bacterial extract on the cells in the culture was possible.

Although in some samples, IL-6 and IL-8 were secreted through 24-hour incubation, the growth of Lewis a and Lewis b in both groups was significantly increased by stimulating the culture cells with Staphylococcus aureus.

**Conclusion:** Repeated tests and cytokine assays identified that there was no significant difference between PBMCs in individuals with Lewis a and Lewis b regarding IL-6 and IL-8 production capacity.

**Keywords:** Interleukin-6 and Interleukin-8, Blood group Lewis a and Lewis b, PBMCs, Staphylococcus aureus bacteria , blood group A

# Immunology of Chemical Victims and Environmental Pollution

## Oral Presentation

ICIA2018\Immunology of Chemical Victims and Environmental Pollution\Oral\7858
**7858**

# TCDD effects on induction of regulatory T cells in peripheral blood mononuclear cells in endometriosis patients

Mahsa Tanha[1], Shohreh Nikoo[2], Tahereh Naji[3], Amir-hassan Zarnani[4]

*1. Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences & Technology Pharmaceutical Sciences Branch, Islamic Azad University, Tehran - Iran (IAUPS)*
*2. Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran.*
*3. Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences & Technology Pharmaceutical Sciences Branch, Islamic Azad University, Tehran - Iran (IAUPS)*
*4. Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*

**Background:** Endometriosis is a benign and chronic inflammatory disease defined by the presence and development of endometrial tissues outside the uterine cavity. 2,3,7,8-Tetrachlorodibenzo-p-dioxin(TCDD), commonly referred to as dioxin, is considerd as one the main a risk factors involved in development of endometriosis. TCDD exerts its impacts through binding the aryl hydrocarbon receptor (AHR). On the other hand, Regulatory T cells (Tregs), have also been suggested to play a part in the immunopathogenesis of endometriosis. Here, we investigated TCDD impacts on Treg induction in peripheral blood mononuclear cells (PBMCs) of endometriosis patients.

**Methods:** The effect of TCDD on induction of Tegs was assessed by flow cytometry. In addition, the expression of FoxP3 and AhR was evaluated using real-time PCR.

**Results:** TCDD treatment significantly increased FoxP3 expression in CD4+ T cells of endometriosis as compared with the non-endometriosis group. Furthermore, an increase in the expression of FoxP3 and AhR in the presence of dioxin in the endometriosis group was significant compared to the non-endometriosis group. Interestingly, we found a significantly decreased AhR expression in the endometriosis group, however; we observed no marked differences between the endometriosis and non-endometriosis groups with respect to FoxP3 expression.

**Conclusion:** Our findings demonstrated, for the first time, that TCDD is able to increase the level of FoxP3, a key Treg transcription factor, in peripheral blood T cells of endometriosis patients compared to the non-endometriosis samples. This suggests the existence of an intense feedback to inflammatory responses in endometriosis patients. Collectively, PBMCs of the endometriosis patients respond differently to dioxin in comparison with that of the non-patient individuals.

**Keywords:** Endometriosis, Dioxin, AhR, Regulatory T cells

ICIA2018\Immunology of Chemical Victims and Environmental Pollution\Oral\7982
**7982**

# Investigation on the effects of aflatoxin $B_1$ on activation of caspases and ATP depletion in astrocytes

Jalil Mehrzad[1], ParisaVahidi[2], Amir Mohammad Malvandi[3], Saman Hosseinkhani[2]

*1. Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
*2. Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran*
*3. International Center for T1D, Pediatric Clinical Research Center Fondazione Romeo edEnricaInvernizzi, Department of Biomedical and Clinical Sciences L. Sacco, University of Milan, Milan, Italy*

**Background:** Aflatoxin $B_1$ ($AFB_1$) is a poisonous substance which is classified as group 1 carcinogenic agents by International Agency for Research on Cancer (IARC). Although $AFB_1$ is implicated as a carcinogen in hepatocellular carcinoma, brain autopsies in affected areas shows its presence in 81% of cases. Considering the importance of $AFB_1$ impact on the astrocytes, there still exists a scarcity of research on this issue within the literature.

**Methods:** This research investigates the apoptosis effect of $AFB_1$ on primary mouse astrocytes in vitro. To this aim, after detection of $LC_{50}$ of $AFB_1$ for astrocytes, the astrocytes were exposed to various concentration of $AFB_1$ for 24, 48 and 72 hours,. Concentration of intracellular ATP and caspase-3/7 activity was determined by the phenomenon of bioluminescence and luciferase reactions. Furthermore, cytochrome c release from mitochondria was carried out by western blot and percentage of apoptotic astrocytes was obtained using flow cytometry as well.

**Results:** The results indicated that, environmentally relevant level of $AFB_1$ (32 nM) induces apoptosis in astrocytes through ATP depletion and caspases activation. $AFB_1$-mediated apoptosis in astrocytes occurs through activation of caspase-3/7 with typical cytochrome c release.

**Conclusion:** Such in vitro effects of $AFB_1$ on astrocytes at the organelle and protein levels opens new doors to understanding the biological behavior of $AFB_1$ as well as molecular mechanisms that might translate to in vivo CNS inflammation, infection, and cancer in mammals caused by chronic exposure to $AFB_1$. Indeed, $AFB_1$-astrocytes interactions could trigger neurotoxicity in mammals. Further basic studies on the effect of biologically relevant level of $AFB_1$ on brain immune cells and molecules are in progress.

**Keywords:** Aflatoxin $B_1$, Bioluminescence, Astrocytes, Caspases, Neuroinflammation.

ICIA2018\Immunology of Chemical Victims and Environmental Pollution\Oral\8009
**8009**

# Evaluating the effect of Dioxin mediated aryl hydrocarbon receptor activation on T helper cell subsets in an inflammatory condition

Narjes Soleimanifar[1,2,3], Behrouz Nikbin[1,2,3], Katayoon Bidad[4], Mahdi Mahmoudi[5], Ahmad Reza Jamshidi[5], Zahra Hosseinikhah[2], Shayan Mostafaei[5], Mohammad Hossein Nicknam[1,3]

1. *Molecular immunology research center, Tehran University of Medical Sciences, Tehran, Iran*
2. *Department of Molecular Medicine, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran*
3. *Department of Immunology, Tehran University of Medical Sciences, Tehran, Iran*
4. *Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran*
5. *Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Dioxin as an omnipresence environmental toxin is a mane activator of aryl hydrocarbon receptor (AhR). AhR is a cytoplasmic transcription factor that regulates toxin-metabolizing enzymes such as cytochrome P450. The widespread expression of this factor in the immune system has shifted the point of AhR studies toward the immunologic role of it. The aim of our study is surveying the effect of Dioxin mediated aryl hydrocarbon receptor activation on T helper cell subset.

**Methods:** Peripheral blood mononuclear cells were obtained from 12 healthy people, and cultured before and after treatment with dioxin and antagonist for 72 hours. The percentage of Th17, Th22, and Tfoxp3 + cells was measured by flow cytometry. IL17 and IL10 cytokine levels were evaluated in the supernatant of culture media by ELISA method. The rate of expression of genes were evaluated using Real-Time PCR.

**Results:** Th22 cells were significantly increased by dioxin treatment, whereas Dioxin decreased the percentage of Th17 cells and expression of IL17 in the healthy subjects. The expression level of Aryl hydrocarbon receptor in dioxin treated cells was increased.

**Conclusion:** AhR can effect on fine regulation of the immune responses by altering the Th17 and enhancing of the Th22 cells.

**Keywords:** Dioxin, AhR, Th17,Th22.

ICIA2018\Immunology of Chemical Victims and Environmental Pollution\Oral\8618
**8618**

# Seborrheic dermatitis and its relationship with Serum immunoglobulins levels in Sardasht chemical victims (20 years after sulfur mustard exposure)

Sara Parsapour[1]

*1. Department of imonology,Faculty of Medicine, Shahed University, Tehran, Iran*

**Background:** Seborrheic dermatitis is a very common skin complication that affects seborrheic dermatitis in general, 4% of the world's population, and half of adults also have dandruff, which is actually a mild form of seborrheic dermatitis in the palms of the head. Dermatitis means skin lesions that are red and inflamed, causing itchy skins; seborrheic means that these skin lesions appear in the areas of the skin's oily skin (the location of the sebaceous stem cells), such as the face, the palatal, and the middle chest. The cause of seborrheic dermatitis is a fungus called malassezia. Sulfur mustard is a cytotoxic and vesicant chemical warfare agent it long-term effects include skin, respiratory and eye complications. The most important skin long-term complications include itching, eczema, xerosis, hyperpigmantasion. Serum IgE and IgG4 levels has a direct relationship with skin problems in allergic diseases, and hypersensitivity such as eczema, itching and etc. In this study, the relationship between serum levels of immunoglobulins with seborrheic dermatitis in Sardasht chemical victims was Studied 20 years after exposure to sulfur mustard.

**Methods:** This research is part of a cohort study of Sardasht chemical victims. A total of 372 male chemical victims exposed to sulfur mustard and 128 male in the control group in age range of 20-60 years old were. Both groups were clinically evaluated by a specialist physician. The sandwich ELISA was used for measure of serum immunoglobulins (ELISA kit : Bethyl, USA). The results were reported Mann-Whitney test and all statistical computing were performed using SPSS version 20

**Results:** Serum IgM level in chemical victims without seborrheic dermatitis was significantly lower than the control group with seborrheic dermatitis (P=0.004).

**Conclusion:** The results of this study showed that no significant relationship between Serum immunoglobulins levels with seborrheic dermatitis caused by sulfur mustard.

**Keywords:** immunoglobulins, seborrheic dermatitis, sulfur mustard

ICIA2018\Immunology of Chemical Victims and Environmental Pollution\Oral\9833
**9833**

# Evaluation of MiR-21b and MiR-15b gene expression in Formalin FixedP araffinE mbedded(FFPE) tissues of sulfur mustard exposed individuals with long-term pulmonary complications

Sahar Salimi[1], Farshid Noorbakhsh[2], Tooba Ghazanfari[1*]

1. *Department of Immunology, Faculty of Medicine, Shahed University, Tehran, Iran.*
2. *Department of Immunology, Faculty of Medicine, Tehran University of Medical Sciences. Tehran, Iran*
3. *Department of research center of regulation the immunology response. Tehran, Iran*

**Background:** Sulfur mustard (SM) is powerful alkylating agent with cytotoxic, carcinogenic and mutagenic features which the toxicity of this substance has been recognized last decade ago. Sulfur Mustard Complications appear in two forms of acute and chronic that affects multiple organs such as lung, eye, skin and etc. Along with, inflammatory cytokines have major roles in acute and chronic inflammatory complications by sulfur mustard. Furthermore, miRNAs has important function in chronic inflammatory diseases. As this regard, miR-21 and miR-15 could potentiate TGF-β signaling pathway and be able to promote fibrosis process in lung tissue. So that, gene expression of miR-21 and miR-15 was evaluated in exposed individuals with sulfur mustard and unexposed people as control group.

**Methods:** The number of exposed individuals with SM is twenty as case group and the number of unexposed control group twenty.

Total RNA was extracted from FFPE tissue samples of exposed individuals to sulfur mustard and unexposed individuals. Extracted total RNA quantity was measured by Nano drop and its quality evaluated by bioanalyzer. Gene expression of miR-21b and miR-15b was evaluated by Real-time PCR and U6 snRNA was utilized as control gene.

**Results:** gene expression of miR-21b was decreased significantly in exposed individuals group as compared to unexposed control group. There was no significant differences in gene expression of miR15-b in exposed individuals in compared to unexposed control group.

**Conclusion:** In regard to decrease expression of miR-21b, it seems that miR-21 have a role to down-regulate TGF-β signaling pathway.

ICIA2018\Immunology of Chemical Victims and Environmental Pollution\Oral\9995
**9995**

## Circulating MSCs in Sulfur Mustard-exposed Patients with Long-term Pulmonary Complications

Sara Ghaffarpour[1], Tooba Ghazanfari[1, 2], Eisa Salehi[3], Seyed Mahmoud Hashemi[4], Sussan K. Ardestani[5], KeivanGohari Moghadam[6], Ensieh Sadat Mirsharif[1], Razieh Dilmaghanian[1], S.Faghihzadeh[7]

1. *Immunoregulation Research Center, Shahed University, Tehran, Islamic Republic of Iran*
2. *Department of Immunology, School of Medicine , Shahed University, Tehran, Islamic Republic of Iran*
3. *Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Islamic Republic Iran*
4. *Department of Immunology, School of Medicine, ShahidBeheshti University of Medical Sciences, Tehran, Islamic Republic of Iran*
5. *Institute of Biochemistry and Biophysics, Tehran University, Tehran, Islamic Republic of Iran*
6. *Internal Medicine Department, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
7. *Department of Biostatistics and Social Medicine, Zanjan University of Medical Sciences, Zanjan, Islamic Republic of Iran*

**Background:** Sulfur mustard (SM) is a toxic agent that causes acute and long-term pulmonary complications. Recent evidence has shown the impact of SM on mesenchymal stem cells (MSCs). These cells have a critical role in repairing damaged tissues. In this study, we evaluated the mobilization of MSCs in SM-exposed patients with long-term pulmonary complications.

**Methods:** Fifty-nine SM-injured patients with prolonged pulmonary complications and 20 healthy individuals were included. Patients were classified based on taking drugs, having comorbidities, and respiratory consequence severity. MSCs with phenotype of CD45-CD44+CD29+CD105+ were measured in peripheral blood by using flow cytometry.

**Results:** Circulating MSCs were lesser in SM-exposed patients rather than the control group (median = 0.93 and 2.72 respectively, p = 0.005). No significant difference was observed in the MSC population between patients taking systemic corticosteroids or antibiotics and did not taking them, but comorbidities like liver and kidney diseases, and asthma had changed the peripheral level of MSCs in SM-exposed subjects. In addition, circulating MSCs did not show association with long-term pulmonary complication severity.

**Conclusion:** SM exposure causes decline in the circulating MSCs in survivors. Lower level of peripheral MSC population in SM-exposed patients is not affected by taking corticosteroids or antibiotics, but comorbidities are probably involved in MSC trafficking. Decreased circulating MSCs do not associate with pulmonary complications' severity; however, further studies in mustard lung models are required to demonstrate the therapeutic or pathologic role of MSCs in SM injuries.

**Keywords:** Mesenchymal Stem Cells, Mustard Gas, Lung, Flow Cytometry.

# Immunology of Exercise, Aging, and Nutrition

# Oral Presentation

ICIA2018\Immunology of Exercise, Aging, and Nutrition\Oral\7500
**7500**

## Immuno-potential Effects of Donkey's Milk on Human Mononuclear Cells

Seyedeh Forough Sadeghian-Kiadehi[1], Saeid Taghiloo[1, 2], Esmaeil Allahmoradi[1, 2]

1. *Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
2. *Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran*

**Background:** Nowadays, donkey's milk consumption has been reevaluated for their potential benefits to human health. In infants with intolerance to cow's milk, donkey's milk represents a good alternative due to its chemical characteristics similar to those of human milk. In the present in vitro study, the pro- and anti-inflammatory effects of donkey's milk were evaluated on human peripheral blood mononuclear cells (PBMC).

**Methods:** PBMCs were isolated from 12 young and 12 aged normal subjects using Lymphosep media. $2\times10^5$ cells were cultured in 0.2 ml of RPMI medium supplemented with 10µl of each the optimal and non-cytotoxic dose of pasteurized donkey's milk, 100 IU/ml penicillin, 100 µg/ml streptomycin and 10% fetal calf serum in flat-bottom 96-microwell culture plates. PolymyxinB (PMB-50 µg/mL) was added to all wells to inhibit the possible endotoxin contamination. Lipopolysaccharide (LPS-100 pg/mL) was also applied as positive control. All cultures were performed in triplicate and plates were incubated at 37°C in a humidified atmosphere with 5% CO2. After 18 hours, culture supernatants were harvested to measure TNF-α, IL-6, IL-8 and IL-10 by ELISA.

**Results:** Donkey's milk significantly increased TNF-α (p: 0.01), IL-8 (p< 0.0001), IL-6 (p< 0.0001) and IL-10 (p: 0.01) levels in PBMCs. In addition, the levels of IL-6 (p: 0.002), IL-8 (p: 0.002) and TNF-α (p: 0.002) secreted from aged subjects were significantly higher than young subjects. Contrast with these data, the level of IL-10 was markedly reduced in aged subjects (p: 0.02).

**Conclusion:** Our findings highlight the immune-potentiation effects of donkey's milk, and suggest that donkey's milk might be investigated as a beneficial dietary component to up-regulate the immune response in aged people.

**Keywords:** Donkey's milk, Immuno-potential, aged subject, young subjects, LPS, Cytokine

ICIA2018\Immunology of Exercise, Aging, and Nutrition\Oral\7596
**7596**

# The effect of vitamin D supplements on the frequency of CD4+T cell subsets in women with Hashimoto Thyroiditis, a Double-Blind Placebo-Controlled Study

Nodehi M[1], Ajami A[1] , Izad M[2] , Asgarian-Omran H[1] , chardouli R[3], Amouzegar A[3], Azizi F[3] ,Hematabadi M[4] , Esfahanian F[4] ,Yekaninejad S[5] , Saboor-Yaraghi AA[6]

*1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran and Molecular and Cell Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran*

*2. Department of Immunology, School of Medicine, International Campus, Tehran University of Medical Sciences, Tehran, Iran*

*3. Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

*4. Department of Endocrinology, Vali-Asr Hospital, Imam Khomeini Complex Hospital, Tehran University of Medical Sciences, Tehran, Iran*

*5. Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

*6. Department of Immunology, School of Public Health , Tehran University of Medical Sciences, Tehran, Iran*

**Background:** In addition to its known effects on bone metabolism, vitamin D may regulate immune function. Investigations on the pathogenesis of Hashimoto Thyroiditis and the mechanism of vitamin D action have been revealed that vitamin D may be a good choice to reduce damage to the thyroid cells caused by autoreactive immune cells. Considering the important role of CD4+ Tcells in the pathogenesis of Hashimoto's thyroiditis, the effect of this vitamin on the frequency of Th1, Th2, Th17 and Tr1 cells in patients with Hashimoto Thyroiditis was explored.

**Methods:** 34 patients aged 20-45 years were introduced by endocrinologists and entered the study. Patients with history of other autoimmune disease, allergy, malignancy, chronic kidney or liver disease were excluded from the study. They were recruited into two groups including 17 patients in each group. Group I and II were treated weekly with 50.000 IU of cholecalciferol and placebo respectively, for 3 months. Before and after supplementation, the frequency of Th1, Th2, Th17 and Tr1 cell was evaluated using flowcytometry method.

**Results:** The results showed that cholecalciferol supplementation caused a significant decrease in the Th17/Tr1 ratio, however, had no significant effect on percentages of Th1, Th2, Tr1 and Th17 cells in Hashimoto's thyroiditis patients.

**Conclusion:** In this study that conducted on women with Hashimoto Thyroiditis, supplementation by cholecalciferol for 3 months could change the phenotype of CD4+ T cell lymphocytes. Therefore, this vitamin can be considered as a supplement to prevent and treat the disease.

**Keywords:** Cholecalciferol, Hashimoto Thyroiditis, Th17 Cells, Tr1 Cells, Th1 cells, Th2 cells

ICIA2018\Immunology of Exercise, Aging, and Nutrition\Oral\8228
**8228**

# Investigating the ratio of Th1/Th2 cells after three sessions of endurance exercise as an adjuvant in HSV-gd1 DNA vaccine

Mohammad Taher Afshounpour[1], Mahdieh Molanouri Shamsi[1], Afsaneh Jamali[1], Sara Soudi[2], Hoorieh Soleimanjahi[3], Reza Gharakhanlou[1]

1. *Department of Physical Education and Sports Science, University of Tarbiat Modares, Tehran, Iran*
2. *Department of Immunology, University of Tarbiat Modares, Tehran, Iran*
3. *Department of Virology, University of Tarbiat Modares, Tehran, Iran*

**Background:** The relations between the vaccination, exercise and adjuvant effects of exercise are a new perspective in the exercise immunology, but there are many questions about it and needs extensive research. Therefore, the aim of this study was to investigate the ratio of Th1/Th2 cells after three sessions of endurance exercise as an adjuvant in HSV-gd1 DNA vaccine.

**Methods:** Balb/c mice (6 to 8 weeks) were randomly assigned into six groups, 1- Endurance exercise, 2- Vaccine, 3-PCDNA, 4- Control, 5- Endurance exercise + Vaccine and 6- Endurance exercise + PCDNA. Endurance exercise was performed sequentially for three days on the animal treadmill. Immediately after the third session, HSV-gD1 DNA vaccine was injected into the tibialis muscle of mice. 12 days after the second vaccine, in spleen INF-γ and IL-4 cytokines were evaluated by ELISA method. One-way ANOVA was used to analyze the data.

**Results:** The results showed that three sessions of endurance exercise could significantly increase the ratio of Th1/Th2 cells in the HSV-gD1 vaccine model. Also, there is a significant difference between this group and other groups, especially in the vaccinated group.

**Conclusion:** According to the findings, three sessions of endurance exercise act as an adjuvant in HSV-gd1 DNA vaccine and enhance the immune response.

**Keywords:** Th1/Th2 cells, endurance exercise, adjuvant, DNA vaccine.

ICIA2018\Immunology of Exercise, Aging, and Nutrition\Oral\8506
**8506**

# Effects of vitamin D on the expression of Th17 cell-related cytokines in experimental autoimmune encephalomyelitis

Sayyed Vahab Azizi[1], Rayhaneh Ahangar-Parvin[1], Marziyeh Mohammadi-Kordkhayli[2], Mohammad-Taghi Rezayati[1], Maryam Nemati[3], Fatemeh Ayoobi[4], Zahra Taghipour[5], Hossain Khoramdelazad[1], Abdollah Jafarzadeh[*2]

1. *Department of Immunology, Medical School, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.*
2. *Department of Immunology, Medical School, Kerman University of Medical Sciences, Kerman, Iran.*
3. *Department of Laboratory Sciences, Para-Medicine School, Kerman University of Medical Sciences, Kerman, Iran.*
4. *Department of Physiology, Medical School, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.*
5. *Department of Histology, Medical School, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.*

**Background:** A broad of immunomodulatory properties is attributed to vitamin D. The aim was to evaluate the vitamin D effects on the expression of some Th17 cell-related cytokines in an experimental autoimmune encephalomyelitis (EAE) model.

**Methods:** The C57Bl/6 mice used for EAE induction by immunization with myelin oligodendroglial glycoprotein mixed with Freund's adjuvant. From day +3 to +30, the mice were administrated by Intraperitoneal injection of PBS or olive oil in control groups and vitamin D in treatment group. At day 31, mice were scarified and the expression of IL-17A and IL-23 in the spinal cord and serum were measured by real time-PCR and ELISA, respectively.

**Results:** The expression of IL-17, IL-23 P19 and IL-23 P40 in spinal cord and serum IL-17 and IL-23 levels in PBS-administrated EAE mice was significantly higher than healthy group ($P<0.02$, $P<0.001$, $P<0.001$, $P<0.04$ and $P<0.05$ respectively). In EAE mice treated with vitamin D, the expression of aforementioned parameters were significantly decreased as compared with control group ($P<0.05$, $P<0.001$, $P<0.001$, $P<0.04$ and $P<0.02$ respectively).

**Conclusion:** Vitamin D downregulates the Th17 cell-related cytokines in EAE mice. The possible therapeutic potential of vitamin D for treatment of MS can be consider in future investigation.

**Keywords:** Experimental autoimmune encephalomyelitis, Vitamin D, IL-17, IL-23

ICIA2018\Immunology of Exercise, Aging, and Nutrition\Oral\9771
**9771**

# Comparative study of the combinatory effects of Docosahexaenoic acid (DHA) and Linoleic acid (LA) with Taxol on the expression of metastasis related genes and microRNAs in triple-negative metastatic breast cancer cell line

M. Javadian[1,2], N. Shekari[1], M. Soltani [1], A. Mohammadi [1], B. Baradaran[1], D. Shanehbandi[1], T. Kazemi[1]

1. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Involvement of some genes and microRNAs (miRNAs) in metastatic breast cancer has been extensively investigated. However, there are limited numbers of study regarding the effects of standard anti-cancer therapeutic agents and also common dietary supplements on the expression of such genes and miRNAs. In this study, we aimed to investigate the effects of DHA and LA fatty acids on the expression of matrix metalloproteinase (MMP)-9, 2, Talin2, HIF-1α and vimentin genes, and tumor-suppressor miR-194 and 106b oncomiR, and also their possible synergistic effects with Taxol.

**Methods:** MDA-MB-231 Cells were cultured under normoxic and hypoxic conditions, and treated for 24 hours with taxol in IC50 concentration, 100 µM DHA and 50 µM LA, alone or in different combinations. Cells were harvested, and RNA/miRNA extraction and cDNA synthesis were performed using standard methods. Expression levels of studied genes and miRNAs were analysed using quantitative real-time PCR. Western blotting and scratch test were also performed to confirm findings.

**Results:** Taxol and DHA significantly down-regulated MMP-9, MMP-2, HIF-1α and vimentin expression and up-regulated Talin2 in normoxic and hypoxic condition ($p<0.05$). But our results showed that LA induced up-regulation of MMP-2 and MMP-9, vimentin expression in these conditions ($p<0.05$). miR-194 and miR-106b, showed up- regulated and down-regulated expression in all treated respectively. The result of western blotting for Talin2 didn't confirm the effects of different treatments, but scratch test showed inhibitory effect of DHA alone and in combination with Taxol.

**Conclusion:** Antimetastatic effects of DHA and Taxol, alone or in combination, were confirmed in this study. MMP-2, MMP-9, HIF-1α and vimentin showed down-regulation after treatment. Consistent result were found about down-regulation of miR-106b oncomir and up-regulation of tumor-suppressor miR-194. But their role in altered expression of Talin-2 needs for more investigation.

**Keywords:** Docosahexaenoic acid, Breast cancer, MicroRNA, Metastasis.

# Immunology of Infectious Diseases

# Bacteria and Fungi

# Oral Presentation

ICIA2018\Immunology of Infectious Diseases Bacteria and Fungi\Oral\7815
**7815**

# Comparison the percentages of Th1 and Th17 cells in untreated and antimicrobial treated patients with brucellosis

Mina Rahmanpour[1], Sanaz Jourghasemi[1], Ghasem Solgi[1], Fariba Keramat[2], Mehrdad Hajilooi[1*]

*1. Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
*2. Department of Infectious Diseases, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran; Brucellosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran*

**Background:** Brucellosis is a worldwidely zoonotic infection caused by brucella, facultative intracellular Gram-negative coccobacilli bacteria. Host protection against brucella relies on cell-mediated immunity. T helper type 1 (Th1) cells play a critical role in immunity to brucellosis, while role of T helper type 17 (Th17) cells against the infection remained unknown. In this study aim to compare the percentages of Th1 and Th17 cells in untreated and antimicrobial treated patients with brucellosis.

**Methods:** Peripheral blood mononuclear cells (PBMCs) isolated from 15 untreated brucellosis patients (new cases) and 15 treated brucellosis patients with antimicrobial treatment (30 study subjects) using ficoll density gradient centrifugation. PBMCs cultured with phorbolmyristate acetate (PMA, 50 ng/ml), ionomycin (1 µg/ml) and brefeldin A (5 µg/ml) for 5 h at 37℃ in 5% CO2. Subsequently, flowcytometry analysis performed for $CD4^+IFN\text{-}\gamma^+$ Th1 and $CD4^+IL\text{-}17^+$ Th17 cells.

**Results:** The percentage of Th1 cells were increased in new cases in comparison to patients under treatment (Th1: 3.57±1.86% vs 2.79±2.25%, P=0.34). Also, there was an increase in the count of Th17 cells among untreated and treated patients (Th17: 0.35±0.14% vs 0.25±0.14%, P=0.07).

**Conclusion:** In this study, we demonstrated the increase percentages of Th1 and Th17 cells in untreated patients. This results indicate that immune responses related to this cells are important in patients with brucellosis.

**Keywords:** Brucellosis, Th1 cells, Th17 cells

ICIA2018\Immunology of Infectious Diseases Bacteria and Fungi\Oral\8085
8085

**PBMCs of patients with abdominal aortic aneurysm in co-culture with endothelial cells produce lower levels of IL-9 in comparison to control group**

Hamid Aria[1, 2], Mehdi Kalani[3], Hossein Hodjati[4], Mehrnoosh Doroudchi[1]

1. *Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.*
2. *Bioinformatics and Computational Biology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.*
3. *Prof. Alborzi Clinical microbiology research center, Nemazee hospital, Shiraz University of Medical Sciences, Shiraz, Iran.*
4. *Department of Vascular Surgery, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.*

**Background:** Cytokines are major players in inflammatory condition leading to diverse diseases. Abdominal Aortic Aneurysm (AAA) is the dilatation of abdominal aorta, which happens in some of the patients with atherosclerosis. In an attempt to mimic the interaction of blood cells with wounded endothelium, we investigated the effect of CagA+ and CagA- Helicobacter pylori extracts in co-culture of PBMCs with endothelial cells (EC).

**Methods:** PBMCs were isolated from 5 men with diagnosed AAA and 5 men with normal/insignificant angiography, CT-scan and ultrasonography results. EC cells were extracted from umbilical cords by collagenase method (HUVEC). PBMCs were cultured in plates with bacterial extract of CagA+ and CagA- Helicobacter pylori in co-culture with HUVEC cells for 48 hrs. Then, the supernatant were removed to measure IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F, IL-21, IL-22, IFN-γ and TNF-α using a commercial fluorescent-labeled bead assay.

**Results:** IL-9 production by patients' PBMCs without antigenic stimulation in co-culture with HUVECs (1403.07 ± 2856.07 Pg/ml) was lower than controls (12412.00 ± 7399.47 Pg/ml) (P=0.05). Production of IL-22 by PBMCs in response to CagA- H. pylori extract in co-culture with HUVECs (22.82 ± 11.37 Pg/ml) was lower compared to controls (37.21 ± 10.76 Pg/ml) (P=0.05).

**Conclusion:** The difference in the cytokine production pattern in patients with AAA and controls suggest the importance of these factors in the initiation/progression of AAA.

**Keywords:** Abdominal aortic aneurysm, cytokines, Helicobacter pylori, endothelial cells

ICIA2018\Immunology of Infectious Diseases Bacteria and Fungi\Oral\8415
**8415**

# The capability of recombinant Hcc fragments in the activation of T cells was assessed by CD69 expression

Saeedeh Torabi Goudarzi[1], Mehdi Yusefi. PhD[1, 2], Jafar Majidi. PhD[1, 2]

1. *Department of Immunology, Faculty of Medicine Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** A protective response against tetanus toxin and toxoid demands efficient specific T cell and B cell responses. Tetanus neurotoxin (TeNT), a 150 kDa polypeptide, is the main cause of tetanus disease. TeNT consists of two structurally distinct chains, a 50 kDa N-terminal light (L) and a 100 kDa C-terminal heavy (H) chain. C-terminal heavy (H) chain (fragment C) has two sub-domains named as proximal HCN and carboxyl sub-domain or HCC. Beside neural binding property, HCC has been recently found as an immunodominant part of TeNT. In the present study, we investigated the effects of recombinant HCC (rHCC) on the capability of recombinant Hcc fragments in the activation of T cells was assessed by CD69 expression.

**Methods:** In the present study, we isolated human T cells by magnetic-activated cell sorting method (MACS), Purified T cells were co-incubated with recombinant Hcc. The concentration of different cytokines were determined by ELISA and then we were analyzed the expression of markers of lymphocyte T activity by flowcytometry.

**Results:** The results showed that rHcc enhanced expression of CD69 on the surface of T-cells and promoted differentiation of $CD4^+$ T-lymphocytes toward a T-helper1 ($T_H1$) phenotype and upregulation of interferon (IFN)-γ secretion.

**Conclusion:** These results indicated that HCC is critical for immunogenic and immunoprotective activity of TeNT and is able to induce T cells and has a stimulatory effect on IFN-γ secretion. This subdomain can propose as an adjuvant and a candidate to stimulate the immune system.

**Keywords:** Tetanus, Tetanus neurotoxin, Immune Respones, Hcc subdomain

ICIA2018\Immunology of Infectious Diseases Bacteria and Fungi\Oral\9779
**9779**

# Aspergillus fumigatus conidia stimulate cytokines release from epithelial cells (TC-1 JHU-1): a study of modulatory effect of propolis extract

Ali Reza Khosravi[1], Hadis Moradjodaki[2]

*1. Department of Microbiology and Immunology, University of Tehran, Tehran, Iran*
*2. Department of Microbiology and Immunology, University of Tehran, Tehran, Iran*

**Background:** Aspergillus fumigatus conidia are the most prevalent indoors fungal allergens. The interaction between Aspergillus antigens and lung epithelial cells result in innate immune functions. The association between Aspergillus conidia and allergic reactions, like allergic bronchopulmonary aspergillosis (ABPA) and asthma have been repeatedly reported. The interaction between Aspergillus antigens and lung epithelial cells result in innate immune functions. Since conventional therapies for allergy and asthma are limited, finding new promising treatments are inevitable.This study was designed to evaluate the effect of Aspergillus fumigates conidia on some cytokine releases from mouse epithelial cells and to investigate the effect of propolis on cytokines modulation.

**Methods:** Cells were divided to two groups, one was exposed to $3\times10^4$ conidia of Aspergillus fumigatus and another group was treated by propolis (25µg/ml) as well as exposure to A. fumigatus conidia. Cytokines IL-13, IL-12, $IFN_\gamma$ and IL-17 were measured at times 0, 6 and 12 hours after exposure using ELISA assay.

**Results:** The results indicated that A. fumigatus could increase the release of understudied cytokines with IL-13 and IL-17 being the most affected one, whilst treatment with propolis decreased the effects of A. fumigatus on IL-13 and IL-17 production. The results showed that propolis has down regulatory effects on Th2 cytokine, IL13, and IL17 production, whereas it caused a significant induction of IL-12, as an important Th1 cytokines by lung epithelial cells (LECs).

**Conclusion:** With respect to the obtained results, propolis extract might be contributed to decrease Th2 responses in allergic asthma phenomenon. However more investigations must be done in future to fully understand its efficacy.

**Keywords:** Aspergillus fumigatus, cytokines, epithelial cells, propolis

ICIA2018\Immunology of Infectious Diseases Bacteria and Fungi\Oral\9892
**9892**

# NRWCFAGRR: A Novel Antimicrobial Peptide with Nitric Oxide Induction Activity

Mehdi Ghahary[1] , Kazem Ahmadi[1], Mahdi Fasihi-Ramandi[1]

*1. Molecular Biology Research Center, system biology and poisonings institute, Baqiyatallah University of Medical Sciences, Tehran, Iran*

**Background:** Bacterial resistance against antibiotic has caused many problems in the procedure of treating humans and animals all around the world. Inappropriate utilization of antibiotics can result to resistance in the target microorganism population. Antimicrobial peptides have been introduced as new effective strategies that kill bacteria quickly and cause less antibiotic resistance.

**Methods:** In the present research we have been studied the cytotoxic effect of NRWC peptide on eukaryotic and prokaryotic cells. Twelve bacterial strains were selected to study the antimicrobial effects of NRWC peptide. MIC and MBC assay were used to study the inhibitory and killing activity of these peptides respectively. Peptide cytotoxic effects were assayed on HeLa cell line and human RBC using MTT assay and Hb release measurement respectively. J774 macrophage cell line was used to measure the amount of nitric oxide production in response to the peptide.

**Results:** The results showed that NRWCFAGRR peptide in different concentrations had bactericidal and inhibitory effect on the growth of all 12 bacterial strains in a dose dependent manner. It has also been proven that the toxic effect of peptide on human cells is inevitable at the MIC and MBC concentration. The highest amount of nitric oxide produced under the influence of this peptide was observed in a period after 48 hours treatment.

**Conclusion:** Considering the researches have been conducted in the field of microbial peptides, the peptide introduced in this research has anti-microbial properties that kills some microbes directly and theoretically has the ability to kill some organisms indirectly via induction of nitric oxide in the macrophages.

**Keywords:** Antibiotics resistance, Nitric oxide, MBC, MIC, Antimicrobial peptide

# Immunology of Infectious Diseases

## Viruses

## Oral Presentation

ICIA2018\Immunology of Infectious Diseases Viruses\Oral\6358
**6358**

# Designing a DNA Vaccine for Therapeutic Goals in Cancer Mouse Model of Human Papilloma Virus Type-16

Ali Ganji[1,2], Hamid Abtahi[1,2], Ghasem Mosayebi[1,2], Iman Farahani[1,2], Ali Ghazavi[1,2], Maryam Moazamigoodarzi[1], Mostafa Ejtehadifar[1], Saeed Babaie[3]

*1. Molecular and Medicine Research Center, Arak University of Medical Sciences, Arak, Iran.*
*2. Department of Microbiology and Immunology, School of Medicine, Arak University of Medical Sciences, Arak, Iran.*
*3. Department of Anatomy, Arak University of Medical Sciences, Arak, Iran*

**Background:** Infection with human papillomavirus type 16 (HPV 16) causes cervical cancers. Although, many attempts have been made to treat of these infections, but further studies are necessary to find new therapies. So, the aim of this study was setting light on therapeutic potencies of HPV 16-derived DNA vaccines to introduce a treatment based on improving the immune system functions.

**Methods:** DNA vaccine for optimum expression in bacteria was designed and the recombinant protein was expressed. The designed DNA vaccine was injected via intramuscular route in mouse model of HPV in compare with intra-peritoneal injection of recombinant protein with adjuvant. The induced mice were monitored based on animal survival, tumor size, and immunologic responses.

**Results:** Comparisons illustrated meaningful decrease in tumor size ($P < 0.05$) of treated with a mixture of DNA vaccine and protein in contrast to the other groups. Leukocyte infiltration and proliferation were significantly showed increased number in the treated group with DNA in comparison with control group ($P < 0.05$). The level of IgG2a showed an increasing in DNA vaccine and mixture of DNA vaccine and protein in comparison with control group ($P < 0.05$). Stimulated splenocytes of the treated mice with mixture of protein and DNA vaccine expressed significantly higher interferon gamma (INF-γ) than the untreated group ($P < 0.05$). The meaning percentage of cytotoxic T lymphocytes in treated group with DNA vaccine was more than other groups ($P < 0.05$).

**Conclusion:** The designed DNA vaccine is suitable candidate for therapeutic goals and results of present work proved that DNA prime/Protein boost approaches or injection of primary doses of DNA vaccine and booster doses of protein can be an impressive way to treatment of those cancers which may be a result of papilloma infections.

**Keywords:** Recombinant proteins, DNA vaccine, Vaccine therapy, Human papilloma virus

ICIA2018\Immunology of Infectious Diseases Viruses\Oral\7444
**7444**

# Increase of CD4+ CD25+ CD127low FoxP3+ regulatory T cells, but not CD4+ CD25+ FoxP3+ cells in active hepatitis B

Mousa Mohammadnia-Afrouzi[1], Mehdi Shahbazi[1], Hiva Mohammadizad[1], Mojgan Bagherzadeh[1]

*1. Department of Immunology, School of Medicine, Babol University of Medical Sciences, Babol, Iran*

**Background:** To investigate immune mechanisms underlying the difference between active and inactive hepatitis B, we analyzed regulatory T cell frequency in chronic hepatitis B patients.

**Methods:** 66 chronic hepatitis B patients were included in the study. Patients were divided in active (32 patients) and inactive (34 patients) groups based on laboratory parameters. Isolation of peripheral blood mononuclear cells (PBMCs) was carried out by using Ficoll-Paque density centrifugation. The frequency of Treg cells and FoxP3 expression level were evaluated by multicolor flowcytometry, after staining PBMCs with fluorochrome-conjugated antibody against CD4, CD25, CD127 and FoxP3. Mean Florescence intensity (MFI) was used as index of FoxP3 expression in Treg Cells.

**Results:** The frequency of CD4+ CD25+ CD127low FoxP3+ Treg cells was higher in active hepatitis compared to inactive patients. Additionally, there was a positive correlation between HBV viral load and frequency of CD4+ CD25+ CD127low FoxP3+ Treg cells in active patients. There was not any significant differences in other subtypes of Treg cells between groups. However, MFI of FoxP3 was directly correlated with HBV viral load.

**Conclusion:** Results of this study suggest that dynamic changes in CD4+ CD25+ CD127low FoxP3+ Treg cell frequency and FoxP3 expression show different trends in active and inactive hepatitis B patients.

**Keywords:** Treg, Hepatitis B, Active, Inactive, FoxP3

ICIA2018\Immunology of Infectious Diseases Viruses\Oral\7488
**7488**

## TIM-3 as a marker of exhaustion in CD8+ T cells of active chronic hepatitis B Patients

Hiva Mohammadizad[1], Parizad Bavandpour-Baghshahi[1], Mousa Mohammadnia-Afrouzi[1], Mehdi Shahbazi[1], Mojgan Bagherzadeh[1]

*1. Department of Immunology, School of Medicine, Babol University of Medical Sciences, Babol, Iran*

**Background:** Chronic hepatitis B is the most important chronic viral infection affecting 5 percent of the world population. Distinguish of the inactive patients from active, play an important role in treatment of patients. Virus-specific $CD8^+$ T cells lose effector function during chronic HBV infection and can express inhibitory receptors, TIM3, PD-1, and CD39, that are key feature of $CD8^+$ T Cells exhaustion in humans. In this study, we analyzed the level of PD-1, Tim-3 and CD39 expression in CD8+ T cells isolated from peripheral blood mononuclear cells of patients with active and inactive chronic hepatitis B.

**Method:** In the study, 34 inactive and 32 active chronic hepatitis B patients were included. Isolation of peripheral blood mononuclear cells (PBMCs) was done by Ficoll-Paque density centrifugation. The level of PD-1, Tim-3 and CD39 expression in CD8+ T cells of Patients determined by multicolor flo cytometry after staining PBMCs using fluorochrome-conjugated antibody against CD39, Tim3, PD1, CD8.

**Results:** CD8+ T cells frequency was significantly lower in patients with active chronic B hepatitis than inactive hepatitis B ($p = 0.0001$). CD8+ TIM3+ T cells and also CD8+TIM3+PD1+ T cells were significantly higher in patients with active chronic hepatitis B than inactive ones. However, PD1 and CD39 markers did not show significant differences. In active chronic phase, there was a positive correlation between AFP, ALT, and HBV viral load with CD8+ TIM3+ T cells frequency.

**Conclusion:** In active chronic hepatitis B, the number of exhausted T cells, due to the virus involvement, are more than the inactive and TIM3 seems to be a marker of exhaustion in CD8 T cells of these patients.

**Keywords:** exhausted, active, hepatitis B, CD8, TIM3

ICIA2018\Immunology of Infectious Diseases Viruses\Oral\7757
**7755**

# Blocking of opioid receptors in experimental formalin-inactivated respiratory syncytial virus (FI-RSV) immunopathogenesis: from beneficial to harmful impacts

Vahid Salimi[1], Habibollah Mirzaei[2], Ali Ramezani[2], Alireza Tahamtan[1], Fatemeh Adjaminejad[1], Ghazal Fatemi-Nasab[1],Fazel Shokri[3], Talat MokhtariAzad[1].

1. *Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
2. *Department of Virology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz*
3. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Opioid system plays a significant role in pathophysiological processes, such as immune response and impacts on disease severity. Here, we investigated the effect of opioid system on the immunopathogenesis of respiratory syncytial virus (RSV) vaccine (FI-RSV)-mediated illness in a widely used mouse model.

**Methods:** Female Balb/c mice were immunized at days 0 and 21 with FI-RSV ($2\times10^6$ pfu, i.m.) and challenged with RSV-A2 (3×106 pfu, i.n.) at day 42. Nalmefene (NL) as a universal opioid receptors blocker administered at a dose of 1 mg/kg in combination with FI-RSV (FI-RSV+NL), and daily after live virus challenge (RSV+NL). Mice were sacrificed at day 5 after challenge and bronchoalveolar lavage (BAL) fluid and lungs were harvested to measure airway immune cells influx, T lymphocyte subtypes, cytokines/chemokines secretion, lung histopathology, and viral load.

**Results:** Administration of nalmefene in combination with FI-RSV (FI-RSV+NL-RSV) resulted in the reduction of the immune cells infiltration to the BAL fluid, the ratio of CD4/CD8 T lymphocyte, the level of IL-5, IL-10, MIP-1α, lung pathology, and restored weight loss after RSV infection. Blocking of opioid receptors during RSV infection in vaccinated mice (FI-RSV-RSV+NL) had no significant effects on RSV immunopathogenesis. Moreover, administration of nalmefene in combination with FI-RSV and blocking opioid receptors during RSV infection (FI-RSV+NL-RSV+NL) resulted in an increased influx of the immune cells to the BAL fluid, increases the level of IFN-γ, lung pathology, and weight loss in compared to control condition.

**Conclusion:** Although nalmefene administration within FI-RSV vaccine decreases vaccine-enhanced infection during subsequent exposure to the virus, opioid receptor blocking during RSV infection aggravates the host inflammatory response to RSV infection. Thus, caution is required due to beneficial/harmful functions of opioid systems while targeting as potentially therapies.

**Keywords:** Respiratory syncytial virus, Formalin-inactivated RSV, Immunopathogenesis, Nalmefene.

ICIA2018\Immunology of Infectious Diseases Viruses\Oral\8181
**8181**

# Measles virus infected dendritic cell migration in a 3D in vitro human airway test system

Shaghayegh Derakhshani[1], Andreas Kurz[2], Sibylle Schneider-Schaulies[1]

1. *Department of biophysics and biotechnology,University of Würzburg, Germany*
2. *Department of Virology and Immunobiology,University of Würzburg, Germany*

**Background:** Parameters important in Measles Virus (MV) transmission from dendritic cells (DCs) to epithelial cells such as cytoskeletal or receptor dynamics were determined in 2D systems. It remains unclear whether and to what extent these apply in a more complex environment such as the respiratory tract epithelium. Therefore we analyze these parameters in 3D models of the airway mucosa with high in vitro/in vivo correlation.

**Methods:** 3D models Fibroblasts are seeded apically onto a decellularized porcine intestine derived scaffold, and H358 epithelial cells are subsequently added; models are cultivated under airlift conditions. Cellular composition of the 3D models was evaluated by immunohistochemistry (IHC) and immunofluorescence (IF). DCs infected with MV (IC323-eGFP) for 24 h were applied basolaterally to the collagen scaffold. DC trafficking towards epithelial cells was recorded by time lapse microscopy. Spread of MV infection to epithelial cells was microscopically monitored and quantified by flowcytometry.

**Results:** IHC/IF stainings of paraffin embedded models performed 24 h following basolateral DC application allowed for distinction of layers of fibroblasts and epithelial cells with DCs interspersed in the epithelial layer. To visualize DC migration through the models, cells were tracked 30 min following their basolateral application in a 3h 3D time series. Remarkably, MV infected DCs migrated more efficiently towards the apical surface of the models than uninfected cells indicating that MV-infection enhances DC tissue motility. In support of this hypothesis, pAkt levels were found elevated in MV-infected DCs. In addition to that of proteins, lipid sorting is important in membrane dynamics and thereby, in viral transmission. Accordingly, pharmacological inhibition of the acid sphingomyelinase (ASM) in DCs efficiently modulated viral transmission to epithelial cells in 2D and 3D conditions.

**Conclusion:** In the current study we showed that MV infection enhanced DCs motility in a 3D environment. Moreover, sphingolipid turnover is of obvious importance in viral transmission to epithelial cells. Current analyses address subcellular redistribution of ceramides in infected DCs to further unravel their role in this process.

**Keywords:** Measles virus, dendritic cells, cell migration, 3D tissue models

ICIA2018\Immunology of Infectious Diseases Viruses\Oral\8255
**8255**

# Induction of inflammatory mediators by Influenza A virus protein PB1-F2 in J774.1 macrophage

Zahra Farzaneh[1], Nasser Gholijani[2], Kurosh Kalantar[1]

1. *Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Autoimmune Disease Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background**: Influenza A virus (IAV) has potential to cause mortal pandemics with considerable health and socio-economic burdens. Some of IAV express virulence factor PB1-F2. The PB1-F2 protein has pro-apoptotic activity and contribute to viral pathogenesis by delaying in viral clearance and inducing inflammation. Macrophages are susceptible to IAV infection and produce high levels of inflammatory cytokines and chemokines.

**Methods:** The cytotoxic effect of different concentrations of PB1-F2 protein on J774.1 macrophage was determined by MTT assay. Then, the pro-inflammatory effect of PB1-F2 on the expression of iNOS, COX-2, IL-6 and TNF-α genes were evaluate by the real-time PCR. Production of key inflammatory mediators including IL-6 and TNF-α were assessed using ELISA assay. In addition, PB1-F2 treated macrophages examined for nitric oxide (NO) production by means of griess reagent.

**Results:** Treatment of cells with 0.22 and 0.45 µmol/ml of peptide increased NO production to 105.8 ± 1.24% and 119.5 ± 12.8% of the LPS-only treated cells, respectively. Peptide at 0.9 µmol/ml concentration significantly increased gene expression of iNOS and COX-2. Gene expression increased to 2.02 ± 0.25 RFC ($p< 0.001$) (iNOS) and 3.81 ± 0.74 RFC ($p<0.0001$) (COX-2) of the LPS-only treated cells (positive control). Treatment of cells with concentrations 0.45 and 0.9 µmol/ml of peptide significantly induced gene expression of pro-inflammatory cytokines (IL-6, TNF-α). Peptide at these concentrations increased IL-6 gene expression to 2.19 ± 0.73 RFC and 3.65 ± 0.3 RFC. At the same concentrations of peptide, TNF-α gene expression showed an increasing level to 4.15 ± 0.86 RFC and 5.55 ± 0.44 RFC of positive control, respectively. Both of cytokines in protein level also increased.

**Conclusion:** These finding suggest that PB1-F2 protein is a potent inducer of inflammatory mediators in macrophages that may contribute to diseases severity.

**Keywords:** Influenza A virus, PB1-F2, Inflammation, Macrophage

ICIA2018\Immunology of Infectious Diseases Viruses\Oral\8539
**8539**

# Investigating the Effects of different vaccination schedules on Hepatitis vaccine induced antibody titers in ICU Personnel of Kowsar Hospital of Sanandaj, 2018

Pejman Allahveisey[1], Jalil Adabi[2], Asrin Safari[2], Elahe Amani[2]

1. *School of nursing, University of medical sciences Iran*
2. *School of nursing, University of medical sciences Kurdistan*

**Background:** Hepatitis type B has been recognized as one of the infections, which can be easily transformed through infected blood. The rate of this kind of infection transmission was reported to be under 1% in the 1970s. The risk of transmission of Hepatitis infection through infected blood has never been completely eliminated, due to the fact that factors such as Window Period and Low Titer of HBV can cause infection transmission. Vaccination is the key step to provide immunization coverage for individuals exposed to Hepatitis.

**Method:** The participants of this study were the personnel of ICU ward in Kowsar Hospital of Sanandaj. Their antibody titer was measured; afterwards, a checklist was developed for the vaccination project. Twenty-five of the personnel agreed to participate in the present study. The collected data was analyzed using SPSS 18.

**Result:** The collected data revealed that 64% of the participants had done the vaccination schedule completely for three times, 20% of them only completed the first and the second phases of the schedule, and 16% of them just did the first phase. The results showed that 81.25% of the participants, who had done the vaccination for three times, indicated antibody titer which was over 200.

**Conclusion:** Completed vaccine schedule can often guarantee healthy individuals in 95% of cases. It can be concluded that personnel of hospitals, in particular those who work in ICU wards, need to check their antibody titer regularly.

**Keywords:** Hepatitis, Personnel of ICU ward, Antibody Titer

# Immunology of Mycobacterium Tuberculosis

# Oral Presentation

ICIA2018\Immunology of Mycobacterium Tuberculosis\Oral\9873
**9873**

# The analysis of exosomal miRNAs released from the human macrophages after infection with bovis bacillus Calmette-Guerin (BCG)

Esmaeil Mortaz[1], Shamila D Alipoor[2], Payam Tabarsi[1], Mehdi Mirsaeidi[3], Johan Garssen[4], Masoud Movassaghi[5], Ian M Adcock[6]

*1. Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Institute of Medical Biotechnology, Molecular Medicine Department, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran*
*3. Division of Pulmonary, Critical Care, Sleep and Allergy, Department of Medicine, University of Miami, FL, USA*
*4. Nutricia Research Centre for Specialized Nutrition, Utrecht, Netherlands.*
*5. Department of Pathology and Laboratory Medicine, University of California, Los Angeles (UCLA), Los Angeles, CA, USA*
*7. Airways Disease Section, National Heart & Lung Institute, Imperial College London, London, UK*

**Background:** Tuberculosis (TB) remains a significant global health concern and its diagnosis is challenging due to the limitations in the specificity and sensitivity of the current diagnostic tests. Exosomes are bioactive 30-100nm vesicles that are produced by most cell types and are found in almost all human body fluids. Exosomal microRNAs (miRNAs) can transfer biological information between cells and tissues and may act as potential biomarkers in many diseases. In this pilot study we assessed the miRNA profile of exosomes released from human monocyte-derived macrophages upon infection with Mycobacterium bovis Bacillus Calmette-Guerin (BCG).

**Methods:** Human monocytes were obtained from the peripheral blood of 3 healthy subjects and driven to a monocyte-derived macrophage (MDM) phenotype using standard protocols. MDMs were infected with BCG or left uninfected as control. 72h post-infection, exosomes were collected from the cell culture medium, RNA isolated and RNA-seq performed. The raw reads were filtered to eliminate adaptor and primer sequences and the sequences were run against the mature human miRNA sequences available in miRBase. MicroRNAs were identified using an E value <0.01. miRNA network analysis was performed using the DIANA miRNA tool, miRDB and functional KEGG pathway analysis.

**Results:** Infection of MDMs with BCG leads to the release of several exosomal miRNAs. These included miR-1224, -1293, -425, -4467, -4732, -484, -5094, -6848-6849, -4488 and -96 all of which were predicted to target metabolism and energy production-related pathways.

**Conclusions:** This study provides evidence for the release of specific exosomal miRNAs from BCG-infected MDMs. These exosomal miRNAs reflect host-pathogen interaction and subversion of host metabolic processes following infection.

**Keywords:** Mycobacterium, Exosome, miRNA, Macrophage, Biomarker

ICIA2018\Immunology of Mycobacterium Tuberculosis\Oral\9869
**9869**

# miR-1224 Expression is Increased in Human Macrophage After Infection With Bacillus Calmette-Guerin (BCG)

Mohammad Varahram[1], Shamila D. Alipoor[2,3,4], Esmaeil Mortaz[2], Payam Tabarsi[2], Majid Marjani[2], Gert Folkerts[5], Johan Garssen[5,6], Ian M. Adcock[7,8]

*1. Mycobacterilogy Research Center (MRC) National Research Institute of Tuberculosis and lung diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Institute of Medical Biotechnology, Molecular Medicine Department, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran*
*4. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*5. Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, Netherlands*
*6. Nutricia Research Centre for Specialized Nutrition, Utrecht, Netherlands*
*7. Airways Disease Section, National Heart & Lung Institute, Imperial College London, London, UK*
*8. Priority Research Centre for Healthy Lungs, Hunter Medical Research Institute, The University of Newcastle, Newcastle, New South Wales, Australia*

**Background:** Tuberculosis (TB) remains a major threat to human health. Understanding the strategies mycobacterium takes to overcome immune defense is important to control the infection. miRNAs are master regulators of most pathways in the human body. Infection with mycobacterium affects upon host metabolic pathways to obtain the nutrition for its intracellular survival. In this study, we aimed to investigate the effect of BCG infection on the expression of three miRNAs (miR-1224, -484 and -425), which have been previously demonstrated to be important in infection and metabolic pathways.

**Methods:** Peripheral blood monocyte derived macrophage (MDM) cultures were prepared and infected with BCG at a multiplicity of infection (MOI) =10 or left uninfected as a control. 72h post-infection, the cultured cells were subjected to RNA extraction, cDNA synthesis and real-time PCR. Expression levels miRNAs were normalized to the levels of U6 snRNA (Rnu6) using the $2^{-\Delta\Delta Ct}$ method.

**Result:** Infection with BCG resulted in a highly significant increase in miR-1224 expression (24.4±3.8-fold induction) in human MDMs. The induction of miR-484 (1.8±0.3-fold increase) and of miR-425 (1.2±0.2-fold increase) was less increased compared to miR-1224. Mycobacterium tolerates a hostile microenvironment by escaping from lysosomal degradation and providing a lipid-rich niche by trigger with and re-pattering host metabolism.

**Conclusion:** This study highlighte the potential roles of miRNAs in host responses upon mycobacterium infection.

**Keywords:** Macrophages, MDM, TB, miR-1224, miR-484, miR-425

ICIA2018\Immunology of Mycobacterium Tuberculosis\Oral\8625
**8625**

# Assessment of the CC and CXC chemokine family's amounts in bronchoalveolar fluid and peripheral blood samples from patients with pulmonary tuberculosis in Mashhad Persian date Farvardin 1391 to 1392

Marzie Motaghy[1], Amin Seyfaddiny[2], AmirReza AghebatKhayr[3], Gholam Hossien HasanShahi[4]

1. *Kerman University of Medical Sciences*
2. *Medical Student, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
3. *Medical Student, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
4. *Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

**Background:** Pulmonary tuberculosis is one of the most common infectious diseases, the causative agent of the disease is acid fast bacilli called Mycobacterium tuberculosis. Approximately one-third of the world's population is infected with the bacteria. This disease despite extensive control programs in the past two decades, is still one of the most dangerous infectious diseases as it is estimated that around the world people develop TB disease every 15 minutes and 6 people die of the disease. For prevention of TB, early detection of the disease may be one of the most important strategies.

**Methods:** This cross-sectional study was conducted on a group of 150 patients with pulmonary tuberculosis, were involved. Peripheral blood and BAL was performed for patients as well as controls carried out in coordination with the Mashhad and 150 healthy individuals were collected and blood was obtained. Demographic data were collected through clinical history and methods, and were matched for age and sex. The chemokine family of CC and CXC in peripheral blood samples and bronchoalveolar lavage samples were measured and the results, along with demographic information by SPSS software and statistical method T-Test and Mann - Whitney were analyzed.

**Results:** 150 patients were involved in the scheme, were matched for age and sex. The serum level mean were measured every 3 households CC chemokines in peripheral blood and BAL in patients with pulmonary tuberculosis had increased significantly and statistical differences were significant ($P < .0001$). The serum level mean of 4 CXC chemokine family in peripheral blood and BAL in patients with pulmonary tuberculosis in peripheral blood than the control group also showed significant difference except one(The compare of serum level mean of CXCL9 (Mig) in peripheral blood between patients and controls was not significant ($P = 0.092$)).

**Conclusion:** Our study showed that all CC and CXC chemokine families in the study of BAL (bronchoalveolar lavage) sample in pulmonary tuberculosis patients was significantly associated with the disease, and suggests CCL11 as a new chemokine associated with pulmonary tuberculosis in bronchoalveolar fluid that could serve as a diagnostic marker from alveolar fluid to be used .well as all the items in the peripheral blood of patients with pulmonary tuberculosis in chemokine significantly related to disease and are discussed as tuberculosis diagnostic markers in blood, except CXCL9 (Mig) that local nature of this chemokine probably is production in bronchial epithelial cells, thus making it suggestive tuberculosis marker only in BAL.

**Keywords:** Pulmonary tuberculosis (TB), CC chemokine, CXC chemokine, Broncho alveolar lavage (BAL)

ICIA2018\Immunology of Mycobacterium Tuberculosis\Oral\7603
**7603**

# Comparing effects of route of immunization on the cellular immune responses induced by Mycobacterium tuberculosis recombinant antigen (ESAT-6/CFP-10)

Mozhdeh Namvarpour[1], Majid Tebianian[2], Reza Mansouri[1]

*1. Department of Medical Immunology, Shahid Sadoughi University of Medical Sciences and Services, Yazd, Iran*
*2. Department of Biotechnology, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran*

**Background:** Tuberculosis, as one of the main health problems of the present century, affects about 10 million people annually. The BCG vaccine is the only available vaccine against this disease in the recent 75 years and its protective responses can make enormous changes. Therefore, study on development of a new vaccine against tuberculosis is the new topic of many investigations. Route of vaccination is one of the most important factors in creating a good immune response. Recently, intranasal administration route has increased vaccines efficiency against respiratory infections. In this study, the effects of vaccine administration routes including intra-nasal, subcutaneous, and intramuscular routes on creating protective humoral immunity against tuberculosis has been compared and examined.

**Methods:** Recombinant antigen ESAT-6/CFP-10 was administered by three routes of IM, IN and SC to 8 weeks Balb/C mice with or without adjuvant for three times at two-week intervals. For IM and SC administration routes, adjuvant MF59 and for IN route adjuvant CTB were used. Then, the case-control study was carried out to evaluate the cellular responses elicited against ESAT-6/CFP-10, that were evaluated by mice spleens' immune cells culture proliferation and cytokine assay

**Results:** The results showed that vaccinated mice with recombinant antigen have higher titers of IFNγ, IL5 and significant increase in immune cells proliferation compared to the control groups. In the comparison between the other routes of injection, although SC and IM routes, were both able to make a good immune responses, IN route could activate high cellular immune responses that had fewer side effects than other aggressive injection routes.

**Conclusion:** This study emphasize that immunization of mice with recombinant ESAT-6/CFP-10 protein by IN route are durable and protective.

**Keywords:** Mycobacterium tuberculosis, Intramuscular, Subcutaneous, Intranasal administration

ICIA2018\Immunology of Mycobacterium Tuberculosis\Oral\11084
**11084**

# The Immunomodulatory Changes in Patients with Tuberculosis

Rafal J. Al-Saigh[1], Hussam Al-Humadi[2]

1. *Department of Clinical Laboratory Sciences, Faculty of Pharmacy, University of Babylon, Babylon, Iraq*
2. *Dean of Faculty of Pharmacy, University of Babylon, Babylon, Iraq*

**Background:** Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis. The aim was to investigate the levels of immunomodulatory markers like interluekin-6 (IL-6), tumor necrotizing factor-α (TNF- α), cell differentiation-4 (CD4) and CD8 levels in those patients with active tuberculosis (TB) disease in comparison with control group.

**Method:** 41 adults diagnosed with TB were included in comparison to 32 healthy individuals at Babylon health center for pulmonary diseases and TB. Descriptive data for patients and control group were collected by well-trained researcher following a structured questionnaire. In parallel, peripheral blood collected to determine IL-6, TNF- α, CD4 and CD8. Then the assessment for the association between clinical and descriptive data and immunomodulatory markers levels was investigated statistically.

**Results:** The majority of TB patients were males (56%) and 71% were resident in rural areas; 47% of them were living in middle socio-economic state, moreover, 47% of TB cases had diabetes, furthermore, 51% had chronic obstructive pulmonary diseases, 12% had hypertension and 39% of them had chronic anemia with 47% smokers with no significant difference versus control. Following to that, there was highly increased in IL-6 and TNF-α levels in TB patients versus control ($P<0.001$), with low CD4 level versus control ($P<0.001$). While there was no significant change shown in CD8 levels versus control and this might highly be correlated with 30% of abnormal liver function tests among TB patients.

**Conclusion:** A high proportion of TB patients have low CD4 level mostly associated with active disease. Moreover, the increase of IL-6 and TNF-α levels suggests an inverse impact on CD4 level which closely associated with the outcome of the disease.

**Keywords:** Tuberculosis, Immunomodulatory markers, IL-6, TNF-α, CD4, CD8.

# Immunology of Rheumatic Diseases

# Oral Presentation

ICIA2018\Immunology of Rheumatic Diseases\Oral\7790
**7790**

# Effect of Lactobacillus rhamnosus and Lactobacillus delbrueckii on the expression levels of miR-181a and miR-155 in the PBMCs of systemic lupus erythematosus patients

ZohrehVahidi[1], Mahmoud Mahmoudi[2], Zahra RezaeiYazdi[3], Maryam Sahebari[3], AlirezaEsmaeili[4], NafisehTabasi[5], Maryam Rastin[6*], Morteza Samadi[7*]

*1. Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

*2. MD-Ph.D of immunology. Immunology Research Center, BuAli Research Institute, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

*3. MD of Rheumatology. Rheumatic Diseases Research Center, Ghaem Hospital, Internal Medicine section, Mashhad University of Medical Sciences*

*4. Ph.D. Student of Immunology. Immunology Research Center, BuAli Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran*

*5. MSc of Immunology. Immunology Research Center, BuAli Research Institute, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

*6. Ph.D of immunology. Immunology Research Center, BuAli Research Institute, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

*7. Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

**Background:** Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease described by the production of autoantibodies and activated immune cells against self-antigens. The expression patterns of some miRNAs in PBMCs were shown to be associated with SLE development and activity. Probiotics showed anti-inflammatory and modulatory effects on the immune responses. In this study, we evaluated the effect of probiotics Lactobacillus rhamnosus and Lactobacillus delbrueckii on the expression levels of miR-155 and miR-181a in the PBMCs of SLE patients.

**Methods:** A total of 20 newly diagnosed SLE patients and 20 healthy controls were enrolled in the study. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood and were treated with probiotics Lactobacillus rhamnosus ($10^7$ Bac/ ml) and Lactobacillus delbrueckii ($10^5$ Bac/ ml) and cultured in RPMI-1640 for 48 hrs. After that, total RNA containing micro-RNAs were extracted. cDNA was synthesized for miRNAs and gene expression of miR-181a and miR-155 was analyzed by real-time PCR method.

**Results:** Findings showed expression levels of miR-181a and miR-155 in SLE patients were higher than those of healthy controls. Expression of miR-181a and miR-155 were down-regulated in the PBMCs of SLE patients after treatment with Lactobacillus rhamnosus (*P < 0.1,**P < 0.02) and Lactobacillus delbrueckii (*P < 0.01,**P < 0.001). Also, Expression of miR-181a and miR-155 were significantly down-regulated in the PBMCs of SLE patients after treatment with a mixture of lactobacillus delbrueckii and rhamnosus (*P < 0.004, **P < 0.03).

**Conclusion:** Treatment of PBMCs with Lactobacillus rhamnosus and Lactobacillus delbrueckii and with a mixture of 2 probiotics, downregulate the expression rate of miR-181a and miR-155 in treated cells in compared with untreated cells. Lactobacillus rhamnosus and Lactobacillus delbrueckii modulate the expression of miR-181a and miR-155 in SLE patients, in result they should have beneficial in the management of SLE patients.

**Keywords**: miR-155, miR-181a, systemic lupus erythematosus, autoimmunity, probiotic

ICIA2018\Immunology of Rheumatic Diseases\Oral\8217
**8217**

# The effect of DNA methylation on expression of adhesion molecules In peripheral blood mononuclear cells of patients with limited and diffuse scleroderma

Navid Dashti[1, 2], Mohammad Vodjgani[1], Mahdi Mahmoudi[2], Farhad Gharibdoost[2], Hoda Kavosi[2], Ahmad Reza Jamshidi[2]

1. *Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
2. *Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Scleroderma is an autoimmune rheumatic disease that may result in damages to the skin, endothelium of blood vessels, muscles, and internal organs through inflammation and fibrosis. Products of L-selectin (SELL) and β2-Integrin (ITGB2) genes participate in several functional pathways of immune system. The aim of this study was to survey the mRNA expression level of SELL and ITGB2 genes as well as methylation status of CpG sites in promoter region of differently expressed gene in PBMCs of patients with limited and diffuse scleroderma.

**Methods:** PBMCs were isolated from whole blood of 50 scleroderma patients and 30 healthy controls. After the extraction of total RNA and DNA contents from PBMCs, complementary DNA (cDNA) was synthesized. Afterwards, quantitative analysis of SELL and ITGB2 messenger RNA (mRNA) was conducted by real-time polymerase chain reaction (PCR) using the SYBR Green PCR Master Mix. To evaluate the methylation status of CpG sites in the promoter region of gene, nested-PCR products of bisulfite-treated DNA from scleroderma patients and controls were sequenced via Sanger difficult sequencing method.

**Results:** ITGB2 gene in PBMCs of scleroderma patients was overexpressed significantly in comparison to healthy controls. However, no altered SELL expression was observed. Three CpG sites of 12, 13 and 14 were significantly hypomethylated in patients group, despite overall methylation status of ITGB2 gene promoter revealed no significant difference between study groups. There was no statistically significant correlation between methylation status of ITGB2 promoter and the gene expression in patients.

**Conclusion:** Regarding to lack of correlation of increased expression of ITGB2 with its promoter hypomethylation in scleroderma patients, our study suggests that upregulation of ITGB2 in PBMCs from scleroderma patients is probably due to another mechanism other than methylation alteration.

**Keywords:** Scleroderma, SELL, ITGB2, DNA methylation, CpG site

ICIA2018\Immunology of Rheumatic Diseases\Oral\8428
**8428**

# The effect of endothelial reticulum stress and autophagy pathways on IL-23 production of peripheral blood mononuclear cell-derived macrophages in patients with ankylosing spondylitis

Alireza Rezaiemanesh[1,2], Mahdi Mahmoudi[3], Ali Akbar Amirzargar[2,4], Mahdi Vojdanian[3], Ahmad Reza Jamshidi[3], Mohammad Hossein Nicknam[2,4]

1. *Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*
2. *Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
3. *Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran*
4. *Molecular Immunology Research Center, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Interleukin (IL)-23/IL-17 pathway involves in the pathogenesis of ankylosing spondylitis (AS). The exact mechanism implicated in overexpression of IL-23 and activation of the IL-23/IL-17 axis has not been understood. The current study aimed to evaluate the role of endothelial reticulum stress and autophagy pathways in the IL-23 production of peripheral blood mononuclear cell-derived macrophages in patients with AS.

**Methods:** Peripheral blood monocyte isolated from 10 HLA-B27$^+$, 5 HLA-B27$^-$ patients, 10 HLA-B27$^+$,and 5 HLA-B27$^-$ normal subjects were differentiated to macrophages by macrophage-colony stimulating factor (M-CSF) for 7 days. Selected samples were treated with IFN-γ to up-regulate HLA–B expression prior to stimulation with LPS for 24 hours. Also, thapsigargin and 3-Methyladeninewere used for ER stress and unfolded protein response (UPR) induction and autophagy inhibition, respectively. Flowcytometry was used to detect monocyte purity and expression of macrophage markers. Analysis of mRNA expression for HLA-B and B27, UPR-associated genes (BiP, XBP1, PERK, ATF6, and CHOP) and autophagy genes (Atg5, Atg12, and Atg16L) was performed using RT-qPCR .IL-23 was assessed by RT-qPCR and ELISA methods.

**Results:** Data showed significant overexpression of HLA-B, HLA-B27, UPR genes (BiP, XBP1, CHOP, and PERK) and IL-23 levels in M-CSF-derived macrophages, also after treatment with IFN-γ± LPS from AS patients compared to controls. Thapsigargin significantly increased thelevel of IL-23 in macrophages from AS patients in comparison to healthy macrophages. No variations found in autophagy genes before and after treatment. It is notable that autophagy inhibition with 3-Methyladenine does not affect IL-23 production.

**Conclusion:** Our data suggest that UPR activation occurs in macrophages either before treatment with IFN-γ ± LPS or after that in AS patients and is accompanied by overexpression of IL-23. UPR appears has a regulatory role in theIL-23 production.

**Keywords:** Ankylosing spondylitis, Autophagy, Endothelial reticulum stress, Interleukin-23, Macrophage

ICIA2018\Immunology of Rheumatic Diseases\Oral\8443
**8443**

# Inhibition of ERAP1 represses of HLA-B27 free heavy chains expression and NK cells responses in Ankylosing Spondylitis

Farhad Babaie[1,2], Hamed Mohammadi[1, 2], Sorayya Salimi[1, 2], Alireza Rezaiemanesh[3], Behzad Baradaran[1, 2], Zohreh Babaloo[1, 2]

1. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
3. *Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*

**Background:** Ankylosing spondylitis (AS) is a type of arthritis that is referred to a group of chronic immune mediated inflammatory diseases termed as seronegative spondyloarthro pathies. It typically affects the joints of the spinal and axial skeleton and exhibits typical clinical features and genetic factors such as HLA-B27 and ERAP1. The ERAP1 trim amino acid residues at the N-terminal to optimize their length for binding to MHC class I molecules and can potentially alter surface expression of HLA-B27 free heavy chains (FHCs). We assessed the effects of ERAP1 polymorphism/chemical inhibition on HLA-B27 FHC expression and NK Cells responses in AS.

**Methods:** To measure the expression levels of cell surface HLA-B27 FHCs in monocyte-derived macrophages from patients with AS carrying rs30187 (K528R) and rs27044 (Q730E) ERAP1 genotypes, we used flowcytometry. ERAP1 inhibited monocyte-derived macrophages were cocultured with AS NK cells. NK cells responses were analyzed by IFN-γ, CD69, and CD107a by flow cytometry.

**Results:** The expression of surface HLA-B27-(FHCs) in monocyte-derived macrophages from patients with AS was reduced in the AS-protective ERAP1 polymorphisms. ERAP1 inhibition in monocyte-derived macrophages downregulated HLA-B27 FHC surface expression, reduced IFN-γ production, and also CD107a and CD69 expression by AS NK cells.

**Conclusion:** ERAP1 plays an important role in determining the expression levels of cell surface HLA-B27 FHCs and potentially upregulated NK cells responses in AS by binding to HLA-B27 FHCs.

**Keywords:** Ankylosing spondylitis, ERAP1, HLA-B27, NK cell.

ICIA2018\Immunology of Rheumatic Diseases\Oral\8484
**8484**

# Inhibition of ERAP1 suppresses free heavy chain expression and CD8+ T cell responses in Ankylosing Spondylitis

Hamed mohammadi[1,2], Maryam Hemmatzadeh[1,2], Farhad Babaie[1,2], Alireza Rezaiemanesh[3], Jafar Majidi[1,2], Behzad Baradaran[1,2]

1. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
3. *Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*

**Background:** Ankylosing spondylitis (AS) is a chronic immune-mediated inflammatory disease that affects both axial and peripheral skeletons and soft tissues. Evidence offers that disease pathogenesis is strongly associated with human leucocyte antigen (HLA)-B27 and endoplasmic reticulum aminopeptidase 1 (ERAP1) .ERAP1 is a key aminopeptidase in HLA class I presentation and can potentially alter surface expression of HLA-B27 free heavy chains (FHCs). We evaluated the effects of ERAP1 variations/inhibition on HLA-B27 FHC expression and TCD8 responses in AS.

**Methods:** To evaluate the expression of FHC cell surface in monocyte-derived macrophages from patients with AS carrying rs30187 and rs27044ERAP1 genotypes, we used flow cytometry. ERAP1 inhibited monocyte-derived macrophages were co-cultured with AS CD8+ T cells. CD8+ T cells responses were evaluated by IFN-γ, CD69, and CD107a by flow cytometry.

**Results:** The expression of surface FHCs in monocyte-derived macrophages from patients with AS was decreased in the AS-protective ERAP1 variants. ERAP1 inhibition in monocyte-derived macrophages suppressed HLA-B27 FHC surface expression, reduced IFN-γ production, and also CD69 and CD107a expression by AS CD8+ T cells.

**Conclusion:** ERAP1 activity determines the surface expression of HLA-B27 FHCs and potentially enhance TCD8 responses in AS by binding to HLA-B27 FHCs.

**Keywords:** Ankylosing spondylitis, ERAP1, TCD8, HLA-B27 free heavy chains

ICIA2018\Immunology of Rheumatic Diseases\Oral\8667
**8667**

# Increased inflammatory Responsiveness of Peripheral Blood Mononuclear Cells (PBMCs) to in vitro NOD2 Ligand Stimulation in Patients with Ankylosing Spondylitis

Negar Vanaki[1, 2*], Taghi Golmohammadi[1*], Ahmadreza Jamshidi[2], Maryam Akhtari[2], Mahdi Vojdanian[2], Shayan Mostafaei[2], Shiva Poursani[2], Nooshin Ahmadzadeh[2], Mahdi Mahmoudi[2*]

1. *Department of Biochemistry, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
2. *Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Ankylosing spondylitis (AS) is a common debilitating rheumatic disease in which the innate immune responses play important role in its pathogenesis. The Interleukin (IL)-23/IL-17 axis related genes have critical role among the factors that predispose the individuals to AS. Nucleotide binding oligomerization domain (NOD)-2 as an innate receptor is critical for IL-23 production in cells. Therefore, we aimed to stimulate NOD2 signaling and study its effects on cytokine production of peripheral blood mononuclear cells (PBMC) in these patients.

**Methods:** PBMCs from 18 patients with active AS and 18 healthy individuals were separated by ficoll-hypaque density gradient centrifugation and cultured in the presence of muramyl dipeptide (MDP), as NOD2 ligand. Quantitative expression analysis of NOD1, NOD2, RIPK2, SLC15A4, NLRP1, NLRP3, IL23A, IL17A, IL1B and TNFA genes was performed using Real Time PCR. Finally, protein changes of IL23A and IL17A expression were validated using enzyme linked immunosorbent assay (ELISA).

**Results:** Apart from NOD1 that tend to be downregulated in the controls, all the selected genes showed overexpression in response to MDP in cells from the studied groups. Except RIPK2, all the genes had higher expression changes upon MDP stimulation in the AS population. Overexpression of IL23A and IL17A were confirmed at protein levels using ELISA.

**Conclusion:** This study indicated that AS PBMCs were hyper-responsive to MDP stimulation. This observation implies an important role of NOD2 in the pathogenesis of inflammatory diseases including AS.

**Keywords:** Ankylosing spondylitis, NOD2, PBMC

ICIA2018\Immunology of Rheumatic Diseases\Oral\10039
**10039**

# The expression of CCN1 and its specific integrins in lungs of mouse model of systemic sclerosis

P. Safari[1], F. Vafashoar[1], H. Poormoghim[2], P Jorbozedar[1], M. Nadji[3] , T. Mousavi[4], N. Mojtabavi[1]

1. *Immunology department, Iran University of medical sciences, Tehran/Iran*
2. *Scleroderma study group Firuzgar Hospital, Iran University of medical sciences*
3. *Institute of Immunology and Infectious disease, Antimicrobial resistance Center*

**Background:** Systemic sclerosis is an autoimmune disease with vascular abnormalities, skin and internal organs fibrosis. This disease results from deposition of extracellular matrix in tissues. Member of CCN family perform their different functions through interactions with their specific integrins and proteoglycans in health and disease. Concerning the role and interaction of CCN1/ CYR61 with its specific integrin receptors in fibrosis exist paucity of information. Therefore we aimed to evaluate the expression of CCN1 and its related receptors in lungs of mouse model of systemic sclerosis.

**Method:** Mice received bleomycin for 28 days for induction of fibrosis. On days 10, lungs were removed from lethally anesthetized mice for measurement of hydroxyl proline content, histochemistry staining with H& E, Masson trichrome and RNA extraction. Specific primer for CCN1, $\alpha_v$, $\beta_3$, $\alpha_6$, $\beta_1$were designed and qPCR was performed.

**Results:** Hydroxyproline assay and Masson trichrome staining of mice, indicated fibrosis on day 28 and even more fibrosis on day 35. The overall infiltrated cells to lungs were significantly increased on day 28 qPCR results showed increment of CCN1 and $\alpha_6$ $\beta_1$integrin genes on day 10 and their decline to normal level on day 28. The expression of integrin $\alpha_v$ didn't change at any of the assessment days.

**Conclusion:** The increase of CCN1 and its integrins transcript on day 10 prior to establishment of fibrosis on day 28, may indicate the role of CCN1 as a profibrotic factor in the lung, however further investigation at the level of protein seems to be necessary.

**Keywords:** CCN1, integrins, systemic sclerosis.

# Immunomodulation and Immunoregulation

# Oral Presentation

ICIA2018\Immunomodulation and Immunoregulation\Oral\8189
**8189**

## Generation of Tolerogenic DC by Lactobacillus Rhamnosus and Delbruki in Healthy and SLE Donors

Seyed-Alireza Esmaeili[1,2,3], Mahmoud Mahmoudi[1,2], Fatemeh Mardani[1,2,3], Sahar Khorasani[1,2,3], Zohreh Vahidi[1,2,3], Nafiseh Tabasi[1], Mahdieh khazaee[1], Maryam Rastin[1,2*]

1. *Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
2. *Immunology Department, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
3. *Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** Systemic lupus erythematous (SLE) is a complicated autoimmune disease. Loss of tolerance against self-antigen and excessive inflammatory immune responses happen in SLE patients. Dendritic cells (DC) induce and promote immune responses in innate and adaptive immune system. Tolerogenic DCs regulate priming of immune response and prevent from inflammatory responses. Tolerogenic probiotics could produce regulatory monocyte-derived DC in culture media. The aim of this project was evaluating the effect of two tolerogenic probiotics (Lactobacillus delbrueckii and lactobacillus rhamnosus) for production of the regulatory DC in healthy and SLE donors.

**Methods:** in order to immature DC (IDC) production, Monocytes from healthy and SLE donors cultured with optimize concentration of GM-CSF and IL-4 for 5 days; followingly in order to mature DC (MDC) production, IDC of SLE and healthy donors treated for 48 h with cytokines plus L. delbrueckii, L. rhamnosus and mix probiotics. FITC-uptake assay performed for uptake of IDC. Surface marker expression and gene expression of IDC and MDC were evaluated by Flowcytometry and Real-Time PCR methods.

**Results:** we observed the significant reduction in the expression of co-stimulatory molecules (HLA-DR, CD86, and CD80) also CD83, CD1a, and CD14 in probiotics-derived DC of both healthy and SLE donor in compression with LPS -derived DC. Also in probiotics-derived DC in compared to LPS-derived DC Indoleamine 2, 3-dioxygenase (IDO) and IL10 were increased but was declined the expression of IL12.

**Conclusion:** our finding revealed that live probiotics could change the phenotype of DC to the regulatory and increase expression of IDO and IL10 during the differentiation process. Therefore, these modulatory cells probably could be used in treatment of SLE patients.

**Keywords:** Systemic lupus erythematosus (SLE), Probiotics, Dendritic cell(DC), Lactobacillus delbrueckii and lactobacillus rhamnosus

ICIA2018\Immunomodulation and Immunoregulation\Oral\8219
**8219**

# Regulation of Th17/ Treg Balance by Mesenchymal Stem Cell-Conditioned Medium in Mouse Model of Chronic Colitis

Sedigheh Pouya[1], Maryam Heidari[1], Kaveh Baghaei[2], Hamid Asadzadeh Afgdaei[2], Afshin Moradi[3], Saeed Namaki[1], Mohammad reza Zali[3], Seyed Mahmoud Hashemi[1]

*1. Department of Immunology, School of medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Basic and Molecular Epidemiology of Gastrointestinal Disorder Research Center, Research Institute for Gastroenterology and Liver Disease, Shahid Beheshti University of Medical Science, Tehran, Iran*
*3. Gastroenterology and Liver Disease Research Center, Research Institute for Gastroenterology and Liver Disease, Shahid Beheshti University of Medical Science, Tehran, Iran*

**Background:** Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gastrointestinal tract. Among the factors that cause IBD the role of immune response in manifestation and progression of IBD has been strongly emphasized. On the other hand, the role of mesenchymal stem cells (MSC) and mesenchymal stem cell-conditioned medium (MSC-CM) in the regulation of immune responses (inhibition of T lymphocytes proliferation and induction of regulatory T cells) also has been proved. In this study, the effects of MSC-CM on the Th17/ Treg cell balance in the chronic model of colitis have been investigated.

**Method:** In this study after induction of chronic colitis in female C57BL/6 mice, intraperitoneal injection of MSC-CM (500µl) was done for 6 times. Briefly, for colitis induction, the mice were received dissolved 2% dextran sulfate sodium (DSS) in water for 4 days that followed by only water consumption for 7 days. This step repeated in 3 cycles. After euthanizing the mice the serum and mononuclear cells of mesenteric lymph nodes and spleen were isolated. Then serum and the cells were cultured with and without PHA for 72 hours. At the end of the experiment the levels of IL-10, IL-17 and TGF-β in serum and cell culture supernatant were evaluated (ELISA method).

**Results:** The results showed that after MSC-CM injection, IL-17 production was reduced. Also, the levels of anti-inflammatory cytokines, IL-10 and TGF-β, were increased both in the serum and in the cell culture supernatant.

**Conclusion:** According to the results mesenchymal stem cell- conditioned medium injection, can make a balance between Th-17 and regulatory T cells function and can ameliorate inflammatory responses in colitis.

**Keywords:** Inflammatory bowel disease, Mesenchymal stem cells, Conditioned medium, Dextran sulfate sodium.

ICIA2018\Immunomodulation and Immunoregulation\Oral\8227
**8227**

# Oleoylethanolamide improves inflammation and oxidative stress in obese people. A randomized double-blind, placebo-controlled trial

Payahoo L[1], Khaje-Bishak Y[2], Asghari M[3], Ostadrahimi A[4]

1. *Talented student center, Student Research Committee, Nutrition Research Center, Faculty of Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Student Research Committee, Faculty of Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran*
3. *Road Traffic Injury Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
4. *Nutrition Research Center, Faculty of Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Oleoylethanolamide (OEA) is structurally similar to the endocannabinoid system components, but, it does not bind to the cannabinoid receptors. Obesity as a chronic low-grade inflammation disorder has been prevailed dramatically, in recent times. The aim of the present study was to determine the effects of Oleoylethanolamide supplementation on the markers of inflammation and oxidative stress in obese people.

**Methods:** This randomized, double-blind, placebo-controlled clinical trial was carried out on 60 healthy obese people in 2016 in Tabriz, Iran. Eligible subjects were randomly divided into two interventions (received daily two 125 mg OEA capsules) and control groups (the same amounts starch) and treated for 8 weeks. Blood samples (5 ccs) were taken in fasting state at baseline and at the end of the study. The concentrations of malondialdehyde (MDA) and total antioxidant status (TAS) were measured by spectrophotometer method. High sensitive-C reactive protein (hs-CRP) level measured by Immunoturbidimetry assay using the commercial kits and IL-6 and TNF-α levels were assayed by ELISA method. The differences between groups were assessed by analysis of covariance (ANCOVA) and $p<0.05$ defined as statistically significant.

**Results:** The analysis was done on 56 samples that completed the study. OEA decreased significantly serum levels of IL-6 and TNF-α ($p<0.001$). The levels of hs-CRP decreased significantly in the intervention group ($p=0.011$), however, ANCOVA test didn't reveal significant amounts. Changes in other variables were not detectable ($p>0.05$).

**Conclusion:** Use of Oleoylethanolamide as a complementary and alternative approach could be effective in attenuating inflammation in obesity and related disorders.

**Keywords:** Inflammation, Endocannabinoids, Obesity, Oleoylethanolamide, Oxidative stress.

ICIA2018\Immunomodulation and Immunoregulation\Oral\8303
**8303**

# Dendritic cell migration to skindraining lymph nodes is controlled by dermatan sulfate and determines adaptive immunity magnitude

Reza Nadafi[1], Jasper J. Koning[1], Henrike Veninga[1] , Xanthi N. Stachtea[2], Tanja Konijn[1], Antonie Zwiers[1], Anders Malmström[2], Joke M. M. den Haan[1], Reina E. Mebius[1], Marco Maccarana[2], Rogier M. Reijmers[1,]

*1. Department of Molecular Cell Biology and Immunology, VU University Medical Center, Cancer Center Amsterdam, Amsterdam, the Netherlands*
*2. Department of Experimental Medical Science, Lund University, Lund, Sweden*

**Background:** For full activation of naïve adaptive lymphocytes in skindraining lymph nodes (LNs), presentation of peptide: MHC complexes by LNresident and skinderived dendritic cells (DCs) that encountered antigens, is an absolute prerequisite. To get to the nearest draining LN upon intradermal immunization, DCs need to migrate from the infection site to the afferent lymphatics, which can only be reached by traversing a collagendense network located in the dermis of the skin through the activity of proteolytic enzymes.

**Methods:** Using in-vitro experiments, in-vivo mouse model and performing multicolor FACS staining, we could showed impaired DCs migration due to altered collagen fibrillogenesis in skin.

**Result:** Here, we show that mice with altered collagen fibrillogenesis resulting in thicker collagen fibers in the skin, display a reduced DC migration to the draining LN upon immune challenge. Consequently, the initiation of the cellular and humoral immune response was diminished. Antigenspecific CD8+ and CD4+ T cells as well as antigenspecific germinal center B cells and serum immunoglobulin levels were significantly decreased.

**Conclusion:** Hence, we postulate that alterations to the production of extracellular matrix, as seen in various connective tissue disorders, may in the end affect the qualitative outcome of adaptive immunity.

**Keywords:** skindraining lymph node, adaptive immunity, DCs, dendritic cells, dermatan sulfate epimerase, collagen, antigen.

ICIA2018\Immunomodulation and Immunoregulation\Oral\8347
**8347**

# Investigating the involvement of microRNA-146 family in the pathogenesis of multiple sclerosis using EAE animal model

Farideh Talebi, Samira Ghorbani, Farshid Noorbakhsh

*Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** MicroRNAs have recently emerged as a new class of modulators of gene expression. miRNAs are small non-coding RNA molecules which control protein synthesis through translational repression or degradation of mRNA transcripts. Recent studies have shown that dysregulation of immunity-related miRNAs might contribute to autoimmune disorders such as multiple sclerosis. Herein, we investigated the contribution of miR-146 isoforms and their target gene in the pathogenesis of multiple sclerosis using EAE animal model.
**Methods:** The expression levels of isoforms of miR-146 as well as their potential target genes were measured in the CNS tissue from EAE mice and stimulated lymphocytes by real-time RT-PCR. The miR-146a-5p/b-5p mimic sequences were transfected into T CD4+ cells and the role of them were then investigated in T cell differentiation by flowcytometric analysis of intracellular cytokines. Luciferase assays using vector containing the 3′UTR of predicted target was performed to confirm the interaction of miRNA sequences with target genes transcripts.
**Results:** Expression of both isoforms miR-146 were significantly increased in acute and chronic phases of EAE as well as activated lymphocytes in comparison with control groups whereas the levels their predicted target gene decreased. CD4 T cell differentiation was skewed towards Th17 subset in miR-146a-5p and miR-146b-5p transfected cells. Luciferase assays revealed SMAD4 as direct target of both of miR-146 isoforms and overexpression of miRNA mimic sequences suppressed the expression of this target transcripts in lymphocytes.
**Conclusions:** Our findings suggest that increased expression of miR-146 isoforms in the CNS tissue, as well as in lymphocyte might be involved in the pathogenesis of multiple sclerosis through altering the process of differentiation of these cells into the inflammatory phenotype. Our findings suggested that targeting and suppression of protective gene in multiple sclerosis such as SMAD-4 by miR-146 is a possible mechanism for miRNA effects.

**Keywords:** Multiple Sclerosis, miR-146, SMAD4

ICIA2018\Immunomodulation and Immunoregulation\Oral\9749
**9749**

## Association between serum cytokine concentrations and the presence of hypertriglyceridemia

Seyed Reza Mirhafez[1,2], Mohammad Tajfard[2], Amir Avan[2], Alireza Pasdar [2,3], Reza Nedaeinia [4,5], Malihe Aghasizade[2], Hafezeh Davari [2], Mostafa Manian [6,7], Adeleh Mahdizadeh[8], Zahra Meshkat[9], Ali Movahedi[1], NahidGhaedAmini[10], NahidEskandari[11], RasoulSalehi[12], Gordon A. Ferns[13], Majid Ghayour-Mobarhan[14]

*1. Department of Basic Medical Sciences, Neyshabur University of Medical Sciences, Neyshabur, Iran.*
*2. Department of Modern Sciences and Technologies, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*3. Division of Applied Medicine, Medical School, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK*
*4. Student Research Committee, Department of Medical Biotechnology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*5. Department of Microbiology, Deputy of Food and Drug, Isfahan University of Medical Sciences, Isfahan, Iran*
*6. Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran*
*7. Neurosciences Research Center, Alzahra Hospital, Isfahan University of Medical Sciences, Isfahan, Iran*
*8. Cardiovascular Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
*9. Antimicrobial Resistance Research Center,Mashhad University of Medical Sciences,Mashhad, Iran*
*10. Department of Biology, Payame Noor University of isfahan, Isfahan, Iran;*
*11. Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
*Applied Physiology Research Center, , Isfahan University of Medical Sciences, Isfahan, Iran*
*12. Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.*
*13. Brighton & Sussex Medical School, Division of Medical Education, Falmer, Brighton, Sussex BN1 9PH, UK.*
*14. Biochemistry of Nutrition Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** Hypertriglyceridemia is an established risk factor for coronary-heart-disease. Inflammatory cytokines are known to be important mediators of atherogenesis; however the relationship between the concentrations of specific inflammatory cytokines and the presence of hypertriglyceridemia has not be well established. The purpose of this study was to investigate the relationship between the serum levels of several pro-and anti-inflammatory cytokines and the presence of hypertriglyceridemia. **Methods:** Four hundred and eighty-four subjects with/without established hypertriglyceridemia were recruited. Anthropometric-parameters and biochemical-analysis (including a full fasted lipid profile) were determined. The serum levels of several cytokines and growth factors including IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, MCP-1, IFN-γ, EGF, and VEGF were measured followed by univariate and multivariate analyses. **Results:** Individuals with hypertriglyceridemia had a significantly higher body mass index, total cholesterol and triglyceride, compared to the group without hypertriglyceridemia. Serum levels of MCP-1, TNF-α and IL-8 were significantly higher in subjects with hypertriglyceridemia [e.g., IL-8 from 7.8ng/L (95%CI: 4.6-18.9) versus 5.7ng/L (95%CI: 3.6-11.9), P<0.05]. The multivariate analysis showed that the increased serum concentration of TNF-α was independently associated with high-density lipoprotein cholesterol (HDL-C), while the serum levels of IL-8 and MCP-1 were associated with hypertriglyceridemia. **Conclusion:** Subjects with serum triglycerides of ≥ 2.25 mmol/L had an altered cytokine-profile, particularly with respect to serum IL-8, MCP-1 and TNF-α, which might partially account for its adverse clinical-consequences. Further-investigations in a large multi-center setting are warranted to unravel the potential functional-importance of these cytokines in individuals with hypertriglyceridemia.

**Keyword:** cytokines, hypertriglyceridemia, dyslipidemia, triglyceride

ICIA2018\Immunomodulation and Immunoregulation\Oral\9853
**9853**

# A new module combined therapy using of azoximer bromide and laciumin treatment irritable bowel syndrome

Sheikh Sajjadieh Mohammadreza

*Department of clinical laboratory immunology and allergy, National medical academy for post graduate education, Kiev, Ukraine*

**Background:** Some evidence suggests that irritable bowel syndrome (IBS) is affected by the immune system. This study focused on the effect of Azoximer bromide in complex with Lacium as a probiotic in compare with classical therapy of IBS.

**Methods:** Our study included 22 children with IBS with diarreha treated by probiotic and azoximer bromide (study group), and 19 children treated only by classical therapy (control group). Lymphocytes subset was analyzed by flow cytometry, phagocytic activity was assessed by latex article, IgE and serum cytokine were evaluated by ELISA. The intestinal bacterial microbiota was assessed by medical microbiologic method.

**Results:** The change of immune status in IBS patient is the holistic description of our articles. After therapy in group study the percentage of all lymphocyte phenotype subsets, phagocytic activity, IgE, cytokine level and intestinal microbiota were near of healthy subjects compared with control group. In control group the mean percentage of CD3 and CD4 were less than in healthy subjects ($P<.05$), the mean percentage of CD8, CD16 and CD22 were more than in healthy subjects ($P<.05$). The mean level of IL-4 and TNF-α were significantly lower when compared with before therapy ($P<.001$). No significant difference between before and after therapy was observed in serum concentration of the mean level of IFN-c, IL-1 and phagocytic activity. Intestinal dysbiosis is corrected after therapy in both groups.

**Conclusions:** Immunomodulator azoximer bromide when contributed with probiotic can be used to elicit or amplify an immune response in patient having suppressed immune system. Probiotic is a prescription medication for treatment of irritable bowel syndrome and in addition for correct immune status may be prescribed azoximer bromide.

**Keywords:** Probiotic , Immunomodulator , Irritable bowel disease

# Immunoparasitology

# Oral Presentation

ICIA2018\Immunoparasitology\Oral\5318
**5318**

# Immunohistochemical observation of the inflammatory cell infiltration in adventitia of human hydatid cysts

Rasoul Jafari[1], Behnam Sanei [2], Azar Baradarn[3], Hossein Yousofi Darani[1]

1. *Department of of parasitology and mycology, Isfahan university of medical sciences, Isfahan, Iran*
2. *Department of Surgery, Isfahan university of medical sciences, Isfahan, Iran*
3. *Department of pathology, Isfahan university of medical sciences, Isfahan, Iran*

**Background:** Human cystic echinococcosis (CE) is a parasitic disease with many immunological aspects which needs to be studied. The aim of the present study was to evaluate the local immune cell infiltration pattern in human CE caused by the Echinococcusgranulosus using immunohistochemistry (IHC) method.

**Methods:** In this study 50 surgically removed hydatid cysts samples and their surrounding tissue were collected from patients referred to Al Zahra Hospital, Isfahan, Iran. IHC was performed on the pre-cyst of 44 liver, five lung and one kidney hydatid cysts using anti human CD3, CD19, CD8, CD4, CD68, CD56, Ki-67 and Foxp3 antibodies.

**Results:** There were positive cells for CD3+ T cells, CD19+ B cells, CD8+ cytotoxic T cells, CD4+ helper T cells, CD68+ macrophages, and Ki-67+ proliferating cells, yet in some cases, there was no positive cells for CD56+ natural killer cells (7 CE patients) and Foxp3+ regulatory T cells (one CE and one chronic hepatitis), and also there was no eosinophils in non-CE samples.

**Conclusion:** Based on the results of the present study, the presence of regulatory T cells and a considerable number of proliferating B cells maybe the cause of persistence of hydatid cyst in human cases for long period of time.

**Keywords:** Hydatid cyst, immune cells, Immunohistochemistery

ICIA2018\Immunoparasitology\Oral\6317
**6317**

# Evaluation of serum concentration of AFP marker in toxoplasmosis pregnant women with high level of IgG & IgM toxoplasma antibody by ELISA assay

Hamideh Rahimi[1], Ehssan Shariat bahadori[2]

1. *Department of Immunology of medical University of mazandaran.*
2. *Department of parasitology University of Tarbiatmodares of Tehran.*

**Background:** Toxoplasmosis is a parasitic disease which may cause some laboratory symptoms in infected individuals. One of the main ways to transmit this organism is placenta to fetus pathway. If this transmission occurs in the 3th month of pregnancy the abortion, central nerve system and ocular disorder will happen. Because of this issue, the precise technique for detection of Toxoplasma Antibody such as IgG and IgM is important, that contain ELISA to detect Toxoplasma Antibody such as IgG and IgM and AFP.

**Methods:** In this survey the main sample is serum that can be collected from 255 pregnant women infected with toxoplasma gondii in avesina center. Then we detected serum concentration of AFP in toxoplasmosis pregnant women with high level of IgG & IgM toxoplasma antibody by ELISA assay.

**Results:** The results of this survey showed that, in these total pregnant women the infection by toxoplasma gondii is occurred and 13% of them had high levels of AFP in their serum. The statistical survey was done by SPSS18.

**Conclusion:** in some pregnant women with high level of IgG & IgM toxoplasma antibody had high levels of AFP in their serum and this index correlates with NTD in their fetus.

**Keywords:** Toxoplasma gondii, ELISA ,IgG antibody, IgM antibody, AFP

ICIA2018\Immunoparasitology\Oral\7543
**7543**

# Efficacy and mechanism of action of selected antimicrobial peptides on Leishmania major parasite: in vitro and in vivo assays

Farnaz Zahedifard[1], Hyeryon Lee[3], Joo Hwan No[3], Mona Salimi[2], Ahmad Asoodeh[4], Sima Rafati[1]

1. *Immunotherapy and Leishmania Vaccine Research Department, Pasteur Institute of Iran*
2. *Physiology & Pharmacology department, Pasteur Institute of Iran*
3. *Leishmania Resaerch Lab, Pasteur Institute of Korea*
4. *Departement of Chemistry, Ferdowsi University of Mashhad*

**Background:** Leishmaniasis as a vector born and neglected disease affects tropical and subtropical areas in the world. The only ideal available drug, Amphotericin B is expensive for majority of infected communities and need patients to be hospitalized. Immergence of having a new drug in leishmaniasis made researchers to find new candidates. Antimicrobial peptides nowadays are in major concern to battle infectious agents. In this study two different antimicrobial peptides ( Brevinin 2R and Jellein) and their Lauric acid conjugated version were studied against L. majorin vitro and in vivo. Also peptides' mechanism of action on promastigotes was defined.

**Methods:** We measured effective concentration 50 (EC50) of antimicrobial peptides on leishmania promastigotes and amastigotes. Hemolysis assay and toxicity in THP1 cells were performed for the peptides. Membrane permeability with SYTOX green and membrane potential changes with bisoxonol fluorescence probes were investigated. SEM views of parasite-treated peptides were captured. Caspase 3 and 7 activation in promastigote were measured. Changes in mitochondrial potential were also studied in promastigotes. Apoptosis induction was also investigated through flow cytometry assay of Annexin/PI dyes. In vivo studies are now going on in Balb/c mouse model of cutaneous leishmaniasis.

**Results:** Lauric acid-Brevinin( L- Brevinin) had the best effect on L. major promastigotes but simultaneously had toxic effect on THP1 cells and erythrocytes. Jellein destroyed promastigote in higher concentrations. Both peptides can change membrane permeability and potential fast after exposure to promastigotes. Peptides couldn't activate caspases 3 and 7 in promastigotes but they could change mitochondrial membrane potential effectively. Flowcytometry assay showed necrosis versus apoptosis in peptide-treated promastigotes. SEM results vividly showed changes in the size and membrane of parasites in EC50 concentration of peptides ( L-Brevinin and Jellein). Infected Balb/c mice treatment is now being done.

**Conclusion:** L-Brevinin and Jellein both are effective against L.major promastigotes. They damage parasites through membrane disruption.

ICIA2018\Immunoparasitology\Oral\7888
**7888**

## Transcriptome profiling of the Leishmaniatropica infected cutaneous lesion

Nasrin Masoudzadeh[1], Amir Mizbani[2], Yasaman Taslimi[1], Sima Habibzadeh[1], Vahid Mashayekhi[3], Hossein Mortazavi[4], Housein Malekafzali Ardekani[1], Sima Rafati[1]

1. *Immunotherpy and Leishmania Vaccine Research Dept., Pasteur Institute of Iran, Tehran, Iran*
2. *Zurich, Switzerland*
3. *Cutaneous Leishmaniasis Research Center, Emam Reza Hospital, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
4. *Department of Dermatology, Razi Hospital, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Leishmania (L.) tropica is the main causative agent of anthroponotic cutaneous leishmaniasis (CL) in Iran. CL caused by L. tropica infection show a longer healing time, often resulting in disfiguring scars and are more difficult to treat compared to CL caused by L. major. Despite these problems, there is still a lack of data describing the host immune pathways that active in localized L. tropica infection in human patients. To address this issue, we applied RNA sequencing technology in skin biopsies to characterize indetail the transcriptome of the human host at the lesion during L. tropica infection.

**Methods:** Skin biopsies were obtained from confirmed L. tropica infected patients with acute lesion using a 4-mm punch. The CL diagnosis was based on both clinical examination and PCR. Normal skin samples were taken from volunteers without a history of leishmaniasis. All participantsprovided written informed consent. Total RNA isolated from frozen tissues and sequencing libraries were prepared following Ribosomal RNA depletion. Sequencing of single-end 125-nucleotide sequences was performed on an Illumina HiSeq-2500 platform. Transcript quantification and differential expression analysis were done using Bioconductor package edgeR.

**Results:** Comparing the transcription profile of the L. tropica skin lesions with normal skin identified 5396 differentially regulated genes. Forty-seven of the up-regulated genes encode immunoglobulin fragments, highlighting the remarkable involvement of B cells in the infection site. Gene set enrichment analysis indicated that cytotoxic $CD8^+$, inflammasome, B and T cell activationare associated with acute lesions.

**Conclusion:** We observed prominent role for B cells and immunoglobulins in lesions, suggesting that B cells may be correlated with disease severity rather than protection or cure. Defining the host inflammatory pathways within L. tropica lesions will be critical for development of new therapeutic interventions. In addition, dual RNA-seq of host and parasite genes is highly recommended for in-depth investigation.

**Keywords:** Cutaneous leishmaniasis, Leishmaniatropica, RNA sequencing, Transcriptome.

ICIA2018\Immunoparasitology\Oral\8087
**8087**

# The Role of Toxoplasma gondii Lysate, Excretory/Secretory Antigens and a Combination of them on the expression of IFN-γ and IL-4 and mice surveillance

Ali Fattahi Bafghi[*1], Aghdas Mirjalili[2], Mohammad Kazemi Arababadi[3]

*1. Associate professor, Medical Parasitology and Mycology Department, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
*2. MSc Histologist, Morph-anatomy Department, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
*3. PhD in Immunology, Immunology of Infectious Diseases Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.*

**Background:** Toxoplasma gondii, an obligate intracellular parasitic protozoan, is capable of infecting a wide range of warm-blooded vertebrates. This survey was designed to The Role of Toxoplasma gondii Lysate, Excretory/Secretory Antigens and a Combination of them on the expression of IFN-γ and IL-4 and mice surveillance.

**Methods:** The above-mentioned antigens were prepared, encountered with the isolated murine peritoneal leukocytes and then the IFN-γ and IL-4 levels were measured in the stimulated leukocytes culture supernatants using ELISA technique. Moreover, the effect of these antigens on the mice survival time was examined.

**Results:** The results of one-way ANOVA showed that there was a significant difference among the testing groups under study (TLAs, ESAs and TLA: ESA) on the basis of IL-4 and IFN-γ concentrations ($P < 0.05$). In addition, the result of Turkey's HSD test showed only a significant difference between the TLA: ESA and TLA groups ($P < 0.05$). The results of two-tailed t-test for pair-wise comparison of the mice survival time in the immunized and challenged groups showed a significant difference between the two testing groups ESAs as well as TLA: ESA and the control groups ($P < 0.05$). The Pearson's correlation coefficient results showed that there was a significant correlation between the mice survival time and the spleen size ($P < 0.01$).

**Concussion:** This study discussed on the host-parasite interaction with the involvement of IFN-γ and IL-4 and how these interactions possibly help the parasite to establish its life-cycle in nature.

**Keywords:** IFN-γ, IL-4, Toxoplasma gondii, host-parasite interaction.

ICIA2018\Immunoparasitology\Oral\8241
**8241**

# Preparation of Theileria annulata infected Hyalomma anatolicum ticks stabilate for use in efficacy of bovine Theileriosis vaccine

Asghar Afshari[1], Gholamreza Habibi[1], Kasra Esmaeilnia[1], Hossein Modirrousta[2]

*1. Department of Parasite vaccine research and production of Razi vaccine and serum research institute, Agriculture Research, Education and Extension Organization (AREEO), Karaj, Iran*
*2. Department of Wildlife research of Razi vaccine and serum research institute, Agriculture Research, Education and Extension Organization (AREEO), Karaj, Iran*

**Background:** Tropical theileriosis caused by the protozoan parasite Theileria annulata, is one of the most important tick-borne disease that affect cattles, accompanied with economic loses in endemic area such as Iran. Theileriosis is a vector-borne disease that is transmitted by Hyalomma ticks (Acari-ixodidae). Control and prevention of tropical theileriosis are carried out by both acaricides and administration of live attenuated vaccine. Efficacy of vaccine needs to be challenged by live virulent parasite preserved in tick stabilate that infected with acute strain of Theileria annulata. This study was carried out in order to preparing of tick infected stabilate to applying in vaccine efficacy test.

**Methods:** The experimental theileriosis was achieved by inoculation of previously infected blood sample to a naïve healthy calf. Different stages of Hyalomma anatolicum tick life cycles including eggs, larvae, nymph and adult gained by incubation and feeding on white rabbit in laboratory conditions. The unfed nymph ticks fed on experimentally infected calf. Theileria annulata infection in calf and ticks was detected by PCR using specific primers. The tick stabilate was prepared by grinding infected ticks in normal saline and preserved in deep freeze using 15% of glycerol as cryoprotectant. Bioassay was performed to confirm the infectivity of prepared stabilate by subcutaneous injection to intact susceptible 4 months old calf.

**Result:** Hyalomma anatolicum ticks successfully reared and complete life cycle lasted about 90 days. Theileria annulata infection was confirmed by clinical signs, microscopic observation of blood smears and specific PCR test in both calf and ticks specimens. Incubation period of disease by injection of blood and stabilate lasted 14 and 8 days, respectively.

**Conclusion:** Severity of clinical signs and low incubation period of disease caused by injected stabilate revealed its acuteness and so it can be used in vaccine efficacy test and other molecular, serological and challenge studies.

**Keywords:** Theileria, Tick, Stabilte, Vaccine efficacy

ICIA2018\Immunoparasitology\Oral\8262
**8262**

# Evaluation of Natural Killer Cells Function Exposed to Recombinant Leishmania major Lipophosphoglycan-3 Antigens

Sanaz Rasolzadeh Nori[1,4], Arash Pourgholaminejad[3], VahidYounesi[2], Mehdi Yousefi[1,4]

1. *Stem Cell and Regenerative Medicine Institute, Tabiz University of Medical Sciences, Tabriz, Iran*
2. *Pishtaz Teb Zaman Diagnostics, Tehran, Iran*
3. *Department of Immunology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran*
4. *Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Leishmaniasis is one of the widespread infectious diseases that caused by intracellular parasite, Leishmania major. Nowadays, there is not effective vaccination procedure and immune-booster strategy for this disease. Among many antigenic molecules in the structure of Leishmania major, lipophosphoglycan-3 (LPG-3) antigen and its fragments are critically immunogenic and play major role in the pathogenicity of the parasite. It has been hypothesized that LPG-3 enhances immune response against Leishmanisasis through natural killer (NK) cells activation and can increase immune response of the host. However, in the current study, we analyzed the effects of LPG-3 in activation of human NK cells.

**Methods:** Human NK cells were isolated from healthy volunteers (n=10) by magnetic-activated cell sorting (MACS). NK cells purity was checked by Flowcytometry. Then, purified cells were co-incubated with recombinant LPG-3 in presence/absence of TLR-2 inhibitory-monoclonal antibody for 72 hours. Afterward, the gene expression and protein secretion of IFN-γ and TNF-α were measured in treated-NK cells and cell-supernatants by real-time PCR and ELISA methods, respectively.

**Results:** Our findings indicated that recombinant LPG-3 increases the expression of IFN-γ and TNF-α significantly ($p<0.01$) in NK cells after three days of co-incubation in compare with untreated-NK cells. Also, the concentration of IFN-γ and TNF-α in cell-supernatants increased ($p<0.01$).

**Conclusion:** We concluded that Leishmania major LPG-3 antigen can activate NK cells toward enhancement of inflammation, and enhances immune response against leishmaniasis. Our findings suggest immunization with LPG-3 may enhance innate immune system against leishmaniasis.

Keywords: NK cell, LPG-3, IFN-γ, TNF-α, Innate immunity

ICIA2018\Immunoparasitology\Oral\8316
**8316**

# The Evaluation Effect of fucose-mannose ligand of Leishmania infantum on murine peritoneal macrophages in vitro

Namdar Ahmadabad Hasan[1], Shafiei Reza[1], Nezafat Ferizi Maryam[1], Kalantar Kurosh[2], Hatam GholamReza[3], Bakhshi Joibari Fahimeh[4]

*1. Department of Pathobiology and Laboratory Sciences, School of Medicine, North Khorasan University of Medical Sciences, Bojnourd, Iran*
*2. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
*3. Department of parasitology and Mycology, school of Medicine, Shiraz University of Medical Sciences*
*4. Student Research Committee, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran*

**Background:** Fucose-mannose ligand (FML) is glycoprotein, which is present both on promastigotes and amastigotes of Leishmania infantum. It has potential use as a tool for prognosis, diagnosis, and vaccine development of visceral leishmaniasis. In present study we evaluated the effect of FML on murine peritoneal macrophages in vitro.
**Methods:** We treated macrophages and LPS stimulated macrophages with different concentration of FML purified from Leishmania infantum and measured the concentration of TNF-α, IP-10, IL-12p70, and IL-10 production in culture supernatant of these cells by sandwich ELISA. We also measured culture supernatant levels of nitric oxide (NO) by Griess reaction.
**Results:** Our findings showed that that FML significantly increases NO, IL-10, IL-12p70 and IP-10 production in macrophages, but doesn't have any effect on TNF-α production (*P< 0.05).We also found that FML significantly increases the IL-12p70/IL-10 ratio in culture supernatants of macrophages(*P< 0.05).
**Conclusion:** We concluded that FML can polarize macrophages to an M1-like phenotype, although our results showed that FML enhances IL-10 production from macrophages and doesn't increase TNF-α production from them.

**Keywords:** Fucose-mannose ligand, Macrophage, Nitric oxide, Visceral Leishmaniasis

ICIA2018\Immunoparasitology\Oral\8503
**8503**

# Evaluation of stability of the attenuated line of Leishmania major transfected with PSA gene in BALB/c mouse

Sahar Mashayekhi [1], Fahime Aeine negin[1], Hamid Daneshvar[1]

*1. Departmant of Immunology, Medical School of Kerman University*

**Background:** Prostate cancer is the second most common cancer among men worldwide. The attenuated leishmania can be a good candidate for live recombinant vaccine to express proteins and a tumor-associated antigen (TAA). In this study, attenuated line of Leishmania major (H-line) was used to express the PSA gene. The aim of this study was to evaluate the expression of the PSA gene in this parasite and to investigate the stability of transfected parasite in BALB / c mice.

**Methods:** In this study, we cloned PSA gene into attenuated leishmania major. To examine the safety of the transgenic parasite, BALB/c mice were injected subcutaneously with stationary-phase L. major wild, H-line, recombinant promastigotes. The lesion volume was measured weekly. To investigate the ability of promastigotes to infect macrophages and their survival within macrophages, peritoneal macrophages were exposed to stationary-phase promastigotes. The culture slides then were incubated in 5% CO2, 95% air at 37˚C. At the end of the incubation period, the infection status of the macrophages was determined by microscopy.

**Results:** The PSA gene cloned into leishmania major H-line successfully for first time and we could detect the expression of PSA gene in leishmania major H-line. Safety evaluated by 2 criteria: first, the promastigotes of the recombinant leishmania major could enter but not survive in macrophages, and second, that mice inoculated subcutaneously with attenuated parasites did not develop skin lesions.

**Conclusions:** These finding represent that leishmania major H-line could be used as a new vector vaccine for expression of TAAs and other proteins.

**Keywords:** L.major H-line, vector vaccine, prostate cancer, PSA

ICIA2018\Immunoparasitology\Oral\8641
**8641**

# Effect of Lavandula angustifolia extract on the antigenemia and WBC responses to acute infection with Toxoplasma gondiiin mice

Mohammadreza Alijani[1], Yaser Piralii[2], Rasool Rahimi Junqani[3]

*1. DVM Student of Veterinary Medicine, Shahrekord University, Shahrekord-Iran*
*2. Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord-Iran*
*3. DVM Graduated Student of Veterinary Medicine, Shahrekord University, Shahrekord-Iran*

**Background:** Toxoplasmosis, caused by the protozoan parasite Toxoplasma gondii, is one of the most common parasitic infections of man and other warm-blooded animals. To control toxoplasmosis, a combination of antifolates, such as pyrimethamine and sulfadiazine, has been used, but a large percentage of undesirable side effects such as hematological toxicity (caused by pyrimethamine), cutaneous rash, leukopenia and thrombocytopenia (caused by sulphonamides) are often reported. The purpose of this study was to determine the effect of Lavandula angustifolia extract on the antigenemia and WBC responses of mice to acute infection with Toxoplasma gondii.

**Methods:** 30 female mice were infected by intraperitoneal (IP) injection of 104 tachyzoites of the virulent RH strain, and divided in 3 groups as follows: 1 control group. 2 infected mice that received extract of Lavandula angustifolia50 mg/kg. 3 infected mice that received extract of Lavandula angustifolia100 mg/kg.for6 days after infection blood sample were collected and toxoplasma antigen were tested by dot-ELISA. Also white blood cell count (neutrophil, lymphocyte, eosinophil and monocyte) were measured. All data were analyzed via ANOVA, with a significant level $P<0.05$.

**Results:** We observed that toxoplasmosis cause lymphopenia, neutrophilia and eosinophilia in all groups. Although extract of Lavandula angustifolia can decrease total WBC, but the different observed is not significant ($p>0.05$). Circulating antigens were detected early on day 2 PI in control group and day 3PIin treatment groups, and the titers increased from day 4 PI in all groups. In other factors such as body weight, splenomegaly and hepatomegaly there was no significant difference between groups received extract of Lavandula angustifolia and the control group ($p>0.05$) Acutely infected mice died within 7 to 10 days' post-infection as a function of the strain and inoculum size.

**Conclusion:** According to the results of the present study Lavandula angustifolia extracthas no effect on Toxoplasma gondii infection in mice.

**Keywords:** Withania somnifera, antigenemia, Toxoplasma gondii, mice

# Immunopharmacology and Medicinal Plants

# Oral Presentation

ICIA2018\Immunopharmacology and Medicinal Plants\Oral\5269
**5269**

# A Comparison of the Immunosuppressive Effects of Silymarin and Cyclosporine A on the Proliferation of Regulatory T Cells and Function

Niloufar Keyhanmehr[1] , Hossein Motedayyen[1] , Nahid Eskandari[1]

*1. Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background:** Immunosuppressive agents are necessary to enhance allograft tolerance after transplantation and the treatment of autoimmune disorders. Regulatory T cells (Tregs), a subset of T cells, play a pivotal role in improving allograft tolerance and determining the fate of transplanted organs. Therefore, the specific aim of this study was to investigate the immunomodulatory effects of cyclosporine A (CsA) and silymarin on Treg proliferation and their cytokine production.

**Methods:** Tregs were isolated from peripheral blood mononuclear cells (PBMCs) from healthy donors and phenotypic characteristics of Tregs were determined by flow cytometry. Tregs were expanded and then cultured with different concentrations of CsA and silymarin. Immunomodulatory effects of CsA and silymarin on the proliferation of Tregs and function were determined after 3 and 5 days of culture.

**Results:** Our results showed that CsA significantly decreased Treg proliferation in a dose-dependent fashion. However, CsA did not have the ability to induce Treg function through the production of transforming growth factor-beta 1(TGF-β1). In contrast, silymarin significantly increased the proliferation of Tregs. A statistically significant increase was also observed in the function of Tregs through TGF-β1 production.

**Conclusion:** The results of this study for the first time show that silymarin, unlike CsA, possess the ability to increase the proliferation and function of Tregs and may be beneficial in treatment of autoimmune disorders and improvement of Treg-dependent allograft tolerance after transplantation.

**Keywords:** Silymarin, Cyclosporin A, Regulatory T cells, TGF-β1

ICIA2018\Immunopharmacology and Medicinal Plants\Oral\7391
**7391**

## An in vitro study on cytotoxic effects of Androctonus crassicauda scorpion venom on K562 cell line

Golnaz Rashidi[1,2], Ata Ghadiri [1], Babak Vazirianzade [3], Nazanin Fathi [1,2], Ali Khodadadi [*1,2], Mohammad Rashno[1,2], Mohammad Ghasemi[1,2]

*1. Department of Immunology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
*2. Cancer Petroleum and Environmental Pollutants Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
*Infectious and Tropical Diseases Research Center, Health Research Institute" and "Medical Entomology and Vector Control Department, Public Health Faculty", Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

**Background:** Today, cancer is posed as one of the major problems in the world. The incidence of cancer and its associated mortality rates are increasing and in spite of diverse medical treatments in this field none of them were deemed thoroughly effective. Therefore using the existing rich resources in the nature for therapeutic purposes is considerable. The objective of the present study was to measure the ability of Androctonus crassicauda scorpion venom on inhibition of cell proliferation in K562 cell line derived from human Chronic Myeloid Leukemia.

**Methods:** Crude venom of Androctonus crassicauda scorpion was obtained by electrical stimulation of telson. Bradford assay was employed to measure the protein concentration. K562 cell line was cultured in RPMI-1640 with 10% heat inactivated fetal bovine serum, 100 units/ml penicillin and 100µg/ml streptomycin. Measurement of cell viability and metabolic activity was performed by MTT assay.$5\times10^4$cells/well of K562 cells were seeded in a 96-well tissue culture plate. The cells were treated with increasing concentrations of A.crassicauda venom from 40 to160 µg/ml for 24h. The absorbance was read at 570 nm in a micro-plate reader.

**Result:** Protein concentration of scorpion venom in this study was calculated 0.5mg/ml. This study showed that the scorpion venom inhibits the growth of K562 cells with IC50=111.46µg/ml.

**Conclusion:** The results of this study proved that A.crassicauda venom induces growth inhibition in K562 cell line. According to other studies as well as the current study, which demonstrated the cytotoxic effect of natural toxins on cancer cells, apoptotic effects induced by A.crassicauda in K562 cell line will be dealt with in our future research, which is hoped to be a step in the improvement of cancer treatments using natural products.

**Keywords:** Androctonus crassicauda, Scorpion venom, K562 Cell Line, MTT, IC50

ICIA2018\Immunopharmacology and Medicinal Plants\Oral\7817
**7817**

# The in vitro impact of Glycyrrhizic acid on CD4+ T lymphocytes through OX40 receptor in the patients with allergic rhinitis

Saloomeh Fouladi[1], Nahid Eskandari[1], Mohsen Masjedi[1]

*1. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background:** Glycyrrhizic acid(GA) the major bioactive component of glycyrrhiza possess a great variety of pharmacological properties such as anti-inflammatory, anti-allergic and immunomodulatory activities. In the present work, the in vitro anti-allergic effect of GA through OX40 receptor was investigated in patients with allergic rhinitis.

**Methods:** Purified CD4+ T cells from patients with allergic rhinitis (n=12) were activated with anti-CD3/ anti-CD28 and anti-OX40 mAb agonist antibody and then treated with different concentration of GA and dexamethasone. Then, cells were incubated (72 hr) for proliferation assay. Protein expression of OX40 in anti-OX40 mAb stimulated CD4+ T cells were evaluated by flow cytometry. mRNA expression of the T-bet, GATA3 and forkhead box P3 (Foxp3) transcriptional factors were measured by quantitative polymerase chain reaction.

**Results:** GA significantly inhibited the augmented T cells proliferation induced with anti-OX40 mAb ($p < 0.05$-$p < 0.01$at100 µM and 200 µM GA compared to control). OX40 expression was also significantly decreased ($p<0.05$). Dexamethasone and GA showed inhibitory effect on T-bet and GATA3 gene expression, but this inhibition was only significant in the case of GATA3 ($p <0.05$-$p <0.01$at dexamethasone and 200 µM GA).In contrast, enhanced gene expression of Foxp3 was seen using both of them ($p<0.05$).

**Conclusion:** We suggest that GA may have a therapeutic effect on allergic rhinitis partly by regulating the Th1/ Th2 balance through suppressing OX40 and increasing the activity of regulatory T cells.

**Keywords:** Glycyrrhizic acid, OX40, Allergic Rhinitis, Th1/Th2 balance

ICIA2018\Immunopharmacology and Medicinal Plants\Oral\7855
**7855**

# Decline in the expression and release of inflammatory mediators by the extracts from Satureja hortensis in macrophages

Zahra Farzaneh[1], Kurosh Kalantar[1], Zahra Amirghofran[1,2]

1. *Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Autoimmune Disease Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Satureja hortensis is a medicinal plant used in traditional medicine for its anti-inflammatory effects. Macrophages are among the main cells involved in the generation of inflammation. This study has investigated the anti-inflammatory effect of Satureja hortensis extracts on macrophages by measuring the expression and release of important inflammatory mediators by these cells.

**Methods:** J774.1 mouse macrophages were stimulated with lipopolysaccharide (LPS) and treated by various extracts of Satureja hortensis. We examined the macrophages for nitric oxide (NO) production using the colorimetric assay followed by real time-PCR for gene expressions and ELISA for cytokine levels. Flowcytometry was used for measuring the surface expression of intercellular adhesion molecule (ICAM)-1.

**Results:** Dichloromethane and hexane extracts more efficiently reduced NO production at non-cytotoxic concentrations compared to the aqueous and butanol extracts. The extracts at 25 µg/ml decreased NO release to less than 43% (dichloromethane) and 53% (hexane) of the control ($p<0.05$). All concentrations of both extracts decreased gene expression of inducible NO synthase (iNOS)(<0.44 fold of control), cyclooxygenase (COX)-2 (<0.29 fold) interleukin (IL)-1β (<0.41 fold), IL-6 (<0.25 fold) and tumor necrosis factor (TNF)-α (<0.2 fold). At 25 µg/ml, dichloromethane extract reduced the IL-6 protein level to 79.2±0.84% and the hexane extract to 49.4±6.8% of the control. The IL-1β level decreased to 62.5±14.6% (dichloromethane) and 36.7±24.3% (hexane). Both extracts reduced ICAM-1 fluorescent intensity of expression in comparison with the control.

**Conclusion:** The inhibitory effects of Satureja hortensis extracts on the expression and release of inflammatory mediators in macrophages suggest the presence of useful anti-inflammatory compound(s) in this plant.

**Keywords:** Satureja hortensis, Anti-inflammatory effect, Macrophages

ICIA2018\Immunopharmacology and Medicinal Plants\Oral\8058
**8058**

# Teucrium polium extract modulates the immune response in Streptozotocin-induced diabetic rats

Hamidreza Safari[1], Mohsen Naseri[2],Gholamreza Anani Sarab[2],Tayebeh Nowras[1], Mahdi Abdollahi[1], Zeinab Rostamy[1]

1. *Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran*
2. *Infectious Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran*

**Background:** Diabetes is a chronic metabolic disorder that is an epidemic disease affecting a large proportion of the population around the world. Recent studies have suggested that proinflammatory cytokines play an important role in the pathogenesis of type1diabetes (T1DM).This study aimed to investigate the Immunomodulatory effects of Teucrium poliumin Streptozotocin-induced diabetic rats.

**Methods:** In this experimental study, diabetes was induced by streptozotocin in male Wistar rats (65 mg/kg). Rats were divided into the following groups: control, untreated diabetic, 2 Teucrium polium (100, 200 mg/kg/d)–treated diabetic groups. Rat's spleen cells cultured, then cell proliferation and inflammatory cytokine release (interleukin-6 (IL-6), interleukin-1 (IL-1)) from cultured spleen cells measured by MTT and ELISA kit, respectively. Data were analyzed using one-way ANOVA and Tukey post-hoc tests in SPSS software and P-values less than 0.05 were considered statistically significant.

**Results:** Proliferation of spleen cells in treatment groups was significantly reduced compared with Control group ($P< 0.05$).There was no significant difference in anti-inflammatory activity between the groups.

**Conclusion:** The result demonstrates that Teucrium polium increased the immune cells proliferation without increasing inflammatory cytokine. Teucrium polium has Immunomodulatory effects, with the goal that the effect of continuous severity due to inflammation can be suppressed.

**Keywords:** Teucrium polium, STZ, Anti-inflammatory effect, Diabetes.

ICIA2018\Immunopharmacology and Medicinal Plants\Oral\8080
**8080**

# Induction of Apoptosis in Raji Burkitt Lymphoma Cell Line by Ferula hezarlalehzarica Extract

Yahya Asemani[1], Maryam Bayat[1], Abbas Azadmehr[2], Reza Hajiaghaee[3], Zahra Amirghofran[1, 4]

1. *Immunology Department, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Immunology Department, Babol University of Medical Sciences, Babol, Iran*
3. *Autoimmune Disease Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
4. *Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran*

**Background:** Ferula plants have recently been considered for their anti-cancer effects. In this study we aimed to determine the anti-tumor and apoptosis-inducing activity of a newly discovered native species, Ferula hezarlalehzarica against cancerous cell lines.

**Methods:** The cytotoxic activity of methanol, hexane, ethyl acetate, butanol and water extracts of F. hezarlalehzarica on Raji, EL-4, HepG2, HeLa, and K562 cell lines was examined by the MTT colorimetric assay. Analysis of apoptosis was performed by Annexin V-PE/7-AAD staining using flow cytometry. Changes in the expression of genes associated with apoptosis including Bax, Bcl-2 and Fas were investigated by real-time PCR. The JC-1 lipophilic cationicdye staining was used for assessment of changes in mitochondrial membrane potential (MMP).

**Results:** The most effective inhibitory activity was observed for the hexane extract of F. hezarlalehzarica with $IC_{50}$value of31.6µg/ml against Raji cell line. A 24-houranalysis of apoptosis in Raji cells showed that this extract induced apoptosis dose-dependently such that at 100 µg/ml of the extract 68.53±1.2% and at 200 µg/ml 96.93±1.45% underwent apoptosis compared to the negative control (5.2±2.02%). Treatment of cells with 75µg/ml of the hexane extract significantly up-regulated the gene expression levels of pro-apoptotic molecules Bax (1.75±0.31-fold,$p<0.01$) and Fas (5.02±0.74-fold, $p<0.01$) and down-regulated the expression level of Bcl-2 (0.23±0.008-fold, $p<0.001$). The ratio of Bax to Bcl-2 expressionsat 75µg/mL was 7.4±0.2. A significant decrease was detected in MMP at concentrations more than100µg/ml (≈0.4, $p<0.05$) of extract.

**Conclusion:** The hexane extract of F. hezarlalehzarica induced apoptosis in Raji cell line by regulating apoptotic and anti-apoptotic genes as well as inducing MMP depolarization. Therefore this extract has the potential to be considered as an anti-cancer agent against Burkitt lymphoma.

**Keywords:** Apoptosis, Burkitt Lymphoma, Ferula hezarlalehzarica

ICIA2018\Immunopharmacology and Medicinal Plants\Oral\8268
**8268**

# Anti-inflammatory Activity of Lactobacillus paracasei on Isoproterenol-Induced Heart Failure in Rat

Bahador Bagheri[1], Zohreh Hesari[2], Mahdi Barati[3], Parviz Kokhaei[4]

*1. Cancer Research Center and Department of Pharmacology, Semnan University of Medical Sciences, Semnan, Iran*
*2. School of Veterinary Medicine, Semnan University, Semnan, Iran*
*3. Department of Immunology, Mashhad University of Medical Sciences, Mashhad, Iran*
*4. Cancer Research Center and Department of Immunology, Semnan University of Medical Sciences, Semnan, Iran*

**Background:** A wealth of evidence has associated heart failure with inflammatory cytokines that can predict clinical outcome. The emerging information has suggested that the certain probiotics have immunomodulatory effects in diseases that have linkages with inflammation. This study was performed to determine whether oral probiotic administration could attenuate or prevent the heart failure induced by isoproterenol in animal model.

**Methods:** Lactobacillus paracasei subsp. paracasei 8700:2 was used. Adult male Wistar rats (n=40, 185±15g), were randomly assigned to five groups, including the control group, probiotic control (0.25 mg/kg/day, oral), isoproterenol control (5 mg/kg/day, subcutaneous), pretreatment group and treatment group were divided. The groups were studied for 30 days. Serum levels of TNF-α and ANP were measured by ELISA and the standard histopathological examination was performed.

**Results:** A significant reduction was seen in the levels of TNF-α and ANP in the treatment group compared to the control group and isoproterenol group ($p<0.05$). Pretreatment with L. paracasei subpp.paracasie 8700:2 could not prevent the development of heart failure induced by isoproterenol. Histopathological analysis showed a marked attenuation of cardiac necrosis and infiltration of immune cells in treatment group.

**Conclusion:** This report indicates that the strain of L. paracasie attenuates inflammatory responses in rats with heart failure. These effects are mediated by reduction in serum levels of the TNF-α and ANP.

**Keywords:** heart failure, isoproterenol, TNF-α, ANP, Lactobacillus paracasei

ICIA2018\Immunopharmacology and Medicinal Plants\Oral\8282
**8282**

# The alteration of human colon cancer, HT29 cell line, after administration of Kombucha, a fermented microbial product

Mahsa Alem[1], Somayeh Ahmadi afshar[1], Norouz Delirezh[1]

*1. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Kombucha is an ancient healing supplement of Asian origin. It strengthens the immune system as a natural medicine. Anti-cancer properties of Kombucha and its derivatives have been shown in many in vivo and in-vitro experiments. It can revive the ionic balance in the cell membrane so prevent many of ailments. The current research was conducted to investigate the apoptotic and cytotoxic effects of administration of Kombucha on some functions of the HT29 as human colon cancer cell line.

**Methods:** The study population consisted, cells were allocated in to different groups such as negative control, treatment with 4 doses (50, 100, 150 and 200 µl/ml) of Kombucha, for 24 h, after cell line culture. All groups were tested via tetrazolium dye (MTT) assay for vitality investigation, natural red (NR) for cell membrane viability investigation, invert microscopy for evaluation of cell's morphology, acridine orange (AO) and propidium iodide (PI) staining for evaluation of necrosis and apoptosis respectively.

**Results:** The cell membrane viability was decreased in HT29 cell line. MTT assay test showed that Kombucha could make significant changes in the vitality of Kombucha treated colon cancer cell line. PI and AO staining manifested that Kombucha induced apoptosis in lower dose and necrosis in upper dose. These results were dose-dependent and statistically significant compared to the control group.

**Conclusion:** Based on these results, it appeared that this agent could be a good candidate for further evaluation as effective therapy acting through induction of apoptosis in human colon cancer cell line.

**Keywords:** HT29-cell line, Human colon cancer, Kombucha

ICIA2018\Immunopharmacology and Medicinal Plants\Oral\8436
**8436**

# Epigallocatechin-3-gallate enhances differentiation in acute promyelocytic leukemia cells via inhibition of PML-RARα and HDAC1

Maliheh Moradzadeh[1], Abazar Roustazadeh[2], Saiedeh Erfanian[2], Alijan Tabarraei[3]

1. *Golestan Rheumatology Research Center, Golestan University of Medical Sciences, Gorgan, Iran*
2. *Research Center for Non-Communicable Diseases and Biochemistry Department; Department of Advanced Medical Sciences & Technologies, School of Medicine, Jahrom University of Medical Sciences (JUMS), Iran*
3. *Infectious Diseases Research Center, Golestan University of Medical Sciences, Gorgan, Iran*

**Background:** Introducing all trans retinoic acid (ATRA) for the treatment of acute promyelocytic leukemia (APL) patients could dramatically improve their survival rate. However, resistance to this drug and their toxicity are major problems in the treatment of APL. Earlier studies suggested that in haematopoietic neoplasms, the green tea polyphenol Epigallocatechin gallate (EGCG) induces cell death without adversely affecting healthy cells.

**Methods:** In the present study, the potential anti-promyelocytic leukemia activity of EGCG and the underlying molecular mechanisms were investigated.

**Results:** EGCG (100 µM) significantly inhibited proliferation and induced apoptosis in HL-60 and NB4 cells. The effect was associated with the decreased expressions of MDR proteins ABCB1 and ABCC1, while the expressions of proapoptotic genes CASP3, CASP8, p21 and Bax/Bcl-2 ratio were significantly increased. EGCG at 25µM concentration, like ATRA (1 µM), induced differentiation of leukemic cells towards granulocytic pattern. Furthermore, EGCG suppressed the expression of clinical marker PML/RARα in NB4 cells, and reduced the expression of HDAC1 in leukemic cells.

**Conclusion:** The results suggest that EGCG can be considered, alone or in combination with ATRA, for preclinical and also clinical testing in APL patients.

**Key words:** APL, EGCG, Apoptosis, Differentiation, MDR

ICIA2018\Immunopharmacology and Medicinal Plants\Oral\10043
**10043**

# Evaluation and comparison of Albumin-curcumin Nano particle and curcumin anti-cancer effects on Sk-BR3 cell line

Zahra Matluobi[1], Parisa Amir Kalvanagh [1], Zuhair Saraf Hasan[1]

*1. Immunology Department, Tarbiat Modares University, Tehran, Iran*

**Background:** The advent of chemotherapy and radiation-resistant cancer cells, as well as severe complications of chemotherapy drugs, has led researchers to try to find solutions to these problems. Among the solutions that are very much considered is the use of some herbal compounds with antioxidant properties as therapeutic supplements along with chemotherapy drugs that reduce the dose of these drugs and thus reduce their side effects. Among the plant antioxidants, curcumin is one of the strongest antioxidants to inhibit the proliferation of cancer cells and induce apoptosis in them. However, curcumin has had very low dissolution in water and its ketone form has been predominant in acidic conditions (the PH of environment surrounding the tumor is acidic). In addition, degradation rate of curcumin by enzyme and its elimination from the bloodstream is very high. So, the antioxidants effects of curcumin in the body have had greatly reduce. To overcome these problems, various compounds of Nano-curcumin have been produced and tested. In this study, curcumin was conjugated with human serum albumin (HSA), and were measured for its effects on breast cancer cell lines (SK-BR3) and were compared with curcumin anti-cancer effects.

**Methods:** After making of curcumin-albumin Nano drug, anticancer effects of its have been examined by MTT and flow cytometry tests on SK-BR3and PBMC cells.

**Results:** FTIR graph and TEM picture confirmed that curcumin and albumin have been combined together. The MTT results showed that in first 24 hours none of the two drugs had a significant effect. After 48 hours curcumin and Nano curcumin had led to 14 % and 24% death in Sk-Br3 cells, respectively. Results of 72 hours showed that Nano curcumin had 52 % cytotoxic effect on cells while the cytotoxicity of curcumin on cells was only 24%. None of the drugs had significant toxicity on PBMC. The results of MTT were approved by Flow cytometry.

**Conclusions:** Nano-curcumin drug increases curcumin solubility in water and its stability in physiological and acidic conditions. These factors had led to a significant increase in the toxicity of curcumin on the SK-BR3 cells without increasing the cytotoxicity of the normal cells.

**Keywords:** curcumin, Albumin, Nano drug, SK-BR3

# Innate Immunity and Inflammation

# Oral Presentation

ICIA2018\Innate Immunity and Inflammation\Oral\7551
**7551**

# IL-17 producing CD4+CD45RO+ T-cells in Atherosclerosis express GITR molecule

Atefe Ghamar Talepoor[1], Negar Behnamfar[1], Mohammad Javad Zibaeenezhad[2], Mehrnoosh Doroudchi[1]

*1. Memory T-cell Laboratory, Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.*
*2. Cardiovascular Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Atherosclerosis (AS) is a chronic inflammatory disease of vessel walls associated with infiltration of immune cells which their function is controlled by different co-stimulatory and co-inhibitory receptors. In this study, we investigated the expression of co-inhibitory molecules on the memory and effector T-cells in patients with Atherosclerosis.

**Methods:** Patients included 9 hypertensive, dyslipidemic, non-diabetic, non-smoker individuals with the diagnosis of coronary artery disease and controls were 8 normotensive, normolipemic, non-diabetic, non-smoker individuals with normal coronary angiography/ insignificant coronary artery disease. PBMCs were separated from the blood and memory T-cell subsets as well as the expression of Glucocorticoid-induced tumor necrosis factor receptor (GITR), Programmed Death-1 (PD-1), IL-17A and IFN-γ were quantified by flowcytometry.

**Results:** CD4+CD45RO+ memory T-cells and CD4+CD45RO- effector T-cells in patients expressed the highest level of GITR molecule. The IL-17 producing memory CD4+CD45RO+ T-cells were enriched in GITR molecule in the patients group (P=0.03). The increased population of GITR+ effector CD4+CD45RO- T-cells in patients, however, did not produce IL-17 (P=0.03). PD-1 expression on memory T-cells of the patients was higher than the controls and was concomitant with the lack of IFN-γ expression (P=0.05). IFN-γ production by effector T-cells was only seen in the PD-1- population in both groups.

**Conclusions:** We provide data on the expression of GITR molecule on IL-17 producing memory T-cells in patients with CAD. A population of memory T-cells, which expressed PD-1 and were not producing IFN-γ, also increased in patients' blood. These data suggest the modified phenotype/function of T-cell subsets in the atherosclerotic inflammation.

**Keywords:** Atherosclerosis, Immunopathology, T cell, inflammation

ICIA2018\Innate Immunity and Inflammation\Oral\7851
**7851**

# Differential pattern of eotaxins (CCL11, CCL24, CCL26) and CCR3 expression in Iranian Parkinson's patients

Hossein Khorramdelazad[1,2]

1. *Molecular Medicine Research Center, University of Rafsanjan, Rafsanjan, Iran*
2. *Department of Immunology School of Medicine. University of Rafsanjan, Rafsanjan, Iran*

**Background:** The Parkinson's disease (PD) is defined as the most frequent human neurodegenerative disorder, after Alzheimer's disease. Recent studies indicated that cytokine/chemokine network and their downstream inflammatory responses are remarkably involved in the PD pathogenesis.

**Methods:** The present study was aimed to examine the expression of eotaxin family members, including CCL11, CCL2, and CCL26 in parallel with their receptor CCR3 in PD patients at both mRNA and protein levels. Blood specimens were isolated from 30 patients suffering from PD and 30 healthy participants as control for analysis in this investigation. Demographic information of patients and controls were also collected via a researcher-designed questionnaire. The enzyme-linked immunosorbent assay (ELISA) was performed for the detection of serum levels of CCL11, CCL24, and CCL26. Flow cytometric and real-time PCR methods were also employed to determine expression of CCR3.

**Results:** We observed that CCL11 and CCL24 were significantly elevated in serum of PD patients, whereas CCL26 was not significant changed whereby compared to control group ($P<0.001$). In addition, results obtained from flow cytometry and RT-PCR showed that there was no remarkable change in the expression of the CCR3 in PD patients compared with control group.

**Conclusion:** According to these findings, it could presumably be reasonable to conclude that CCL11, CLL24 might be play a part in the PD pathogenesis and they can be considered as potential serum-based biomarkers in this disease. Moreover, it is probable that microglial cells are expressing CCR3 more than circulatory eosinophils in response to released eotaxins from astrocytes due to progressed neurotoxicity as well as PD.

**Keywords:** CCL11, CCL24, CCL26, eotaxin, CCR3, Parkinsonism.

ICIA2018\Innate Immunity and Inflammation\Oral\8154
**8154**

# Macrophage repolarization using PLGA-PEG nanoparticles containing Chrysin: possible application in regenerative medicine

Akram Firouzi Amandi[1], Zohreh Babaloo[1], Younes Pilehvar-Soltanahmad[2], Leili Aghebati-Maleki[3], Farhad Jadidi-Niaragh[1], Mehdi Dadashpour[2]

1. *Immunology Department, Medicine Faculty, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Department of Medical Biotechnology, Faculty of Advance Medical Science, Tabriz University of Medical Science, Tabriz, Iran*
3. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** The aim of this study was to evaluate the efficiency of using a natural substance, Chrysin, encapsulated in PLGA-PEG nanoparticles (NPs) for the modulation of macrophage polarity from the pro-inflammatory M1 to anti-inflammatory M2 phenotype.

**Methods**: Characterization of the NPs was monitored using FTIR, DLS and FE-SEM. The effects of Chrysin-encapsulated PLGA-PEGNPs on the viability of LPS/IFN-c stimulated peritoneal macrophages were determined using MTT assay. The cellular uptake of free Chrysin and nano-formulated Chrysin was measured using confocal microscopy. Also, the expression levels of M1 marker (iNOS and SOCS3) and M2 marker (Arg1 and Fizz) and also pro-inflammatory cytokines were determined by real time PCR

**Results:** Data showed that the nano-formulated curcumin with spherical shape, an average diameter of 102.5 nm and high cellular uptake was significantly less toxic to peritoneal macrophages. Furthermore, the nano formulated Chrysin effectively indicated a reduction in iNOS-2 and an increase in Arg-1 levels than free Chrysin. The change in macrophage phenotype by Chrysin-encapsulated PLGA-PEG NPs could suppress the inflammation in LPS/IFN-c stimulated macrophages as verified by a major reduction in pro-inflammatory cytokines.

**Conclusion:** The results proposed that the Chrysin formulation with PLGA-PEG NPs might be a hopeful platform for the treatment of inflammatory diseases.

**Keywords:** Chrysin, PLGA-PEG, nanoparticles, macrophage polarity, inflammation

ICIA2018\Innate Immunity and Inflammation\Oral\8185
**8185**

# Reciprocal role of hBD2 and hBD3 on the adaptive immune response by measuring T lymphocyte proliferation in terms of CD4 and CCR6 expression

Sina Taefehshokr[1*], Pouriya Dadebighlu[2], Arezoo Naderi[3], Maryam Diansaei[1], Saeed Farhadzadeh[1], Farzin Javid[1], Ebrahim Taghizadeh[2], Nima Taefehshokr[4]

1. *Young Researchers and Elite Club, Tabriz Branch, Islamic Azad University, Tabriz, Iran*
2. *Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran*
3. *Faculty of Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran*
4. *Division of Bioscience, Department of Life Sciences, College of Health and Life Sciences, Brunel University, UK*

**Background:** Human β-defensins are small cationic antimicrobial peptides of innate immune system which can act as a barrier against the majority of pathogens, contributing to the host immune defense. The aim of study is to determine whether human β-defensins (hBD2 and hBD3) play a role in development and proliferation of human effector cluster of differentiation 4(CD4) T cells or not. Furthermore, if enhanced proliferation is observed in the presence of hBD2 and hBD3, these data will demonstrate whether CC chemokine receptor 6(CCR6) is required to be present for this activity to occur.

**Methods:** In this study, we examined the effect of hBD2 and hBD3 on $CD4^+$ T cell proliferation in $CCR6^+$ and $CCR6^-$ T cells through co-culture of PBMCs (Peripheral blood mononuclear cells) with anti-CD3 and anti-CD28 stimulation in the presence or absence of hBD2 and hBD3. Proliferation was assessed using flow cytometry.

**Results:** It was demonstrated that, co-culture with hBD2 and hBD3 led to up-regulation of $CD4^+$ T cell proliferation after 72 hours whereas, $CD4^+$ T cell proliferation was suppressed after 96 hours. On the other hand, $CCR6^-$ and $CCR6^+$ T cell proliferation was up-regulated after 72 hours. But, $CCR6^+$ only was down-regulated in the second cycle in the presence of hBD3. In contrast, after 96 hours $CCR6^+$ and $CCR6^-$ T cell proliferation was decreased.

**Conclusion:** Collectively, our data indicated that human β-defensins (hBD2 and hBD3) play a positive and negative regulatory role in development and proliferation of human effector $CD4^+$ T cells which is essential for optimal adaptive immune responses and the control of immunopathology.

**Keywords:** hBD2, hBD3, T cell proliferation, CD4, CCR6

ICIA2018\Innate Immunity and Inflammation\Oral\8381
**8381**

# Xanthine oxidase is elevated and associated with disease activity in Behçet's disease: study on ALGERIAN PATIENTS

Arezki Chekaoui[1], Houda Belguendouz[1], Karima Lahmar-Belguendouz[1], Djanette Hakem[2], Fatma Zohra Mazari[3], Malika Terrahi[3], Chafia Touil-Boukoffa[1]

1. *Cytokines & NO Synthases" team, LBCM-FSB-USTHB, Algiers, Algeria*
2. *Internal medicine department, CHU Bab El Oued, Algiers, Algeria*
3. *Ophtalmology department, CHU Nafissa Hammoud, Algiers, Algeria*

**Background:** Behçet's disease (BD) is a multisystem disease. It stands at the crossroad between autoimmunity and autoinflammatory diseases. Xanthine Oxidase (XOD) is an enzyme that is involved in free radicals production. Several studies have demonstrated that free radicals trigger biological material oxidation leading to severe inflammatory reactions in BD. In the present study, we aimed to assess the XOD activity plasma levels and elucidate its possible association with BD activity in Algerian BD.

**Methods:** We investigated the Xanthine oxidase activity (XOD) and nitrosative stress marker (Nitric oxide (NO)) in Algerian Behçet's Disease patients (n=78): Active Behçet's disease patients (ABP, n=28) and Inactive Behçet's Disease patients (IBP, n=50) and healthy controls (HC, n=41). Mann–Whitney U test and Pearson correlation test were used for statistical analyses.

**Results:** In our study, we showed that ABP displayed significantly higher XOD activity levels ($p<0.0001$, $p= 0.038$) compared to IBP and HC respectively. In contrast, XOD levels in IBP showed no statistical differences ($p=0.45$) versus HC. Nitric oxide levels significantly increased in ABP ($p<0.0001$) when compared to IBP and HC and in IBP ($p < 0.0001$) versus HC.XOD significantly and positively correlated with NO ($r=0.4882$, $p = 0.0002$) in Behçet's disease patients.

**Conclusion:** We emphasize that Xanthine oxidase activity increased and correlated with the disease severity in BD patients. We suggest exploring further this enzyme (XOD) with the aim to serve as additional marker for assessing BD activity and being an eventual target for therapeutics.

**Keywords:** Behçet's disease, disease activity, Xanthine oxidase, chronic inflammatory disorder.

ICIA2018\Innate Immunity and Inflammation\Oral\8589
**8589**

# Berberine attenuates high glucose-induced lipotoxicity and lipid droplet accumulation in RAW264.7, involvement of suppression of skewing RAW264.7 toward M1

Maryam Shabani*[1], Reyhaneh Babaei Khorzoughi[1]

*1. Department of Clinical Biochemistry, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Macrophages are flexible cells and able to play multiple functions in inflammatory conditions. These major components of innate immunity cells secrete various cytokines and a vast number of mediator molecules and CD markers. These processes are tightly regulated and these molecules have both beneficial and detrimental outcome in health. macrophages can acquire specialized phenotypes; M1 (pro-inflammatory phenotype) and M2 (anti-inflammatory phenotype). M1 subset secreted inflammatory mediators such as iNOS, TNF, MCP1, IL-6, and have CD11-c surface marker. In contrast, M2 markers are CD206, CD163, TGFβ, Arginase. M1/M2 balance has a critical role in inflammation. Diabetes Mellitus is an inflammatory condition and polarization pattern of macrophage is going toward M1. Berberine is an active plant compound that is extracted from CoptisChinensis, which has strong anti-inflammatory properties. This study was conducted to evaluate the anti-inflammatory effect of Berberine.

**Method:** RAW 264.7 macrophages were stimulated by high glucose concentration, then we have studied effects of Berberine (10 µM) on high glucose-induced lipotoxicity and macrophage polarization. We have measured high glucose-induced lipogenesis by oil-red O staining. For investigating macrophage polarization, we assessed M1 marker, CD11c, as an inflammatory factor via flowcytometry. Also, inflammatory genes expression include: iNOS, IL-6, and MCP1 were assayed by Real-time PCR.

**Results:** This study suggests that high glucose induce RAW264.7 lipogenesis. Flow cytometry analyses showed that high glucose skew RAW264.7 toward M1 subset, whereas Berberine attenuated M1 marker up to about 70%. RT-PCR confirmed the anti-inflammatory effect of Berberine through attenuation of inflammatory genes expression ($P< 0.05$).

**Conclusion:** Reduction of lipogenesis, M1 surface marker and above inflammatory genes approves that Berberine can be used as a therapeutic drug in inflammatory diseases.

**Keywords:** Inflammation, Berberine, High Glucose, Macrophage polarization

ICIA2018\Innate Immunity and Inflammation\Oral\9847
**9847**

# Increased circulating Chitinase-3-like Protein-1 (YKL-40) concentrations after cardiopulmonary bypass in coronary surgery patients, the effect of Erythropoietin treatment

Mahnoosh Foroughi[1*], Zohre Mohammadi[1, 2], Mostafa Haji molahoseini[3], Mahmood Bakhtiyari[2], Ali Dabbagh[4]

1. *Cardiovascular Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
2. *Clinical Research and Development Center, Shahid Modarres Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
3. *Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
4. *Anesthesioloy Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Cardiopulmonary bypass (CPB) and the imposed global myocardial ischemia induce extensive inflammatory reaction.YKL-40 is a rather new biomarker in acute and chronic inflammation and cardiovascular disease. Among patients undergoing cardiac surgery, the serum level of YKL-40 has not been investigated yet. The discrepancy and gap about the cardio protective effects of erythropoietin (Epo) still exist. The aim of this study was to assess the effects of CPB and Epo treatment on serumYKL-40.

**Methods:** In this pilot trial, 97 patients were admitted for elective coronary surgery with CPB, and randomly allocated to receive one of three treatments: group A (n = 35, infusion of 20000 IU Epo after aortic clamp in 30-45 min), group B (n = 31, the same intervention after anesthesia and before CPB, control group (n = 31).

**Results:** In comparison between the groups, there was a sharp increase in serum YKL-40 with a 24 h delay after CPB in all groups without significant difference. The increase in serum IL-6 was significant in group B compared with both groups A and control (P=0.001 and P=0.001, respectively). Serum NT-pro BNP reached maximum level after 24 h in all groups and group B was significantly higher than group A (P=0.008). CK-MB was significantly increased in all groups (P<0.001), but this change was less prominent in group B compared to control (P=0.003).

**Conclusion:** The inflammatory response induced by CPB has a dramatic effect on YKL-40 level. Serum YKL-40 reached peak level one day after CPB. Epo treatment has no effect on postoperative serum YKL-40.

**Keywords:** Coronary artery bypass, Erythropoietin, Inflammation, IL-6, Reperfusion injury, YKL- 40.

# Monoclonal Antibody Diagnostic and Therapeutic

# Oral Presentation

ICIA2018\Monoclonal Antibody Diagnostic and Therapeutic\Oral\7606
**7606**

# Production of a Mouse Monoclonal Antibody against cell-surface antigen of new established breast cancer cell line

Farzaneh Ghaderi Shabankareh [1, 2], Abbas Ghaderi [1, 2]

1. *Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Breast cancer is the most common malignancy and the leading cause of cancer-related death among women worldwide. In Iran, breast cancer accounts for 24.6% of total cancers with the crude incidence rate of about 22.6 per 100,000 females, which even is increasing annually. Murine monoclonal antibodies (MoAbs), reactive with several human tumor-associated antigens, have been produced and characterized in many laboratories. The purpose of these studies has been to generate effective monoclonal antibodies that could be useful in tumor diagnosis and therapy.
**Methods:** Splenic lymphocytes of BALB/c mice immunized with a new established breast cancer cell line (Pari-ICR cell line, established in Shiraz Institute for Cancer Research) were fused with the mouse myeloma cell line, SP2/0 in the presence of polyethylene glycol, MW 1450. We generated a panel of monoclonal antibodies against the newly established cell line. The hybrid cultures were screened by flowcytometry for the production of antibodies.
**Results:** Hybridomas that produced antibodies to surface antigens of the immunizing cell line and other breast carcinoma cell lines, but not to human fibroblast cells, mesenchymal stem cells and leucocytes isolated from peripheral blood, were selected and cloned by limiting dilution method. One clone designated 1H3, with IgG2a isotype was further analyzed for specificity by flowcytometry. Monoclonal antibody was affinity purified from mouse as cites fluid for further analysis. The target of monoclonal antibody had a molecular weight of approximately 150 kDa as determined by Western blotting. The functional characteristics and the specific target of the produced monoclonal antibody on immunizing cell line are underway.

**Keywords:** monoclonal antibody, breast cancer cell line, hybridoma.

ICIA2018\Monoclonal Antibody Diagnostic and Therapeutic\Oral\7620
**7620**

# Generating a Novel Monoclonal Antibody against CD73 as a Potential Diagnostic Marker in Human Carcinomas

Kimia Zandeih[1], Saeideh Milani[2], Mehrdad Hashemi[1], JavadMohammadi[2, 3], Fatemeh Ghaemimanesh[2], Ali Ahmad Bayat[2], Reza Hadavi[2], Hodjatallah Rabbani[2]

1. *Department of Genetics, Islamic Azad University, Tehran Medical Sciences Branch, Tehran, Iran*
2. *Antigen and Antibody Engineering Department, Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*
3. *Department of Life Science, Faculty of New Science and Technology, University of Tehran, Tehran, Iran*

**Background:** CD73 is an enzyme which catalyzes the hydrolysis of AMP to adenosine. It also has a key regulatory role in cancer cells proliferation, migration and invasion. Over expression of CD73 has been found in many type of cancer. All of these characteristics led scientists to consider CD73 as a favorable marker for diagnosis and assessments of cancer. The aim of this study was to produce a novel and effective monoclonal antibody against CD73 as a diagnostic tool in human epithelial cancer.

**Methods:** A synthetic peptide derived from CD73 protein was conjugated to Keyhole limpet hemocyanin (KLH) and used for immunization of BALB/c mice. The generated antibody was purified from the supernatant of final hybridoma clone using peptide-affinity chromatography column and its ability to recognize the immunizing peptide was measured by ELISA. The reactivity of the antibody with CD73 protein was then evaluated by Western blot, immunocytochemistry and flowcytometry using different epithelial cancer cell lysates.

**Results:** Western blot, immunocytochemistry and flowcytometry analysis using produced anti-CD73 monoclonal antibody showed a specific recognition of this protein in different carcinoma cell lines including MCF-7, SW480 and A431.

**Conclusion:** Our generated mAb has potential to be utilized in three application including W.B., IHC and FACS for detection and assessment of CD73 expression in human carcinomas.

**Keywords:** Monoclonal Antibody, CD73, cancer, detection

ICIA2018\Monoclonal Antibody Diagnostic and Therapeutic\Oral\8019
**8019**

# Introduction of the First Antibody Drug Conjugate (ADC) based on anti-Placenta-specific1 (PLAC1) Antibody as a Novel Immunotherapeutic Tool for Prostate Cancer

Mohammad Reza Nejadmoghaddam[1,2], Rassoul Dinarvand[2,3], Ramin Ghahremanzadeh[1], Roya Ghods[4,5], Jafar Mahmoudian[2], MaryamYousefi[1], Mahboobeh Nazari[6], Mohammad Hossein Ghahremani[2], Maryam Abolhasani[7], Ali Anissian[8], Morteza Mahmoudi[2] , Amir-Hassan Zarnani[9,10]

1. *Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.*
2. *Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences (TUMS), Tehran, Iran*
3. *Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
4. *Oncopathology Research Center, Iran University of Medical Sciences, IUMS, Tehran, Iran*
5. *Department of Molecular Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, IUMS, Tehran, Iran.*
6. *Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.*
7. *Department of Pathology, Hasheminejad Kidney Center, Iran University of Medical Sciences,IUMS, Tehran, Iran*
8. *Veterinary department, Islamic Azad University, Abhar branch, Abhar, Iran*
9. *Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*
10. *Immunology Research Center (IRC), Iran University ofMedical Sciences, IUMS, Tehran, Iran*

**Background:** Our group has recently reported differential expression of PLAC1 in prostate cancer suggesting the potential usefulness of PLAC1 for targeted immunotherapy of prostate cancer. We hypothesized that taking advantages of cancer cell specificity of anti-PLAC1 antibodies and cytotoxic activity of toxic agents could be a platform for generation of an antibody drug conjugate (ADC) that selectively target prostate tumor cells.
**Methods:** The expression profile of PLAC1 in prostate cancer cells, LNCaP, PC3 and DU145, was assessed by PCR, Western blotting, and flow cytometry. To prepare ADC, SN-38, a potent anti-topoisomerase I derivative of camptothecin, was first aminated and characterized by FT-IR and NMR spectroscopies. Modified SN-38 was conjugated to carbohydrate residues of a monoclonal anti-PLAC1 antibody through amine linkage and drug-antibody ratio (DAR) was measured by HPLC. Binding properties, affinity and internalization capacity of ADC were analyzed by ELISA, flow cytometry and immunofluorescent staining, respectively. Cytotoxicity of SN38-anti-PLAC1 on prostate cancer cells was studied by fluorimetry and single cell gel electrophoresis. Cytotoxic ex vivo effect of anti-PLAC1-ADC was further assessed by Annexin V apoptosis assay in human primary prostate cancer cells. Off target effects of the anti-PLAC1-ADC was also tested in vivo in mice.
**Results:** Prostate cancer cells expressed PLAC1 at both gene and protein levels. Anti-PLAC1 binding to the cells induced rapid internalization of the antibody within few minutes which completed within an hour. After SN38 conjugation to antibody, a DAR of about 6 was achieved. Drug conjugation did not negatively affect affinity and binding property of anti-PLAC1 antibody to cell surface antigen. The ADC retained intrinsic antibody activity and showed enhanced and selective cytotoxicity with an IC50 about 15 fold lower compared to free drug. Anti-PLAC1 ADC induced selective apoptosis in human primary prostate cancer cells and cancer cell lines, while exerted no off target effects as judged by histopathology examination of different organs of the injected mice.
**Conclusion:** Our results showed for the first time the potential application of anti-PLAC1-based ADCs as novel immunotherapeutic modality for patients with prostate cancer.

**Keywords:** PLAC1, Prostate cancer, Antibody-drug conjugate (ADC), Immunotherapy

ICIA2018\Monoclonal Antibody Diagnostic and Therapeutic\Oral\8086
**8086**

# Designing, cloning, expression and purification of the bispecific antibody which can retarget oncolytic Newcastle Disease virus to carcinoma

Monireh Kazemimanesh[1], Frank Momburg[2], Mostafa Jarahian[3],Vahid Baniasadi[4], Kayhan Azadmanesh[1]

*1. Department of Virology, Pasteur Institute of Iran, Tehran, Iran*
*2. Clinical Cooperation Unit, Applied Tumor Immunity, Antigen Presentation and T/NK Cell Activation Group, German Cancer Research Center(DKFZ), Heidelberg, Germany*
*3. Department of Toxicology and Chemotherapy, German Cancer Research Center (DKFZ), Heidelberg, Germany.*
*4. Department of Arboviruses and Viral Hemorrhagic Fevers, Pasteur Institute of Iran, Tehran, Iran*

**Background:** Carcinoma is a type of cancer that develops from epithelial cells. Oncolytic viruses such as Newcastle Disease virus (NDV) are a promising class of drugs that preferentially replicate in cancer cells as well as finally kill the malignant cells. For retargeting NDV to tumor tissue, bispecific antibody (biAb) can be used to bind NDV to tumor cells. Our biAb binds with one arm to the hemagglutinin-neuraminidase (HN) molecule of NDV and with the other arm to the epithelial cell adhesion molecule (EpCAM) which is overexpressed on carcinoma cells.

**Methods:** The plasmid which encodes the biAb was designed and constructed. Suitable competent E. coli DH5α cells were transformed using the $CaCl_2$ standard method. A single bacterial colony from a selective plate was used for DNA preparation. Concentration of extracted plasmid was measured by spectrophotometry and confirmed by double digestion with restriction enzymes. This plasmid was stably transfected in HEK 293 cell using lipofectamin LTX-PLUS.The stable cell line was cultured and after 72 hours the medium (supernatant) was collected. For investigating expression of biAb, SDS-PAGE and Western blotting of medium and filtered medium (concentrated using Amicon® Ultra-15 centrifugal filter devices)as well as flow cytometry analysis were performed. Purification of biAb was performed using rProtein A Gravi Trap (GE Healthcare Life Sciences). The ability of purified biAb to attach to HN of different strains of NDV and EpCAM expressed by carcinoma cells was investigated by flow cytometry.

**Results:** The cloned plasmid which encodes the biAb was confirmed by digestion with restriction enzymes. Expression of biAb was confirmed by flow cytometry analysis. These results show that biAb could attach to HN of different strains of NDV and EpCAM.

**Conclusion:** This biAb binding to NDV-HN and the pan-epithelial antigen EpCAM, may be used to retarget oncolytic NDV to carcinomas.

**Keywords:** Bispecific antibody, Carcinoma, Newcastle Disease virus.

ICIA2018\Monoclonal Antibody Diagnostic and Therapeutic\Oral\8184
**8184**

# Contribution of Fc fragment of monoclonal antibodies in tetanus toxin neutralization

Somayeh Ghotloo[1], Mohammad Mehdi Amiri[1], Jalal Khoshnoodi[1], Mahmood Jeddi-Tehrani[2], Forough Golsaz-Shirazi[1], Fazel Shokri[1,2]

1. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.*
2. *Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*

**Background:** Monoclonal antibodies (MAbs) against neurotoxin of Clostridium tetani are considered as a novel source of immunoglobulins for passive immunotherapy of tetanus. Fab and F(ab')2 may be more effective in neutralizing neurotoxin than intact antibodies due to their faster and higher tissue distribution. In addition, removing Fc portion from the mouse antibodies reduces HAMA response in human. We generated Fab and F(ab')2 of three mouse MAbs, which previously showed neutralizing activities either individually or in combination and compared their neutralizing activities with those of their intact antibodies.

**Methods:** Fab and F(ab′)2 fragments were generated by papain and pepsin digestion, respectively. Immunoreactivity of the fragments was compared with their intact forms by ELISA. Toxin neutralizing activity of the antibodies was evaluated in an in vivo toxin neutralization assay in which various amounts of MAbsor their fragments were mixed with 20MLD of the toxin and injected intraperitoneally to BALB/c mice. Human polyclonal immunoglobulins (tetabulin) and their fragments were used as the control.

**Results:** Enzymatic digestion of MAbs did not affect reactivity of the antigen binding sites. While intact MAbs were able to fully protect the mice even at the lowest dose, none of the mice, which received F(ab′)2 or Fab fragments, survived longer than 14 days even at the highest administered dose. All mice receiving tetabulinor their fragments were fully protected.

**Conclusion:** Loss of protective activity of MAbs fragments is not due to the attribution of Fc region in toxin neutralization, since the mice receiving tetabulin fragments were fully protected. It may be explained by the removal of steric hindrance of Fc region on the receptor binding site of the toxin. It seems that the polyclonal pool of tetabulin contains some molecules with variable regions recognizing receptor binding site of the toxin leading to toxin neutralization.

**Keywords:** Tetanus toxin, Monoclonal antibody, Toxin neutralization, Fab, F(ab′)2

ICIA2018\Monoclonal Antibody Diagnostic and Therapeutic\Oral\8211
**8211**

# Development and Characterization of a Camelid Single Domain Antibody Directed to Human CD22 Biomarker

Fatemeh Faraji[1], Nader Tajik[2], Mahdi Behdani[3], Mohammad Ali Shokrgozar[4], Amir Hassan Zar nani[2], Fatemeh Shahhosseini[4], Mahdi Habibi-Anbouhi[4]

1. *Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*
2. *Immunology Research Center (IRC), Iran University of Medical Sciences, Tehran, Iran*
3. *Biotechnology Research Center, Venom & Biotherapeutics Molecules Laboratory, Pasteur Institute of Iran, Tehran, Iran*
4. *National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran*

**Background:** CD22 is a B-cell-specific trans-membrane glycoprotein which is found on the surface of the most B-cells and modulates their function, survival, and apoptosis. Recently, targeting this cell surface biomarker in B-cell malignancies and disorders has attracted a lot of attention. The variable domain of camelid single chain antibodies (VHH, Nanobody) is a form of antibodies with novel properties including small size (15-17 kDa), thermal and chemical stability, high affinity and homology to human antibody sequences.

**Methods:** In this study, a novel anti-CD22 specific VHH (Nb) has been developed and characterized by the screening of an immunized phage display library and its binding to $CD22^+$ B-cells evaluated.

**Results:** Produced anti-CD22 VHH had a single protein band about 17 kDa of molecular size in western blotting and its binding affinity was approximately 9×10-9 M. Also, this product had high specificity and it was able to recognize the natural CD22 antigen in CD22+ cell lysate as well as on the cell surface (93%).

**Conclusion:** This anti-CD22 VHH with both high affinity and specificity recognizes CD22 antigen well and can be used in diagnosis and treatment of B cell disorders and malignancies.

**Keywords:** CD22, Camelid Single Domain Antibody, VHH

ICIA2018\Monoclonal Antibody Diagnostic and Therapeutic\Oral\8215
**8215**

# A novel method for purification of hyaluronidase from Streptococcus pyogenesis in one step

Amir Shakouri[1], Jalal Abdolalizadeh[2]

1. *MSc of Biotechnology, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *PhD of Clinical Biochemistry, Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Hyaluronidase contains some classes of catalysts that cleave Hyaluronic Acid that exist in mammalian spermatozoa, venom of scorpions and snakes, Leeches, Bacteria and etc, which can digest hyaluronic acid and is also known as a developer factor. It open the way for other active ingredients found in the saliva of leeches to penetrate into deep tissue. The aim of this study is the purification of Bacterial hyaluronidase with affinity chromatography which packed with Anti- Hyaluronidase proteins that produced in rabbit.

**Methods:** The rabbit polyclonal antibody was prepared against the hyaluronidase enzyme. Thus the pure enzyme was injected to rabbit and to evaluation of antibody production, ELISA and double Diffusion tests were done. Antibody purification was done by ion exchange chromatography and protein G affinity chromatography. Then the purified antibody was attached to the CNBr-activated Sepharose and finally it used for purification of hyaluronidase from Bacteria. After the purification, purity of fractions was assessed by SDS-PAGE electrophoresis and proved within Western- Blot.

**Results:** SDS-PAGE analysis showed the purity of protein was up to 98%. Also the single band with a molecular weight of approximately 50 KD is related to Streptococcus Pyogenesis hyaluronidase. Western- Blot analysis demonstrated that single band was related to hyaluronidase.

**Conclusion:** Affinity chromatography using produced antibody would be an economical and safe method for purification of hyaluronidase from Streptococcus Pyogenesis.

**Keywords:** Hyaluronidase, Immunoaffinity Chromatography, Streptococcus Pyogenesis, Polyclonal antibody

ICIA2018\Monoclonal Antibody Diagnostic and Therapeutic\Oral\8295
**8295**

# Phage display-derived antibody fragments against conserved regions of VacA toxin of Helicobacter pylori

FarnazFahimi[1,2], ShamimSarhaddi[2], Mehdi Fouladi[1,2], NaserSamadi[2], JavidSadeghi[4], Asal Golchin [1], MohammadrezaTohidkia[1]*, JalehBarar[1,3], YadollahOmidi[1,2,3]

*1. Research Center for Pharmaceutical Nanotechnology, Biomedicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran*
*2. School of Advanced Biomedical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran*
*3. Department of Pharmaceutics, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran*
*4. Department of Microbiology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz,* Iran

**Background:** Infection with helicobacter pylori, the only carcinogen ı bacterium, is attributed to gastrodudonal disorders and gastric adenocarcinoma. Due to inaccuracy of common diagnostic methods new detection approaches based on monoclonal antibodies are in priority. Among various toxins assisting pathogenesis of H. pylori, VacA is one of the most potential toxins, which is known as the major cause of peptic ulcer in patients.
**Methods:** Solution phase biopanning and screening were performed in order to isolate scFvs against two conserved regions of VacA. Characterization of scFvs was carried out by ELISA, immunoblotting and SPR. Bioinformatic analysis also performed in order to characterize structural and functional properties of isolated scFvs and antibody-antigene interactions.
**Results:** After four rounds of panning and screening, positive colonies in scFv ELISA were harvested for plasmid extraction and sequencing. Among those of which, high diversity of the VacA1 and diversity of 2 colonies for VacA2 were determined. Large scale expression and purification were performed for further complementary tests such as SDS-PAGE, western blot and SPR in order to determine affinity and specificity. Docking results done by bioinformatics analysis determined the interaction of the CDRs with the VacA peptide.
**Conclusion:** In this study, for the first time recombinant antibody fragments were isolated against conserved residues of VacA toxin with high specificity and affinity. New diagnostic approaches based on monoclonal antibody production and combination with drug delivery methods would minimize misinterpretation of the clinical laboratory tests.

**Keywords:** Antibody, Phage antibody display, scFvs, Helicobacter pylori, VacA toxin

# Mucosal and Gastrointestinal Immunology

# Oral Presentation

ICIA2018\Mucosal and Gastrointestinal Immunology\Oral\5257
**5257**

# Diverse profiles of TLR2, 4, 7 and 9 mRNA in Peripheral blood and biopsy specimens of patients with Celiac Disease

Haniye Ghasiyari[1,2], Mohammad Rostami Nejad[3*], Davar Amani[2*], Kamran Rostami[4], Mohammad Amin Pourhosseingholi[3], Mohammad Reza Zali[3]

*1. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*4. Department of Gastroenterology Milton Keynes University Hospital, Milton Keynes, UK*

**Background:** Celiac disease (CD) is an organ-specific autoimmune disease, and both adaptive and innate immunity are involved in its development. The aim of this study was to investigate the TLRs 2,4,7,9 genes expression in both peripheral blood and intestinal mucosa of patients with celiac disease compare to healthy control(HC).

**Methods:** Blood samples from120 confirmed CD patients who had been on a gluten-free diet (GFD) at least for 1 year and 120 age- and sex-matched healthy volunteers served as control group were collected during 2016. Also 20 biopsy specimens from persons who had a clinical manifestation despite GFD were randomly taken. Total RNA was isolated using a standard commercial kit. The mRNA expression of TLRs were quantified by relative qPCR with β2 microglobulin (β2m) as a reference gene.

**Results:** TLR4 and TLR9 mRNA were significantly higher expressed in blood samples from GFD-CD compared to the healthy controls (P=0.02); but not for TLR2. Furthermore, mRNA expression of TLR2 (P=0.03) and TLR4 (P=0.0003) expression level was increased in CD biopsy specimens compared to controls, whereas expression of TLR9 mRNA was significantly decreased in CD patients. There was no significant differences expression of TLR7 in biopsy and blood specimens.

**Conclusions:** It seem that upregulation of TLR4 and TLR9 suggests the contribution of gut microbiota or dysregulation of the immune response to commensal flora in small bowel mucosa in celiac patients.

**Keywords:** celiac disease, Toll like receptor, innate immunity

ICIA2018\Mucosal and Gastrointestinal Immunology\Oral\5306
**5306**

# Generation of recombinant cytokine secreting strains of Lactococcus Lactis to enhance mucosal innate immunity

Sadegh Feizollahzadeh[1], Hossein Khanahmad[3], Ilnaz Rahimmanesh[3], Mazdak Ganjalikhani-hakemi[2], Alireza Andalib[2], MohammadHossein Sanei[4], Abbas Rezaei[2]

*1. Department of Medical Laboratory Sciences, Paramedical faculty, Urmia University of Medical Sciences, Urmia, Iran*
*2. Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
*3. Department of Molecular Biology and Genetics, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
*4. Acquired Immunodeficiency Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background:** Cytokines are secretory mediators of immune system that play a crucial role in innate immunity. Interleukin-18 (IL-18) mainly identified as interferon-γ inducing factor. This cytokine is a pleiotropic cytokine secreted by different types of immune and nonimmune cells. It is involved in defense against viral, intracellular bacterial and protozoan infections and intestinal homeostasis. Lactococcus lactis (L. lactis), is a safe bacterium which increasingly used for heterologous protein expression in therapeutic and industrial applications. In the present study, we engineered L. lactis to express murine mature IL-18 in order to use the new straining the enhancement of intestinal innate immunity.

**Methods:** For a generation of such strain, coding DNA sequence of mature murine IL-18 was optimized for codon usage of L. lactis. It cloned downstream of Nisin system in PNZ8149 plasmid and electroporated to L. lactis NZ3900. Positive clones were selected based on yellow colony formation on Elliker medium and confirmed by polymerase chain reaction (PCR) and restriction digestion. IL-18 production was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), western blotting and ELISA in lysates and medium.

**Results:** Transformed bacteria formed yellow colonies on Elliker. PCR with IL-18 specific primers on extracted plasmids were positive. SDS-PAGE on bacterial lysate showed a thick sharp band around 18kD protein band. The identity of the protein band was then verified using IL-18 primary antibodies in western blot test. IL-18 level in bacterial lysate was 3–4 mg/L as detected by specific ELISA kit. Secreted IL-18 in medium was about 6–7 µg/L.

**Conclusion:** The successfully generated L. lactis strain that expressed active murine IL-18 can be used to evaluate the possible therapeutic effects of IL-18 on mucosal surfaces.

**Keywords:** Cytokine, Innate immunity, Interleukin-18, Lactococcus lactis

ICIA2018\Mucosal and Gastrointestinal Immunology\Oral\7823
**7823**

# Normal total duodenal intraepithelial lymphocytes and CD3+, and CD8+ intraepithelial T cells in celiac patients: first report on cell density counts of the intestinal intraepithelial lymphocytes in Isfahan population, Iran

Hadi Hossein-Nataj[1], Mohsen Masjedi[2], Sayed Mohammad-Hassan Emami Najaffi Dehkordi[2], Mojgun Mokhtari[2], Fereshteh Alsahebfosoul[2]

1. *Mazandaran University of Medical Sciences*
2. *Isfahan University of Medical Sciences*

**Background:** Increased numbers of the duodenal intraepithelial lymphocytes (IEL) are one of the key histological findings in celiac disease (CD). However, this finding may vary from one region to another. There are cases of borderline histology and seronegative CD which might be different from other causes of the duodenal immunological reactions by measuring the mean percentages of iIEL expressing the α/ßTCR, γ/$^{\delta}$TCR, CD3, CD4, and CD8 markers. This study aimed to measure the total CD3+, CD8+ IEL T cells in celiac patients and healthy controls in Isfahan, Iran.

**Methods:** D2 biopsies from definite CD cases (17 cases) and healthy controls (22 cases) were evaluated. H&E staining and several monoclonal antibodies were used in this study by the immunoperoxidase staining method.

**Results:** Immunoperoxidase staining method showed that the mean total IEL was 19 and 40 in the control subjects and CD cases, respectively ($P \leq 0.05$). The upper normal limit of the CD3+ intraepithelial T cells (mean+2SD of IEL T cells counts in the controls) was 20, while it was 14% for the CD8+ T cells. In addition, the H&E staining method showed the cut-off of 34% for IEL in CD.

**Conclusion:** This study suggests that the total IELs are of > 34%, CD3+ IEL > 20% and CD8+ IELs > 14% by the immunoperoxidase staining technique. These might be considered as Marsh I among the general population of Isfahan. Since immunoproxidase staining of IEL by monoclonal antibody has an additional value in CD diagnosis and should be done for interpreting when there is disagreement between the serology and H&E staining methods.

**Keywords:** Celiac disease, duodenal biopsy, intestinal intraepithelial lymphocytes, normal range.

ICIA2018\Mucosal and Gastrointestinal Immunology\Oral\8254
**8254**

## The up-regulation of NRF-2and IL-11 in mild Ulcerative colitis

Milad sabzevary-ghahfarokhi[1], Hedayatollah Shirzad[2]

*1. Student Research Committee, Shahrekord University of Medical Sciences, Shahrekord, Iran*
*2. Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran*

**Background:** Ulcerative colitis is an inflammatory disorder of the colon in which the balance of the intestinal immune system is disturbed. Oxidative stress plays crucial roles in the pathogenesis of UC. Our aim was to measure protein level of IL-11, and characterized the expression pattern of the NRF-2 protein in histological tissues.

**Methods:** 20 patients with mild ulcerative colitis and 21 healthy subjects were enrolled in the study. The IL-11 level in tissues was determined by means of ELISA. NRF-2 protein and mRNA in mucosal tissue were also performed by immunohistochemistry and real-time PCR.

**Results:** The increased level of IL-11 protein and NRF-2 mRNA were shown in the mild patient compared to control subjects. Furthermore, NRF-2 protein dominantly appears in mucosal epithelium.

**Conclusion:** the existence of oxidative stress in UC patients induces NRF-2 expression to resist against damages. The NRF-2 factor is a key regulator transcription factor of IL-11 production. Our data indicated that NRF-2 increased in patients leading to the protection of mucosal tissue. It is suggested that IL-11 act as a regenerator factor which may be a point of research in the novel therapeutic approaches for IBD.

**Keywords:** Oxidative stress; NRF-2; IL-11.

ICIA2018\Mucosal and Gastrointestinal Immunology\Oral\8349
**8349**

# Interleukin-6 gene polymorphism -174 G/C, −572G/C association with susceptibility to Iranian celiac disease population

Zeinab Barartabar[1], Abdolrahim Nikzamir[1], Majid Sirati Sabet[1], Vahid Chaleshi[3], Elham Aghamohammadi[2], Mohammad Reza Zali[3], Mohammad Rostami Nejad[3]

*1. Biochemistry Department, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** polymorphisms of -572G and -174G in the interleukin 6 promoter can effect on the production and secretion of interleukin 6 which may play a role in the inflammation and pathogenesis of celiac disease. The aim of this study was to investigate the relationship between -572G and -174 G polymorphisms with susceptibility to celiac disease in Iranian population.

**Methods:** A total of 105 patients with celiac disease and 106 healthy individuals were recruited in this study during 2016. DNA of both groups were extracted by available kits and gene polymorphism studied by polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP). Also serum levels of Interleukin 6 were evaluated by ELISA method in both groups.

**Results:** The results showed a significant relationship between polymorphism -572Gin CD patients compared with control subjects in genotype (p= 0.001) and alleles (p=0.022), respectively. But there was no significant relationship between polymorphism -174G and frequency of genotype, but the association of this polymorphism with the frequency of allele (p= 0.034), age (p=0.001) and BMI (p=0.003) was seen. On the other hand serum level of interleukin 6 was significantly associated only with rs1800796 (p= 0.000).

**Conclusions:** Our results show that (572- rs1800796) polymorphism may play a role in susceptibility to CD in the Iranians patients.

**Keywords:** Celiac disease, PCR-RFLP, IL-6 gene polymorphism, autoimmune and inflammatory reaction

ICIA2018\Mucosal and Gastrointestinal Immunology\Oral\8615
**8615**

# In vitro activation of Dendritic cells by a novel peptide as gastric cancer antigen-presenting cells

Kaveh Baghaei[1], Ali Asadirad[2], Bahareh Mohebi, Samaneh Beigi[1], FatemehYarian, Seyed Mahmoud Hashemi[2], Maryam Nourizadeh[3], Hamid Asadzadeh Aghdaei[1], Mohammad reza Zali[4]

*1. Basic and Molecular Epidemiology of Gastrointestinal Disorder Research Center, Research Institute for Gastroenterology and Liver Disease, Shahid Beheshti University of Medical Science, Tehran, Iran*
*2. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Immunology, Asthma and Allergy Research Institute, Children's Medical Center Hospital, Tehran University of Medical Sciences, Tehran, Iran.*
*4. Gastroenterology and Liver Disease Research Center, Research Institute for Gastroenterology and Liver Disease, Shahid Beheshti University of Medical Science, Tehran, Iran*

**Background:** Dendritic cells (DC) play an important role in forming the quantity and quality of antitumor immunity. Therefore, DC-based peptide delivery of tumor antigen is becoming a potential approach in cancer immunotherapy. Effective antitumor immunity requires the generation and persistence of functional tumor-specific T-cell responses. The use of peptide-based vaccination has been found to be a promising means to induce antitumor T-cell responses. In current study we showed role of novel gastric cancer drive peptide which present by DCs and activate lymphocytes against tumor cells.

**Methods:** In this approach, autologous DC were generated from their precursors in bone marrow by using conventional cytokine and loaded with specific gastric cancer tumor antigen and then co-cultured with Lymphocyte cells. Immature and mature DCs were confirmed by Flow cytometry. Lymphocyte proliferation was measured by BRDU.

**Results:** Mature dendritic cells were successfully obtained from bone marrow mononuclear cells. The level of CD markers (CD40, CD80, CD83, CD86) were analyzed by flow cytometry. We could show, designed peptide activated DCs to proliferate lymphocytes.

**Conclusion:** Our DC-based peptide vaccination can act as an antigen delivery vehicle, resulting in measurable antitumor immunity in cancer settings in preclinical and clinical after in vivo studies.

**Keywords:** Dendritic cell, Gastric Cancer, Peptide vaccine

ICIA2018\Mucosal and Gastrointestinal Immunology\Oral\8711
**8711**

# Investigation of immunogenic effects of Syphacia obvelata in treatment of experimental colitis mouse model

Niloofar Taghipour[1,2], Nariman Mosaffa[3], Hamid Asadzadeh Aghdaei[4], Mohammad Rostami N ejad[1]

*1. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
*2. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran;*
*3. Department of Immunology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*4. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Many evidences have been suggested a reverse relationship between helminths infections and allergic or autoimmune diseases. In inflammatory bowel disease, excessive Th1 and Th17 cell responses to commensal germs or intrinsic antigens cause progressive inflammation in the tissues.The ability of helminth parasite infections to manipulate the immune system of their host has been proposed to suppress the inflammatory response.

**Methods:** male C57BL / 6 mice were divided into 5 groups: control group, colitis group, Receiving S. obvelata group, Preventive group, Curative group. After creating the experimental model, one group fed the S.obv eggs and then infected with colitis, and the other group was vice versa. A disease activity index score can be assessed to evaluate the clinical progression of colitis. TNF-α, IL-10, IL-17, IFN-γ were evaluated by ELISA in the culture supernatants of MLN and PP cells. The percentage and expression Treg in different study groups were investigated using Real-time PCR and flow cytometry.

**Results:** Treg cells in the colitis group had a significantly decreased in both sites compared to other groups. Significant increase in the population of Foxp3 + cells in MLN and PP was observed in the groups receiving S. obvelata compared to the control and the DSS group. Improvement of symptoms and complications of induced colitis, improvement of histologic inflammation, increased Treg response (IL-10 cytokines) and decreased inflammatory cytokines in both site of mice in the preventive and curative groups were significantly better than the colitis group.

**Conclusion:** Our results showed that S. obvelata, in addition to its role in prevention, has curative potential to reduce inflammatory colitis in mouse models. This is probably due to immunomodulatory effects of S. obvelata the production of anti-inflammatory cytokines and inhibition of inflammatory cytokines.

**Keywords:** Helminth, Colitis, S. obvelata, Treg, Foxp3

ICIA2018\Mucosal and Gastrointestinal Immunology\Oral\9906
**9906**

# Evaluation of the expression level of BANCR Long none coding RNA in the Stool samples of patients with colorectal cancer, advance adenoma and healthy individual

Shadi Sadri[1], Mitra Salehi[2], Ehsan Nazemol hosseini Mojarad[3]

1. *Department of cellular and molecular, North Tehran Branch, Islamic Azad University, Tehran, Iran*
2. *Department of microbiology, North Tehran Branch, Islamic Azad University, Tehran, Iran*
3. *Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Colorectal cancer is mostly diagnosed in the late stages with poor prognosis. CRCis mostly derived from adenoma polyps and using of biomarkers such as long non coding RNA, is recommended for early detection. Long none coding RNA (lncRNAs) are key participants of gene regulations in disease and healthy situation. In this study we evaluation of the expression level of BANCR Long none coding RNA in the Stool samples of patients with colorectal cancer, advance adenoma and healthy individual.

**Methods:** In this study, 20stool samples of CRC patients , 20stool samples of adenoma polyp and 20 stool samples from healthy individual were collected.The clinical information was collected by a questionnaire and clinical reports, mRNA was extracted and cDNA was synthesized. BANCR gene expression was investigated by Real time PCR method and relative quantification.Fold change of genes expression were evaluated by ($2^{-\Delta\Delta ct}$) method. Data were analyzed by 7500 system SDS version 1.3 and Prism version 5 software.

**Results:** The data show that BANCR was significantly over expressed in stool samples of CRC patients comparing to patients with adenoma polyp and healthy individual. However, these changes were not linked with tumor pathological characterizations ($p<0.05$).

**Conclusion:** It seems that, increasing in BANCR expression is highly linked with tumor development and it may be a reason for malignancy progressing.

**Keywords:** BANCR Lnc RNA, Colorectal cancer, early diagnosis

ICIA2018\Mucosal and Gastrointestinal Immunology\Oral\9926
**9926**

## Comparing plasma level of miR-106a to Differentiates Crohn's disease from Ulcerative Colitis

Vahid Chaleshi[1], Mohammad Hossein Ghorbani, Nazanin Amiri, Shabnam Shahrokh, Hamid Asadzadeh Aghdaei, Mohammad Reza Zali

*1. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Inflammatory bowel disease (IBD) comprising Crohn's disease (CD) and ulcerative colitis (UC). Finding new biomarker for detection and monitoring of IBD is importance in diagnosis, prognosis and treatment. miRNAs are a class of non-coding RNAs which regulate gene expression. To date, the majority reports have identified dysregulated miRNAs in IBD. The aim of this study was to evaluate gene expression of plasma miR-106a between ulcerative colitis and Crohn's disease patients and healthy control.

**Methods:** A total of 73 patients with IBD (55 UC, 18CD), and 15 healthy controls were enrolled in the study. Colonoscopy in combination with histological finding confirm the IBD diagnosis. Healthy controls showed no IBD symptoms or any gastrointestinal diseases such as celiac disease, polyp and cancer. miRNA isolated from plasma samples using miRNeasy mini Kit (QIAGEN). cDNA was synthesized from miRNA using miScriptII RT Kit (QIAGEN). miR-106a gene expression examined by q-Real Time PCR using miScript SYBR Green PCR Kit (QIAGEN). All kits were used according to manufacturer's instructions.

**Results:** miR-106a expression was significantly increased in patients with Crohn's disease in proportion of UC patients (P value: 0.0003) and healthy control group (P value: 0.0143). No significant differences were observed between UC patients and control group (P value: 0.7319).

**Conclusion:** According to our results expression of miR-106a between CD and UC maybe used as a new non-invasive factor for diagnosis and differentiation between IBD type.

**Keywords:** Ulcerative colitis (UC), Crohn's disease (CD), Inflammatory bowel disease (IBD), miR-106a

ICIA2018\Mucosal and Gastrointestinal Immunology\Oral\9957
**9957**

# CD8 T cells are more frequent than CD4 T cells in patients suffering from chronic rhino sinusitis with polyp

Farhad Seif[1,2], Majid Khoshmirsafa[1], Hossein Aazami[1], Monireh Mohsenzadegan[3], Babak Ghalehbaghi[4], Mehdi Shekarabi[1,2]

*1. Immunology Department, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*
*2. Immunology Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran*
*3. Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, Iran University of Medical Sciences, Tehran, Iran*
*4. ENT and Head and Neck Research Center and Department, Hazrat Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran*

**Background:** Chronic Rhinosinusitis (CRS) is a common inflammatory disorder influencing sinonasal mucosa. It is classified into two different subtypes referred to as CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). Although multiple immune mechanisms are suspected to be involved in CRSwNPetiology, the impact of T cells is not completely determined. The aim of this research was to evaluate the pattern of T cells (CD8+ and CD4+ T cells) in CRSwNP patients.

**Methods:** In our study, 13 healthy controls and 20CRSwNP patients enrolled. Sinonasal tissues were investigated to find CD4 and CD8 T cells by immune histochemistry method. The diagnosis of CRS was confirmed by the current European Position Paper on Rhino sinusitis and nasal polyps. The Kruskal-Wallis, Mann-Whitney were used in statistical analyses. Gathered data were analyzed using SPSS V.21 and presented by GraphPad Prism V.6 softwares.

**Results:** The median (min-max) age of the groups was: 30 (14-51) for CRSwNP, and 29 (18-47) for healthy controls. The frequency of eosinophils was elevated in CRSwNP patients in comparis on to controls. The frequency of total inflammatory cells in CRSwNPand control groups was 89 (66.5-127) and 11.5 (19-18.5), respectively. The frequency of CD4+ and CD8+ T cells as well as total inflammatory cells were significantly elevated in CRSwNP patients in comparison to healthy controls. CRSwNPgroup showed elevated infiltration of CD8+ T cells in sinonasal mucosa (Pv<0.0001). CRSwNP group showed enhanced infiltration of CD4+ T cells into sinonasal mucosa in comparison to healthy controls (Pv<0.0001).

**Conclusion:** The number of CD8+ and CD4+ T cells was increased in CRSwNP patients. Furthermore, the frequency of CD8+ T cells was higher than CD4+ T cells in sinonasal mucosa of CRSwNP patients. Targeted therapeutic approaches may be accessible by evaluating the pattern of T cells. Therefore, further complementary researches on T cells are needed to achieve this objective.

**Keywords:** Chronic Rhino sinusitis, CRSwNP, T cell, CD4, CD8

ICIA2018\Mucosal and Gastrointestinal Immunology\Oral\10007
**10007**

# Increased expression of IL 17A and IL21 in the small intestine of patients celiac disease

Manizhe Faghih[1], Mohammad Rostami Nejad[2], Davar Amani[1]

*1. Department of Immunology, School of medical science, University of Shahid beheshti, Tehran, Iran*
*2. Department of Gastroenterology and Liver Diseases Research Center, School of medical science, University of Shahid beheshti, Tehran, Iran*

**Background:** Th17 and its related cytokines play an important role in the pathogenesis of celiac disease (CD). Therefore the aim of this study was to evaluate the expression levels of IL-17, IL-21 in treated and untreated celiac disease patients compared to the control group.
**Methods:** In this study, duodenal biopsied were collected from 60 treated CD patients, 60 healthy control and 8 newly diagnosed celiac patients, during 2016. RNA extracted by commercial kit, cDNA synthetized and specific primer pairs were designed for each gene. The expression of genes was investigated by Real-time PCR technique using SYBER Green method.
**Results:** 60 treated CD patients (58.3% female; mean age of 38.8 years old), 60 healthy control (55% female; mean age of 36.5 years old) and 8 newly diagnosed celiac patients (75% male; mean age of 43.3 years old) were involved in the present study. The expression levels IL21 were insignificantly increased in the untreated CD group compared with control group. But the expression of IL-17A in the untreated CD patients was significantly higher than the control group ($p = 0.007$).
**Conclusion:** Our data showed that IL-17A, IL-21 has different expression profile in studied groups. According to the result, the investigated genes can be used as a diagnostic marker for screening patients with celiac disease. However, much more studies are required to confirm our findings and clarify the underlying mechanisms.

**Keywords:** Celiac Disease, IL-17A, IL-21, Gene Expression

# Nanoimmunology

# Oral Presentation

ICIA2018\Nanoimmunology\Oral\7528
**7528**

# Nanocurcumin Influences Regulatory T cell Responses in Ankylosing Spondylitis

Rozita Abolhasan [1, 2], Sanam Dolati[1, 2], Mehdi Yusefi[1, 2]

1. *Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Ankylosing spondylitis is a chronic, inflammatory disease of the axial spine that can apparent with chronic back pain and progressive spinal stiffness. Participation of the spine and sacroiliac (SI) joints, peripheral joints, digits, enthuses are distinguishing of the disease. Regulatory T cells with suppressive effects on inflammation and autoimmunity have been described to implicate in pathology of AS. This present study aimed to explore the effects of nanocurcumin on the frequency of Treg and their associated cytokines.

**Methods:** 24 patients with active AS joined in this study that were divided into two groups: 12 patients received 80 mg nanocurcumin every day for 16 weeks and 12 other patients received placebo as control group. First, the peripheral blood mononuclear cells were collected, and the frequency of Treg was analyzed by flow cytometry. Then, FoxP3 gene expression was appraised by using real-time qPCR. In addition, ELISA was carried out to determine the levels of cytokines such as IL-10, TGF-β and IL-6 in the peripheral blood of AS patients.

**Results:** Our study displayed that nanocurcumin could significantly increase the frequency of peripheral Tregs in AS patients (p value = 0.032). The results of real-time PCR also exhibited that the expression of FoxP3 was significantly increased (p value=0.02 and p value=0.0005, respectively). In addition, nanocurcumin group had higher IL-10 (p value=0.015) and TGF-β (p value=0.043) production and lower IL-6 (p value=0.018) expression than that of placebo group. The changes in the frequency and expression level of cytokines were positively correlated with the disease improvement.

**Conclusion:** Our results proposed that dysregulation of Tregs in peripheral blood of AS patients impacts the AS progress and nanocurcumin therapy through regulation of Tregs and the expression of the related cytokines can be appreciated in the treatment of ankylosing spondylitis and other autoimmune/ inflammatory diseases.

**Keywords:** Ankylosing spondylitis, Nanocurcumin, Regulatory T cells, Cytokines

ICIA2018\Nanoimmunology\Oral\7796
**7796**

**Combination treatment of tumor cells with the STAT3-specific siRNA loaded nanoparticles and BV6**

Afshin Nikkhoo[1,2], Fatemeh Atyabi[4,6],Narges Rostami[1,2], Ali Masjedi[1,2], Ali Rastegari[4], Masoomeh Baghaei[6], Vida Hashemi[7,1,2], Shima Bastaki[3,1], Farhad Jadidi Niaragh[1,2,5]

*1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
*2. Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
*3. Department of cellular and molecular biology, faculty of Basic science, Azarbaijan Shahid Madani university, Tabriz, Iran*
*4. Department of Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 1714614411, Iran*
*5. Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
*6. Nanotechnology Research Centre, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
*7. Department of basic science, faculty of medicine, Maragheh university of medical Science, Maragheh, Iran*

**Background:** Apoptosis resistance is mediated by IAPs that inhibit caspases. Smac mimetics such as BV6 can bind to and inhibit IAPs. Signaling of STAT3 can enhance cancer cell survival. Therefore, combination therapy by BV6 smac mimetic and STAT3 siRNA-loaded nanoparticles (NPs) can be considered as potent anti-tumor therapy.

**Method:** STAT3-specific siRNA loaded NPs were generated by ionic gelation method and applied to downregulate the expression of STAT3 in order to increase apoptosis in4T1 breast cancer cells and B16F10 melanoma cancer cells in combination with BV6smac mimetic. Apoptosis induction was analyzed by MTT assay and expression of apoptosis related genes was assayed by real-time PCR. The expression of angiogenic and metastatic genes was also investigated. The migration of cancer cells was investigated by wound healing assay.

**Results:** Synthesized NPs had about 100 nm size with a polydispersity index below 0.3 and a zeta potential about 13. NPs efficiently encapsulated siRNA, showed high serum stability, and efficient cellular uptake. Expression of STAT3 was significantly decreased in the tumor cells following treatment with nanoformulation. Combination therapy could significantly induce apoptosis in cancer cells which was associated with downregulation of survival molecules. Moreover, tumor cells exhibited lesser expression of angiogenic and metastatic molecules accompanied with decreased migration capacity after combination therapy.

**Conclusion:** Treatment with combination of STAT3 siRNA -loaded ChLa NPsand BV6may be considered as a promising approach for cancer therapy; however, further in vivo investigations are necessary.

**Key word:** STAT3, BV6, NIK, apoptosis, cancer, Trimethyl chitosan

ICIA2018\Nanoimmunology\Oral\7810
**7810**

# The immunomodulatory effect of nanocurcumin on acute Toxoplasmosis in Balb/c mice

Seyed Mohammad Mahdi Ghahari[1], Abolghasem Ajami[2], Mehdi Mahdavi[3], Majid Sadeghizadeh[4], Aahmad Reza Esmaeili Rastaghi[5,*]

1. *Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
2. *Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
2- *Molecular and Cell Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran*
3. *Department of Immunology, Pasteur Institute of Iran, Tehran, Iran*
4. *Department of Genetics, School of Biological Sciences, Tarbiat Modares University, Tehran, Iran*
5. *Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran*

**Background:** One of the controversial infections that most of the warm-blood creatures in the world are involved is related to Toxoplasma gondii. It is an important obligate intracellular protozoan parasite for immune deficient patient and pregnant women who sometimes are caused a death and abortion in individuals, respectively. Herein, immunomodulatory effect of nanocurcumin was evaluated against Toxoplasmosis killed vaccine model in Balb/c mice.

**Methods:** 144 Balb/c mice were included in 8 groups and the mice were administered different regimens of vaccine; vac +30, 20 mg/kg of curcumin and nanocurcumin, vac +Freund, killed vac, vac +Alum and PBS via subcutaneous route for three times with two weeks interval. Two weeks after the last immunization, the sera were assessed for total antibody, IgG1 and IgG2a with an optimized ELISA method. Lymphocyte proliferation was evaluated with Brdu method. The splenocytes culture supernatant was analyzed by ELISA for the presence of IL-4, IFN-γ, IL-2 and TNFα and ratio of IFN-γ/IL4, IFN-γ/ TNFα and IL-2/IL4 cytokines. Then survival rate were determined at 10 days.

**Results:** Our results show that nanocurcumin 20 mg/kg significantly increased IFN-γ/IL4 and IFN-γ/TNFα cytokines but contemplative increased IL-2/IL4 cytokines and lymphoproliferative response versus control group. These results demonstrate that this dose of nanocurcumin could elicit strong Th1 immune responses at the lowest level of inflammation and production IgG antibody titers predominance of IgG2a and finally increased survival rate compared with control groups and P-values less than 0.05 was considered to be statistically significant.

**Conclusion:** This study provides evidence of immunomodulatory effects of nanocurcumin.

**Keywords:** Toxoplasma gondii, nanocurcumin, curcumin, cytokines

ICIA2018\Nanoimmunology\Oral\8500
**8500**

# Enhanced immunological responses with tumor antigen loaded polymeric nanoparticles

Sanaz Sheikhzadeh[1], Nowruz Delirezh[1], Rahim Hobbenaghi[2]

1. *Department of Microbiology, Faculty of Veterinary Medicine, Urmia University*
2. *Department of Pathology, Faculty of Veterinary Medicine, Urmia University*

**Background:** Cancer immunotherapy, the treatment that harnesses the patient's immune system to fight cancer, is now emerging as an important addition to conventional therapies. Cancer vaccines are active immunotherapy. Enhancing the immunogenicity of the vaccines requires efficient targeting of antigens and adjuvants to dendritic cells (DCs), that are the most potent antigen presenting cells for naive T cell activation. Nanoparticles (NP) are new vehicles for delivering vaccines. These particles are efficiently taken up by DCs because of their size and particulate structure which resembles pathogens. This study aimed to investigate the efficacy of a new nanoparticle-based cancer vaccine against breast cancer in mouse model.

**Methods:** Poly (D, L-lactide-co-glycolide) (PLGA) nanoparticles (NPs) containing breast tumor cell lysate were prepared using a double-emulsion solvent evaporation technique. Mice were challenged subcutaneously with 4T1 tumor cells. After induction of palpable tumor, immunotherapy was initiated. The control animals received PBS and the test animals were immunized with PLGA-NPs containing breast tumor cell lysate. One week after the last immunization, mice were euthanized. To evaluate the antitumor effects of vaccine; tumor growth, survival rates and cytokine level were determined.

**Results:** In mice immunized with PLGA-NPs loaded with tumor cell lysate, significantly reduced tumor growth and the survival rate was higher compared to the control group. The production of IFN-γ in spleen cell culture was increased while the production of IL-4 was decreased.

**Conclusion:** The goal of this study was to evaluate the antitumor activity of PLGA-based nanoparticle vaccine in a mouse model of breast cancer and so it is useful to develop a new way for the treatment of cancer.

**Keywords:** Immunotherapy, Nanoparticles, Tumor cell lysate, Cancer vaccine

ICIA2018\Nanoimmunology\Oral\8635
8635

# Improvement of Hematologic and Immunologic Findings in Gamma Irradiated Rats Treated with Selenium Nano-Particles and Selenium Selenite

Siamak Asri Rezaei[1], Ali Asghar Tehrani[2], Arya Sohrabi[2], Zahra Nori Sabzikar[3]

1. *Department of Clinical Pathology, School of veterinary college, University of Urmia*
2. *Department of Pathology, School of veterinary college, University of Urmia*
3. *Department of Clinical Pathology, School of veterinary college, University of Mashhad*

**Background:** The objective of this study was to evaluate the efficacy of the selenium nano-particles and selenium selenite supplementation in boosting the immune response and protecting from oxidative stress in irradiated rats.

**Methods:** 45 mature male albino rats weighing 150 - 200 g were obtained and divided into 3 groups (control, Nano-Selenium and Selenite selenium). Selenium supplementation at dose 0.1 mg/kg was given by IP injection to rats, two weeks before gamma irradiation and then gamma therapy at the dose levels of 0, 2 and 8 Gy was done for two weeks (twice in a week and each time 20 min). After 14 days, blood samples were taken from heart and CBC were done and serum level of MDA, IL-2, TNF-α and IL-6 were determined.

**Results:** The results revealed that total body irradiation induced significant decreases in RBCs, WBCs and lymphocytes, as well as tumor necrosis factor alpha (TNF-α) and interleukin 2 (IL-2) also decrease significantly while interleukin 6 (IL-6) and lipid peroxidation marker malondialdehde (MDA) in serum were elevated. In irradiated animals receiving selenium nano-particles and selenium selenite, values of RBC and WBC significantly increased as compared with the irradiated group, and similarly IL-2, IL-6 and TNF-α were significantly elevated. In contrast MDA levels in rats receiving selenium supplementation was decreased significantly. Also, the results of this study revealed that in immune system protection selenium nanoparticles be more effective than selenium salt.

**Conclusion:** The curative action of selenium nanoparticles enforcing significant innate response could trigger and augment adaptive immune response, thus protecting immune system from radiation induced damage as well as oxidative stress.

**Keywords:** Selenium nanoparticles, Gamma Radiation, Rat, Immune System

ICIA2018\Nanoimmunology\Oral\9965
**9965**

# Gelatin nanoparticle based delivery of Urease and Omp31 in mice protects against Brucella melitensis 16M infection

Morteza Abkar[1], Saeed Alamian[2], Naghmeh Sattarahmady[1]

1. *Nanomedicine and nanobiology research center, Shiraz University of Medical sciences, Shiraz, Iran*
2. *Brucellosis department, Razi vaccine and serum research institute, Karaj, Iran*

**Background:** Brucella(B) species are causative agents of brucellosis, a worldwide zoonotic illness causing Malta fever in humans and abortion in domestic animals. Due to the serious economic and medical consequences of the disease, many efforts are to prevent infection of domestic animals through vaccines.

**Methods:** In this work, we examined the vaccine potential of Gelatin nanoparticles formulation of Urease (Gelatin/Urease) and Omp31 (Gelatin/Omp31) against brucellosis. Gelatin/Urease and Gelatin/Omp31 nanoparticles were separately and in combination administered orally. Particle size and loading efficiency of the nanoparticles were determined. Antibody detection, cytokine measurement, lymphocyte proliferation assay and protection assay were performed. Finally, immunized mice were challenged with the virulent B. melitensis16M.

**Results:** All immunized mice elicited titers of specific immunoglobulin G (IgG). According to cytokine assay and antibody isotypes, oral administration with all vaccine formulations induced a mixed Th1-Th17 immune response. In lymphocyte proliferation assay, spleen cells from all-immunized mice showed a strong recall proliferative response. The combination of Gelatin/Urease and Gelatin/Omp31 nanoparticles conferred protection against B. melitensis infection equivalent to that of vaccine strain B. melitensis Rev.1.In comparison to Gelatin/Omp31 and Gelatin/Urease nanoparticles alone, combination of Gelatin/Omp31 and Gelatin/Urease nanoparticles induced only a low increase in protection level.

**Conclusion:** Altogether these results indicate that Gelatin/Omp31 or Gelatin/Urease nanoparticles could be useful candidates for the development of subunit vaccines against brucellosis. Furthermore, Gelatin nanoparticles are a suitable delivery system for orally-administered Brucella antigens.

**Keywords:** Omp31, Gelatin, Urease, Oral Vaccine

ICIA2018\Nanoimmunology\Oral\9968
**9968**

# Subcutaneous vaccination with aluminium hydroxide/Omp31 and chitosan/Omp31 nanoparticles induces protection against Brucella melitensis infection in BALB/c mice

Morteza Abkar[1], Saeed Alamian[2], Naghmeh Sattarahmady[1]

1. *Nanomedicine and nanobiology research center, Shiraz University of Medical sciences, Shiraz, Iran*
2. *Brucellosis department, Razi vaccine and serum research institute, Karaj, Iran*

**Background:** Vaccination has a high impact on the control and prevention of infectious illnesses. Subunit vaccines, such as recombinant proteins, are promising vaccine candidates because they are nonpathogenic, a virulent, well defined, nonviable and safer for manipulators, and economical. Since recombinant vaccines have low immunogenicity, application of an efficient antigen delivery system and adjuvant is very important. The use of the nanoparticle mediated delivery system is an effective strategy for site specific delivery of antigens.

**Methods:** In this study, we compared the potential of chitosan and aluminium hydroxide nanoparticles (NPs) to assess their ability in the development of new vaccines against brucellosis. Aluminium hydroxide and chitosan NPs were prepared, loaded with the protein antigen B. melitensis Omp31 and characterized. The immunostimulatory capacity of these vaccine delivery systems was examined in vivo.

**Results:** DLS and SEM images showed that most of NPs had a mean size distribution less than 100 nm. The amount of Omp31 loaded onto chitosan and aluminium hydroxide NPs were about 61% and 74%.According to the antibody subclasses and cytokine profile, subcutaneous immunization by both chitosan/Omp31 and aluminium hydroxide/Omp31 NPs induced T helper 1 (Th1) and Th1–Th2 immune responses, respectively. In comparison to aluminium hydroxide/Omp31 NPs, chitosan/Omp31 NPs induced only a low increase in protection level.

**Conclusion:** Altogether, our results indicate that both chitosan and aluminium hydroxide NPs are a potent delivery system for subcutaneous vaccination against brucellosis.

**Keywords:** Brucellosis, Omp31, Chitosan, Aluminium hydroxide.

# Oral Immunology

# Oral Presentation

ICIA2018\Oral Immunology\Oral\7637
**7637**

# Assessment of microRNA-146a in aggressive periodontitis and its association with disease severity

Somayeh Ghotloo [1], Hossein Motedayyen [2, 3], Mandana Sattari [3], Mahmood Saffari[1, 4], Davar Amani[3]

1. *Department of Laboratory Medicine, Kashan University of Medical Sciences, Kashan, Iran*
2. *Department of immunology, Isfahan University of Medical sciences, Isfahan, Iran*
3. *Department of Immunology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
4. *Department of Microbiology and Laboratory Medicine, Kashan University of Medical Sciences, Kashan, Iran*

**Background:** MicroRNA-146a (miR-146a) is a small non-coding RNA that plays a critical role in negative regulation of innate immune response and its dysregulation has been associated with several inflammatory disorders. Aggressive periodontitis is an almost less common but sever form of periodontitis in which chronic inflammation leads to rapid destruction of tissues surrounding the teeth. This study was performed to evaluate miR-146a expression level in gingival tissues of patients with aggressive periodontitis and its association with disease severity.

**Methods:** Gingival samples of 18 patients with aggressive periodontitis and 10 healthy subjects were collected and level of mir-146a and its targets including TNF-α, IL-1β and IL-6 was assessed using real- time PCR assay. Clinical parameters including probing depth (PD) and clinical attachment level (CAL) were measured and their correlations with miR-146a level were determined.

**Results:** Our results demonstrated an elevation in expression level of miR-146a in aggressive periodontitis compared to healthy control (p value< 0.001) which was directly associated with the disease severity (p value< 0.05). Over-expression of miR-146a was accompanied by the reduction of pro-inflammatory cytokines levels.

**Conclusion:** Positive association between miR-146a level and clinical parameter in aggressive periodontitis suggests that miR-146a may contribute to the pathology of the disease and serves as an indicator of disease severity. Elevation of miR-146a in these patients may occur in response to the bacterial components such as LPS leading to the control of pro-inflammatory cytokines level through a negative feedback loop.

**Keywords:** Aggressive periodontitis, MicroRNA -146a, Pro-inflammatory Cytokines

ICIA2018\Oral Immunology\Oral\7706
**7706**

# Correlation Between Expression of Gingival TLR5, TLR2, RANKL in Tissues and Chronic Periodontal Diseases

Ali Saeedi Boroujeni[1], Mandana Sattari[2], Samaneh Delavari[1], Ali Khodadadi[1]

1. *Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
2. *Department of Immunology, Shahid Behashti University of Medical Sciences, Tehran, Iran*

**Background:** Periodontal diseases are the most common chronic infections in human. Inflammatory responses play an important role in periodontal disease. TLRs play a key role in induction of inflammatory responses by periodontal cells. In addition to TLR4, LPS of Porphyromonas gingivalis can stimulate TLR2 and its fimbria can stimulate TLR5, so the aim of this study was to evaluate the gingival gene expression of TLR2 and TLR5 and also RANKL as the most important factor in bone resorption, in periodontal diseases.

**Methods:** Gingival samples were collected from 20 individuals with clinically healthy gingival; 25 patients with moderate to severe chronic periodontitis after homogenizing tissue samples, RNA extraction and cDNA synthesis were done. Then, Real-time PCR technique was used for evaluating gene expression.

**Results:** There were no significant difference between the expression of TLR2 (0.209), TLR5 (0.553) and RANKL (0.597) in control group and chronic periodontitis group. There was also significant correlation between TLR2 and TLR5 gene expression (P≈ 0.000).

**Conclusion:** According to the results, no difference was seen between chronic periodonotitis and control groups in terms of inflammatory responses.

**Keywords:** Periodontitis, Inflammation, RANKL, TLR

ICIA2018\Oral Immunology\Oral\8313
**8313**

## Correlation between TLR5 expression and asymptomatic irreversible pulpitis

Shideh Mehr Mofakham*, Mandana Sattari, Hengameh Akhavan, Sarira Shah Navaz, Aram Mohammmadi

*Shahid Beheshti University of Medical Sciences, Tehran, IRAN*

**Background:** Innate immunity in the pulp uses receptors to recognize molecular patterns common to microbes to initiate inflammation in the dental pulp, which eventually leads to irreversible damage and even necrosis of dental pulps. Since most of studies have been done on the roles of TLR2 and TLR4 in asymptomatic pulpitis, so the purpose of the present study was to compare TLR5 expression between asymptomatic irreversible pulpitis and normal pulps.

**Methods:** Pulps were taken from 20 teeth with asymptomatic irreversible pulpitis and 20 from intact premolars scheduled for extraction as control group. RNAs were collected from homogenized pulps and were subjected Reverse Transcriptase, producing cDNA, which is more stable. Forward and reverse primers were designed using Beacon designer software. Quantitative real time PCR were done by using SYBR Green. Statistical analysis was made by using REST software.

**Results:** The fold change of TLR5 expression in asymptomatic pulpitis was 1.510 but there is not any significant difference between asymptomatic pulpitis and normal pulp ($P \approx 0.852$).

Conclusion: It is concluded that with the progression of dental caries, despite the probable prevalence of some motile bacteria that are labeled with flagellum, but absence of a difference between a healthy pulp and an asymptomatic pulpitis can be a reflection of domination of non-motile bacteria in the pulp.

ICIA2018\Oral Immunology\Oral\8328
**8328**

# The assessment of correlation between salivary matrix metalloproteinase-8, 20 concentration and severe early childhood caries S-ECC

Mina Biria[1], Mandana Sattari[2], Atieh Ehsani[3]

1. *Department of Pediatric Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
2. *Department of Immunology, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
3. *Department of Pediatric Dentistry, Ghazvin University of Medical Sciences, Ghazvin, Iran*

**Background:** Dental caries is a transmissible bacterial disease process caused by acids from bacterial metabolism diffusing into enamel and dentin and dissolving the minerals. Collagen degradations considered to be initiated by endogenous proteolytic enzymes, mainly collagenolytic matrix metalloproteinases (MMPs). Hence the aim of this research was to evaluate the relationship between severe early childhood caries and salivary MMP-8 (or salivary collagenase-2) and MMP-20 (or enamelysin) concentration.

**Method:** 50 children aged 36-72 months were included in this study. They were divided into two groups: caries-free (control) and severe early childhood caries (S-ECC), with 25 children in each group. Standard clinical examinations were performed, and around 1ml of unstimulated and whole expectorated saliva was collected. 3 months after dental restorations, children in S-ECC group were re-sampled and analyzed for salivary concentrations of MMP-8, MMP-20 using an ELISA assay.

**Results**: Before the treatment, subjects in S-ECC group presented with elevated levels of MMP-8 compared with subjects in control group ($p< 0.001$), but salivary concentration of MMP-20 in 2 groups was not statistically significant ($p=0.189$). The difference between salivary concentrations of MMP-8 and MMP-20 before and after 3 months in S-ECC group were statistically significant (0.698 vs 0.331, $P<0.001$; 3.801 vs 3.438, $P=0.024$, respectively).

**Conclusion:** Our data reveal that subjects with severe early childhood caries have elevated levels of salivary MMP-8 compared to subjects with no caries lesions. 3 months after dental restorations, the concentration of MMP-8 and MMP-20 decreased significantly.

**Keywords:** Dental caries, Matrix Metalloproteinase 8, Matrix Metalloproteinase 20, saliva

ICIA2018\Oral Immunology\Oral\8444
**8444**

# Comparison of gene expression of different isoforms of osteopontin in superficial dental caries

Aram Mohammadi[1], Mandana Sattari[1]

*1. Department of Immunology ,School of medicine, Shahid Beheshti University of Medical Sciences*

**Background:** Osteopontin (OPN), a kind of human genum glycoprotein, plays an important role in immune system modulation. OPN can activate osteoclasts, thus causing resorption. Also it may have a protective function against polymicrobial endodontic infection. Because different isoforms of OPN might have diverse roles, so the purpose of the present study was to compare gene expression of different isoforms of osteopontin in superficial dental caries and normal teeth.

**Methods:** Pulps were taken from 20 teeth with superficial dental caries as the case group and 20 pulpal samples from intact premolars scheduled for extraction as control group. RNAs were collected from homogenized pulps and were subjected Reverse Transcriptase, producing cDNA, which is more stable. Forward and reverse primers were designed using Beacon designer software. Finally, quantitative real time PCR were done in order to estimate the expression of different genes (Isoforms of osteopontin and GAPDH as housekeeping gene). Statistical analysis was made by REST software.

**Results:** There was lower expression of OPN2 in superficial caries but there were no significant difference between superficial dental caries and normal teeth regarding expression of OPN, OPN3 and OPN5.

**Conclusion:** It is concluded that probably OPN2 or variant 2 of OPN has a protective role against pulpal inflammation and there is a decrease in its expression by appearance and subsequent progression of dental caries.

**Keywords:** osteopontin, dental caries, pulp.

ICIA2018\Oral Immunology\Oral\8473
**8473**

# Study of the effects of the dental pulp stem cells (DPSCs) on proliferation of peripheral blood mononuclear cells(PBMCs)

Nikoo Hossein-khan nazer[1], Arash Khojasteh[2] , Mandana Sattari[1], Seyed Mahmoud Masiha Hashemi[1]

1. *Department of Immunology, School of medicine, University of Shahid Beheshti*
2. *Department of Oral and Maxillofacial Surgery Shahid Beheshti University of Medical Sciences*

**Background:** Dental pulp stem cells (DPSCs) are multipotent mesenchymal stem cells that have the ability to self-renewand differentiate into a variety of cell types including osteoblasts, chondroblasts and adipocytes. Recent studies demonstrate that these cells exhibit immunomodulatory and anti-inflammatory effects on immune system. DPSCs capable of regulating immune cells which may provide a foundation for clinical use of DPSCs. In this study we determined immunoregulatory effects of DPSCs on peripheral blood mononuclear cell (PBMCs) proliferation using trans well co-culture system.

**Methods:** Dental pulp stem cells extracted from impacted third molars. Cells characterized for differentiation potential to adipogenic and osteogenic lineage and expression of mesenchymal stem cells markers. For transwell co-culture experiment, Stimulated and non-stimulated PBMCs from allogeneic donor cultured in different ratios with or without DPSCs (1:1, 1:5) for 72 hours. After incubation period proliferation of PBMCs evaluated using a cell proliferation BrdU ELISA kit.

**Results:** The BrdU results showed that Dental pulp stem cells reduced allogeneic PBMC proliferation. DPSC could inhibit stimulated and non-stimulated PBMC, 72 hours after incubation. This inhibiting property was more remarkable in 1:1 (MSC: PBMC) ratio.

**Conclusion:** This study demonstrated that Dental pulp stem cells exert anti proliferative effects on proliferation of allogeneic immune cells due to secreting soluble factors.

**Keywords:** PBMC, DPSC, co-culture

ICIA2018\Oral Immunology\Oral\8554
**8554**

# HSP70 and CD184 are not important in tissue destruction in aggressive periodontitis

Sarira Shahnavaz[1], Mandana Sattari[1], Aram Mohammadi[1], Fatemeh Sarlati[2], Farshid Yeganeh[1]

1. *Department of Immunology School of medicine Shahid Beheshti University of medical sciences*
2. *Department of Periodontal School of Dental University of Islamic Azad*

**Background:** Aggressive periodontitis is severe disease that its progression is fast and leads to bone destruction. The aggressive periodontal disease also divides two subtype groups that called local aggressive periodontal (LAP) and generalized aggressive periodontal (GAP). In other hand, CD184 is a chemokine receptor which is involved in the trafficking of leukocytes in to and out of extravascular tissues. CD184 may serve a homeostatic role to prevent excessive inflammation; alternatively, CD184 may be exploited by Porphyromonas gingivalis for suppressing innate immunity. However, the interaction of P. gingivalis with CD184 impairs antimicrobial host defense and it may be one mechanisms of immune evasion. Also, HSP70 family constitutes the most conserved and well-known class of HSPs. HSP70 has some regulatory and cytoprotective functions. So the aim of this study was to evaluate the relationship between expression of CD184 and HSP70 and clinical parameters of tissue destruction [such as CAL (Clinical attachment loss) and PD (Pocket depth)].

**Methods:** For this purpose, gingival tissue samples were collected from 20 individuals with clinically healthy gingiva and 25 patients with aggressive periodontitis. After homogenizing the gingival tissues, RNA was extracted by RNA isolation kit. After synthesis of cDNA, expression of CD184, HSP70 and beta-actin (as housekeeping gene) was evaluated by Real-time PCR.

**Results:** There is not any significant difference regarding expression of CD184 or HSP70 between aggressive periodontitis and healthy groups. In addition there is not any significant correlation neither between CD184 expression and CAL or PD nor HSP70 expression and CAL or PD ($P>0.05$).

**Conclusion:** Based on the results, we cannot suggest any significant role for CD184 in periodontal tissue destruction. Regarding no correlation between HSP70 and clinical parameters we cannot contribute any protective role for HSP70 in aggressive periodontitis. Of course more studies are needed in order to define the precise roles of CD184 and HSP70 in aggressive periodontitis.

**Keywords:** aggressive periodontitis, CD184, CAL, HSP70, PD

ICIA2018\Oral Immunology\Oral\9862
**9862**

# Chemotactic and proliferative responses of human gingival fibroblasts (HGFs) to insulin-like growth factor-1 (IGF-1)

Rokhsareh Sadeghi[1], Christopher A McCulloch[2]

*1. Assistant Professor, Department of Periodontics, Faculty of Dentistry, Shahed University, Tehran, Iran*
*2. D.D.S., Ph.D., F.R.C.D. (C), Canada Research Chair in Matrix Dynamics, Professor, Matrix Dynamics Group, Faculty of Dentistry, University of Toronto, Toronto, Canada*

**Background:** It has been demonstrated the Insulin-like Growth factor-1 (IGF-1) has mitogenic and chemotactic effects on various cell lines. The aim of the present study was to assess the chemotactic and proliferative response of human gingival fibroblasts (HGFs) to IGF-1.

**Methods:** Gingival fibroblasts were obtained from the gingival tissue of medically and periodontally healthy patients. Chemotaxis assay was performed in modified Boyden chambers using Transwell® permeable inserts. The lower chamber of each well was filled with growth medium containing IGF-1at different concentrations (0, 50, 100, 500, 1000 ng/ml). The cell suspension was placed on the top of the filters. Cell migration was assessed after 4 hours of incubation by counting the number of cells on the bottom surface of membranes using confocal microscopy. The proliferative effect of IGF-1 was evaluated by direct cell count. HGFs were plated in 24-well plates in growth medium supplemented with IGF-1 concentrations of 0, 50, 100, 200, 500, and 1000ng/ml. The relative proliferation rate of cells was evaluated after 3, 5 and 7 days, by counting the nuclei of cells using Epifluorescence light microscope.

**Results:** IGF at a concentration of 50 ng and 100ng showed a significant chemotactic effect on HGFs compare to the negative control (P=0.0221, P=0.0009, respectively). Regarding proliferation assay, no statistically significant differences were seen between the groups containing IGF-1 and negative control at any time point (P>0.05).

**Conclusion:** While HGFs showed a dose-dependent chemotactic response to IGF-1, they did not demonstrate any significant proliferative reaction to this growth factor.

**Keywords:** human gingival fibroblast, insulin-like growth factor, chemotaxis, proliferation

# Psychoneuroimmunology and Immunoendocrinology

# Oral Presentation

ICIA2018\Psychoneuroimmunology and Immunoendocrinology\Oral\7479
**7479**

# Frequency and function of regulatory T cells in elderly patients with ischemic stroke

Farzaneh Ghorbani[1, 2], Sanam Dolati[1, 2], Mehdi Yousefi[1, 2]

1. *Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** CD4+CD25+regulatory T (Treg) cells play important role in pathogenesis of ischemic stroke (IS). In the current study, we studied Treg cells frequency and determined the levels of their signature transcription factors and cytokines in the peripheral blood of IS patientswhich is compared between IS patients and control group.

**Methods:** 30 patients with IS and 30 individuals as control group were enrolled in this study. The frequency of Treg lymphocytes, the expression oftranscription factor (FOXP3) and the serum levels of associated cytokine (TGF-β) were assessed by flowcytometry, Real-time PCR and ELISA, respectively.

**Results:** The proportion of Treg cells were significantly reduced in patientscompared with controlsat 1 and 5 days after stroke, from 10.17 ±3.12 to 6.83 ±1.85 ($p<0.0001$) and 8.47 ± 2.06 ($p=0.016$), respectively.FoxP3 transcripts were significantly downregulated in IS patients 1 and 5 days after stroke compared to controlsand TGF-βlevels were diminished in IS patients at 1, 5 and 10 days after stroke compared with the controls from 155.7 ± 32.9to 95.5 ± 28.6 ($p=0.001$), 102 ± 25.4 ($p=0.002$) and 122 ± 32.57 ($p=0.08$), respectively.

**Conclusion:** This study suggests that the reduction of Treg cells contribute to the pathogenesis of IS.Also reduction in levels of TGF-β and FOXP3attributed to that; inflammatory reaction was initiated to decrease and anti-inflammatory function of Tregs was started to increase subsequently 10 days after stroke.

**Keywords:** Ischemic Stroke, Treg, TGF-β, FOXP3

ICIA2018\Psychoneuroimmunology and Immunoendocrinology\Oral\7880
**7880**

## Cytokine profile in autistic patients

Mohammad Mahdi Eftekharian[1], Mohammad Taheri[2,3]

1. *Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran*
2. *Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
3. *Urogenital Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** The etiology of Autism Spectrum Disorders (ASDs) as severe neurodevelopmentalailments is not known. However, several evidences point to dysregulation of immune system as an underlying cause of ASD.

**Methods:** In the present study we evaluated the mRNA expression levels of TNF-α, TGF-β, IFN-γ, CXCL8, IL-1β, IL-2, 1L-4, IL-6, IL-17 in whole blood samples of 30 ASD patients and 41 age and sex-matched healthy subjects with means of real-time PCR.

**Results:** TNF-α, IL-6 and IL-17 have been shown to be significantly up-regulated in ASD patients compared with healthy subjects ($P<0.0001$, $P=0.001$ and $P<0.0001$ respectively). IL-2 has been shown to be significantly down-regulated in total ASD patients ($P<0.0001$). No significant difference has been found in expression levels of other cytokines between patients and healthy subjects.

**Conclusion:** The present study provides further evidences for dysregulation of immune response in ASD patients.

**Keywords:** autism, TNF-α, TGF-β, IFN-γ, CXCL8, IL-1β, IL-2, 1L-4, IL-6, IL-17

ICIA2018\Psychoneuroimmunology and Immunoendocrinology\Oral\8642
**8642**

# Association of major depressive disorder with activation of NLRP3-inflammasome and Total Antioxidant Capacity

Abdolghader Taene[1], Ghazaleh Khalili Tanha[2], Gholamreza AnaniSarab[3]

*1. Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran*
*2. MSc of cellular and molecular biology, Cellular and Molecular Research Center, Birjand university of medical science, Birjand, Iran.*
*3. Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran*

**Background:** Depression is one of the most common, expensive, and devastating psychiatric disorder. Approximately, 16% of males and 25% of females experience depression during their lifetime course. Despite several studies, the pathogenic mechanism of major depressive disorder (MDD) have been poorly understood. It seems that changes in the expression of neurotransmitters, the hypothalamus-adrenal-pituitary (HPA) axis, oxidative stress and immune dysregulation can be a major factor of MDD. The present study examined the hypothesis to determine whether NLRP3-inflammasome is activated in peripheral bloods mononuclear cells from MDD patients. Furthermore, we analyzed the differentiation of Total Antioxidant Capacity (TAC) between MDD patients and healthy groups.

**Methods:** Twenty MDD patients without treatment with a diagnosis of major depression, twenty patients treated with antidepressant drugs and twenty healthy volunteers were enrolled in this study. Samples were collected from Department of psychiatry Emam Reza Hospital, in Birjand. The inflammasome activation was studied bySYBR green real time PCR and TAC of samples were measured by using the determination of ferric reducing ability power (FRAP). The differences in mean expression of NLRP3 and Caspase 1 gene as well as the amount of TCA between groups were analyzed.

**Results:** We found increased NLRP3 (P =0.023) and caspase-1 (P =0.026) gene expression relative to B-actin in bloods mononuclear cells of MDD patients respect to healthy controls. Interestingly, the amount of NLRP3 and caspase-1 gene expression reduced in treated group. Also we found out that serum TCA level in healthy group were significantly higher than both untreated and treated patient groups (P<0/005).

**Conclusion:** We conclude that NLRP3-inflammasome is activated in MDD patients and antidepressant drugs reduce NLRP3-inflammasome in MDD patients. Also reduced levels of TAC were observed in MDD patients.

**Keywords:** Major depressive disorder, Total Antioxidant Capacity, NLRP3-inflammasome

ICIA2018\Psychoneuroimmunology and Immunoendocrinology\Oral\9815
**9815**

# The Effects of D-aspartate in Experimental Autoimmune Encephalomyelitis

Yaser Panahi[1], Mahdi Goudarzvand[2], Kosar Abouhamzeh[3], Antimo D'Aniello[4], Gholamreza Azizi[3*]

*1. North Khorasan University of Medical Sciences, Bojnurd, Iran. Vector-born Disease Research Center, Bojnurd, Iran*
*2. Department of Physiology and Pharmacology, Faculty of Medicine, Alborz University of Medical Sciences, Karaj, Iran*
*3. Non-Communicable Diseases Research Center, Alborz University of Medical Sciences, Karaj, Iran*
*4. Laboratory of Neurobiology, Zoological Station of Naples "Anton Dohrn", Villa Comunale, Napoli, Italy*

**Background:** Experimental autoimmune encephalomyelitis (EAE) is a widely used model for MS. In the present research, our aim was to test the effects of D-aspartate (D-Asp) on remyelination in EAE.
**Methods:** In this study the EAE was induce on C57BL/6 mice and treated by D-Asp. On days 20, brains and cerebellums of mice were removed for histological analysis. Quantitative determination of D-Asp was performed using liquid chromatography-tandem mass spectrometry.
**Results:** Our findings demonstrated that IP injection of D-Asp had beneficial effects on EAE severity than orally gavage.
**Conclusion:** IP injection of D-Asp had more effects on remyelination than orally received EAE mice.

**Keywords:** Experimental autoimmune encephalomyelitis, D-aspartate

# Reproductive Immunology

# Oral Presentation

ICIA2018\Reproductive Immunology\Oral\6337
**6337**

# Immunomodulatory effects of human amniotic epithelial cells on cytokine profile of naïve $CD_4^+$ T cells from women with unexplained recurrent spontaneous abortion

Hossein Motedayyen[1*], Nader Tajik[2], Amir Hassan Zarnani[2], Abbas Rezaei[1]

1. *Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
2. *Immunology Research Center (IRC), Iran University of Medical Sciences, Tehran, Iran*

**Background:** Immune imbalance at the maternal-fetal interface plays a fundamental role in the pathogenesis of unexplained recurrent spontaneous abortion (URSA). Human amniotic epithelial cells (hAECs) possess pregnancy-friendly immunomodulatory effects. Here, we investigated how cytokine profile of naïve T cells from USRA patients is affected by hAECs.

**Methods:** Naive CD4+ T cells were isolated from 18 patients with URSA using immunomagnetic separation method. hAECs were obtained from healthy women during elective cesarean deliveries. hAECs were co-cultured at different ratios(1:1, 1:2, 1:5, 1:10) with naïve CD4+ T cells ($4\times 10^5$) in 24-well plates and stimulated with anti-CD3/CD28A. After 3 and 6 days, the co-culture supernatants were collected and the level of IFN-γ, IL-17A and IL-4 were analyzed using an Enzyme-linked immunosorbent assay (ELISA) kit. In this experiment, hAECsand un stimulated naïve T cells cultured alone served as negative controls, while naïve CD4+ T cells cultured alone and stimulated with anti-CD3/CD28A were considered as positive control.

**Results:** Our results showed that a statistically significant reduction in the level of IFN-γ and IL-17A was observed for all hAECs: naive T cells ratios after 3 and 6 days of co-culture ($p<0.05$-$0.0001$). Moreover, the level of IL-4 was significantly higher at all hAECs: naive T cells ratios after 3 days ($p<0.01$- $0.0001$). This significant increase in IL-4 level was not observed on day 6 for any of the hAECs: naive T cells ratios.

**Conclusion:** These findings suggest that hAECs have immunomodulatory effects on cytokine profile of naive T cells from URSA patients through stimulating Th2 cytokine (IL-4) production and decreasing Th1 and Th17 cytokines (IFN-γ, IL-17A) production.

**Keywords:** Immunomodulatory effects, Amniotic Epithelial Cells, Naïve T cells, Recurrent Spontaneous Abortion.

ICIA2018\Reproductive Immunology\Oral\7494
**7494**

# Th17 and regulatory T cells frequency in RIF's patients treated with IVIG

Zahra Pourmoghadam[1], Majid Ahmadi[1], Mehdi Yousefi[1, 2]

1. *Stem cells and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Failure to achieve successful implantation and clinical pregnancy after transferring at least three good quality embryos in to normal uterus is called recurrent implantation failure (RIF). Several studies have shown the importance of local mother's immune system in the success or failure of implantation. Recent evidence suggests the role of immunotherapy in the treatment of RIF's patients; among them intravenous immunoglobulin (IVIG) has become very important in recent years, with the hope that it will increase the chances of success in in vitro fertilization (IVF). The aim of this study is assessment of immune cells (Th17 and Treg) and their cytokines after IVIG therapy in women with RIF.

**Methods:** 72 patients with immunological abnormalities (elevated Th1/Th2 ratio and natural killer cells frequency) selected and divided into two groups. 40 patients as the IVIG group received aspirin, heparin and IVIG. 32 patients as control group received just aspirin and heparin. 400 mg/kg of IVIG was administered intravenously 2 days prior to embryo transfer. Then Th17 and Treg frequency was evaluated by flow cytometry. RT-PCR and ELISA were used to measure the Th17 and Treg cytokines expression and secretion. RORγt and FOXP3 transcription factors expression were evaluated by RT-PCR.

**Results:** After IVIG treatment elevated Treg frequency (p= 0.186) and FOXP3 expression (p= 0.0004) were detected. TGFβ and IL-10 expression (p= 0.0038, p= 0.0058 respectively) and secretion (p= 0.0156, p=0.0413 respectively) were significantly increased. In comparison with control group no Significant differences were seen in IL-17 and IL-23 expression and secretion as well as Th17 frequency; only RORγt expression was decreased significantly (p= 0.0218).

**Conclusion:** Our study results suggest that treatment with IVIG can be very helpful in successful implantation and pregnancy after IVF by affecting immune regulatory system. So it can be used as a therapeutic option in infertility following IVF.

**Keywords:** Intravenous immunoglobulin, Recurrent implantation failure, Regulatory T cell, T helper 17 cell.

ICIA2018\Reproductive Immunology\Oral\7512
**7512**

## The frequency and function of Follicular T helper in preeclampsia

Haniehheydarlou[1,2], Majid Ahmadi [1,2], Mehdi Yousefi[1,2]

1. *Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Pre-eclampsia is a relatively common multisystem disorder of human, affecting about 3% of pregnancies. It develops in several stages only the last being the clinical illness and causes different maternal and fetal problems. Studies have revealed that immunological changes in the placental microenvironment may have an important role in the initiation of preeclampsia. Whereas, another study show that there is a relationship between TFH and its cytokine (IL-21) in immunological infertility so it may happen through the generation of some antibodies.

**Methods:** 25 preeclampsia women and their control group were assessed at the Alzahra Hospital of Tabriz University of Medical Sciences, Tabriz, Iran. PBMCs were separated from the blood and then RNA was extracted. Afterwards, the frequency of TFH cells was evaluated by flow cytometry and using fluorescent antibodies against CD4, CXCR5 and ICOS markers. The expression of CXCR5 and Bcl6 genes were measured. The TFH related cytokines including IL-6 and IL-21 were assayed by ELISA technology.

**Results:** TFH cells with the CD4+ CXCR5+ ICOS+ phenotypes showed a significant increase in preeclamptic patients compared to normal pregnant women. The expression of CXCR5 and BCL6 were increased. In addition, IL-6 and IL-21 secreted from TFH cells up regulated in patients group. Correlation analysis showed a strong relationship between the TFH CD4+ CXCR5+ ICOS+ and pathogenesis of preeclampsia.

**Conclusions:** These data indicate that CD4+ CXCR5+ BCL6+ TFH cells may participate in the pathogenesis of preeclampsia.

**Keyword:** cytokine, Follicular T helper, Preeclampsia.

ICIA2018\Reproductive Immunology\Oral\7846
**7846**

# Difference in the seminal plasma protein expression in unexplained infertile men with successful and unsuccessful in vitro fertilization outcome

Zahra Kanannejad[1], Behrouz Gharesi Fard[1,2]

1. *Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Unexplained male infertility (UMI) is a condition in which routine semen analysis fails to detect subcellular sperm dysfunctions. In the present research, a comparative proteomics study of seminal plasma (SP) was conducted in men with unexplained infertility whose female partners undergone in vitro fertilization (IVF) treatment to find differences in the SP protein profile.

**Methods:** Five UMI men with successful and eight with unsuccessful IVF outcome enrolled in this study. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) technique was used for protein separation. The differentially expressed proteins were identified using Mass Spectrometry technique.

**Results and conclusion:** indicated that at least three different protein spots, including clusterin, epididymal secretory protein E1, and prostate specific antigen, were differentially expressed in the successful group as compared with the unsuccessful couple (more than 1.5 fold change, $P<0.05$). Considering the role of all three identified proteins in the sperm quality, the results of the present study introduced these proteins as new biomarkers for success of IVF in UMI couples.

**Keywords:** Unexplained male infertility, in vitro fertilization, seminal plasma, Proteomics.

ICIA2018\Reproductive Immunology\Oral\8103
**8103**

# Decidual microenvironments from resorbed and non-resorbed fetuses show divers effects on dendritic cells maturation state and function

Eskandarian Maryam, Yaftiyan Atefeh, Jahangiri Amir Hossein, Moazzeni Seyyed Mohammad

*Immunology Department, Faculty of Medical science, Tarbiat Modares University, Tehran, Iran*

**Background:** Dendritic cells (DCs) constitute a system of antigen presenting cells that are key regulators of immune responses. DCs can acquire immunogenic or tolerogenic properties depending to their microenvironment. In this study we aimed to determine the effects of decidual cells from resorbed and non-resorbed fetuses from abortion prone mice on DC functions.

**Methods:** DCs were differentiated from mouse bone marrow cells in the presence of DC differentiation cytokines, GM-CSF and IL-4. The DCs were then co-cultured with decidual cells from resorbed and non-resorbed fetuses and their immunophenotype and pinocytotic activity were evaluated using the flow cytometric analysis. The obtained DCs were also pulsed with paternal antigens and used for immunization studies. The antigen specific T cell responses were determined in T cells derived from lymph node of immunized animals.

**Result:** Our finding revealed that treatment of dendritic cells with decidual cells from resorbed fetuses significantly increased the MHC-II, CD86 and CD40 expression by DCs compared to DCs treated with decidual cells from non-resorbed fetuses. A remarkable reduction in the endocytic capacity were also observed in iDCs that were co-cultured with decidua of resorbed fetuses. Meanwhile, the ability of DCs to induce lymphocyte proliferation significantly increased following their treatment with decidua of resorbed fetuses.

**Conclusion:** We concluded that in decidua of resorbed fetuses an immune potentiating microenvironment dominancy could be observed which can modulate the DCs phenotype and functions, leading to triggering of an undesirable immune response that is associated with fetal rejection.

**Keyword:** dendritic cells, decidua, microenvironment

ICIA2018\Reproductive Immunology\Oral\8104
**8104**

# The immunosuppressive activity of decidual cells protect the semiallogenic fetus via modulation of dendritic cells functions

Eskandarian Maryam, Yaftiyan Atefeh, Jahangiri Amir Hossein, Mohseni Mahboobeh, Moazzeni Seyyed Mohammad

*Immunology Department, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran*

**Background:** Dendritic cells (DCs) represent a system of antigen presenting cells with a dual property of triggering an immune response or promote cell tolerance, depending on the cellular and molecular context of their microenvironment. This study was done to investigate the immunomodulatory properties of decidual cell from normal pregnancy on DCs.

**Methods:** Immature dendritic cells (iDCs) were prepared from mouse bone marrow progenitors in the presences of IL-4 and GM-CSF cytokines. iDCs were pulsed with paternal antigens. In some cultures of DCs, decidual cells were added during the antigen pulsing process. The immunophenotype and antigen uptake properties of DCs were measured using the flow cytometry. The antigen specific T cell stimulation potency of DCs was also determined using the LTT assay.

**Results:** Our results demonstrated that decidual cells from pregnant mice had inhibitory effect on DCs maturation and function, a significant reduction in the expression of co-stimulatory molecules (MHC-II, CD80 and CD86) and induction of antigen specific T cell proliferative response by DCs were observed. The Ag uptake capability of DCs were remained unchanged after treatment with decidua of the pregnant mice.

**Conclusion:** It seems that decidual microenvironment modulate the function of DCs which result in the induction of immunologic tolerance to the semi allogeneic fetus at the feto–maternal interface.

**Keyword:** BM-derived DC, Decidua, Microenvironment

ICIA2018\Reproductive Immunology\Oral\8159
**8159**

## Menstrual blood-derived stem cells modulate functional features of natural killer cells

Mohammad Reza Shokri[1], Mahmood Bozorgmehr[2,3], Alireza Ghanavatinejad[4], Mehdi Aleahmad[4], Shohreh Nikoo[5], Maryam Tavakoli[2], Somaieh Kazemnejad[6], Reza Falak[1,5], Fazel Shokri[4,7], Amir Hassan Zarnani[2,5]

1. *Department of Immunology, School of medicine, Iran University of Medical Sciences, Tehran, Iran*
2. *Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*
3. *Oncopathology Research Center, Iran University of Medical Sciences, Tehran, Iran*
4. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
5. *Immunology research center (IRC), Iran University of Medical Sciences, Tehran, Iran*
6. *Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*
7. *Department of Hybridoma, Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*

**Background:** Up to now, however, there is a great debate on how functional features of uterine NK cells are regulated during normal pregnancy. We hypothesized that endometrial stem cells could potentially attenuate bona fide cytotoxic features of NK cells.

**Methods:** Menstrual blood stem cells (MenSCs), as surrogates for endometrial stem cells, and Bone marrow mesenchymal stem cells (BMSCs) were isolated and fully characterized. The effects of MenSCs in a co-culture system on various features of NK cells including proliferation, K-562 cytotoxicity, and expression of cytotoxic markers were determined by CFSE, Calcein-AM and flow cytometry, respectively. Secretome of MenSCs was determined by growth factor array. IDO activity in MenSCs upon treatment with IFN-γ was determined by a colorimetric method. The involvement of IL-6, IL-10 and, TGF-β in MenSCs-modulated NK cell proliferation was assessed by neutralizing antibodies.

**Results:** MenSCs exhibited multi-lineage differentiation potential and expressed markers associated with mesenchymal and embryonic origins. Contrary to BMSCs, MenSCs induced proliferation of NK cells, while IFN-γ/IL-1β pre-treated MenSCs significantly inhibited NK cell proliferation. Of 42 growth factors tested, BMSCs produced higher levels of IGFBP 1-4, VEGF-A, b-NGF and M-CSF compared to MenSCs. Both MSCs showed a high activity of IDO upon IFN-γ treatment. The Anti-proliferative potential of IFN-γ/IL-1β-pretreated MenSCs was mediated through IL-6 and TGF-β. Similar to BMSCs, MenSCs impaired the cytotoxic activity of NK cells on K-562 cells, consistent with the lower expression of perforin and granzymes A/B. Interestingly, MenSCs were found to be prone to NK-mediated lysis in an MHC-independent manner.

**Conclusion:** Our findings may imply that NK cell dysregulation in such pregnancy-related disorders as infertility and miscarriage may not be considered as an intrinsic defect, rather stemmed from dysfunction of endometrial stromal stem cells.

**Keywords:** Natural killer cells, Endometrial stem cells, Menstrual blood stem cells, Bone marrow mesenchymal stem cells, Mesenchymal stem cells, Cytotoxicity

ICIA2018\Reproductive Immunology\Oral\8195
**8195**

# Pro-inflammatory Milieu Makes Menstrual blood stem cells a Potent inducer of Function Regulatory T cells

Mehdi Aleahmad[1], Mahmood Bozorgmehr[2,3], Shohreh Nikoo[4], Mohammad-Reza Shokri[5], Alireza Ghanavatinejad[1],Maryam Tavakoli[2], Somaieh Kazemnejad[6], Fazel Shokri[1,7], Amir Hassan Zarnani[2,4]

1. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
2. *Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*
3. *Oncopathology Research Center, Iran University of Medical Sciences, Tehran Iran*
4. *Immunology research center (IRC), Iran university of medical sciences, Tehran, Iran*
5. *Department of Immunology, School of medicine, Iran University of Medical Sciences, Tehran, Iran*
6. *Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*
7. *Department of Hybridoma, Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*

**Background:** Regulatory T cells (Treg) are fundamental for successful pregnancy. There are several mechanisms proposed so far for induction of Tregs at the feto-maternal interface. However, the potential role of endometrial stem cells (ESCs) in this context remained to be elucidated.

**Methods:** Menstrual blood stem cells (MenSCs), as surrogate for endometrial stem cells, were isolated and evaluated for their immunophenotypic characteristics and multi-lineage differentiation capacity. Effect of untreated and IFN-γ/IL-1β-pre-treated MenSCs on CD4+CD25+FOXP3+ Treg generation, proliferation of CD3/CD28-activated CD4+ T cells and IL-10 and IFN-γ production was studied by flow cytometry. Capacity of MenSCs to produce functional IDO was assessed by a colorimetric assay. Involvement of IDO, PGE2, IL-6, IL-10 and TGFβ in MenSCs-modulated T cell proliferation and generation of Tregs was investigated by blocking antibodies. Functional capacity of MenSCs-induced Tregs was studied in an allogeneic MLR system. Bone marrow-derived stem cells (BMSCs) were used as positive control in some settings.

**Results:** Unlike BMSCs, MenSCs induced proliferation of T $CD4^{+}$lymphocytes in a dose dependent manner, while pre-treatment with IFN-γ/IL-1β reversed proliferation promoting effect of MenSCs through induction of PGE2. Both mesenchymal stem cell types showed high IDO activity following IFN-γ treatment. Untreated MenSCs inhibited generation of Tregs, while treatment of MenSCs with pro-inflammatory cytokines resulted in a significantly higher numbers of Tregs mainly through IL-6, IL-10 and TGF-β production. Tregs generated in the presence of MenSCs were found functionally active and significantly inhibited MLR response in a dose dependent manner.

**Conclusion:** The results of this study imply that pro-inflammatory microenvironment, which is established upon blastocyst implantation; may trigger endometrial stem cells to induce generation of T cells with a regulatory phenotype and function.

**Keywords**: Menstrual blood stem cells, Tregs, Pregnancy, MLR inhibition

ICIA2018\Reproductive Immunology\Oral\8478
**8478**

# Adipose-derived mesenchymal stem cells could alter cytokine profile of natural killer cells in abortion prone mice

Fatemeh Rezaei, Seyed Mohammad Moazzeni

*Immunology Department, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

**Background:** Cytokine production by NK cells at the feto-maternal interface play a pivotal role in the maintenance of normal pregnancy and the imbalance of cytokine profile is associated with recurrent spontaneous abortion (RSA). MSCs are shown to have immunomodulatory effects on NK cells and their cytokine production. The purpose of this study is to evaluate the impact of MSCs therapy of RSA mouse model on NK cells cytokine profile.

**Methods:** Adipose derived MSCs were injected (IP) at day 4 of gestation to female CBA/J mice following their mating with DBA/2 male. In RSA control group PBS was injected and CBA/J × BALB/c mating was also used as normal pregnancy control. Decidual cells were isolated at gd12.5 and the production of TGF-β, IL-4, IL-10, and IFN-γ by uNK cells were examined using the flow cytometry.

**Results:** Our results showed that the administration of MSCs could alter the cytokine profile of uNK cells in abortion- prone mice. We demonstrated that MSCs therapy caused the downregulation of IFN-γ and upregulation of IL-4 and IL-10 production by uNK cells at the feto-maternal interface and skewed cytokine production toward a Th2 bias. Also MSCs didn't have any effect on TGF-β production.

**Conclusion:** This finding indicated that MSCs could modulate the uterine NK cells cytokines profile from Th1 predominance to Th2 bias and thereby help to maintain tolerogenic microenvironment at the feto-maternal interface.

**Keywords:** Recurrent Spontaneous Abortion, Mesenchymal stem cell, NK cells, cell therapy, cytokine expression.

ICIA2018\Reproductive Immunology\Oral\9964
**9964**

# MSC therapy reduces complement precipitation at the feto-maternal interface of abortion prone mice

Shahab Shahgaldi[1], Amir Salek Farrokhi[1], Seyed Mohammad Moazzeni [1]

*1. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

**Background:** Recurrent spontaneous abortion, which is quite prevalent among pregnant women, is mainly due to immunological factors and disorders. Complement is involved in a wide and diverse variety of immune and non-immune operations, including the development of miscarriages and has emerged as a common event in recurrent pregnancy loss. Mesenchymal stem cells (MSCs), which possess unique in vivo immunosuppressive properties, reduce the abortion rate through modulation of immune responses. In the present study, the quantity of complement C3 deposition is determined at the feto-maternal interface of abortion prone mice (CBA/J mated DBA/2 males).

**Methods:** MSCs were derived from abdominal fat (AT-MSCs) of CBA/J mice. On the 4.5$^{th}$ day of gestation, the test group (CBA/J × DBA/2) received an IP injection of 1 × 10$^{6}$ of AT-MSCs while the control (CBA/J × DBA/2) and normal pregnancy (CBA/J × BALB/c) groups received an IP injection of PBS. On the 13.5$^{th}$ day of gestation, tissue samples (placentae and deciduae of pregnant mice) were analyzed to measure complement C3 deposition using immunohistochemistry.

**Results:** The resorption rate was significantly reduced following MSC therapy as shown in our previous studies. Furthermore, immunohistochemical analysis of complement deposition demonstrated that MSC administration considerably diminished complement C3 deposition at the feto-maternal interface of abortion prone mice.

**Conclusion:** In the present study, we showed for the first time that adoptive transfer of MSCs reduced abortion rate in abortion-prone mice by adjusting the levels of complement C3 deposition. Our results suggested that diminished complement deposition induced by MSCs could contribute to better pregnancy outcomes.

**Keywords:** Mesenchymal stem cell, Recurrent spontaneous abortion, Cell therapy, Complement deposition, Complement C3, Pregnancy.

ICIA2018\Reproductive Immunology\Oral\9976
**9976**

# The regulatory influence of 1,25-Dihydroxy Vitamin $D_3$ on VEGF related angiogenesis in patients with endometriosis

Zahra Moradi[1,2], Sepideh Khodaverdi[3], Mahdi Shekarabi[1], Tahereh Mohammadi[2], Ali Akbar Delbandi[1,2]

*1. Immunology Research Center, Immunology and Infectious Disease Institute, IRAN University of Medical Sciences*
*2. Immunology Department, School of Medicine, IRAN University of Medical Sciences*
*3. Endometriosis Research Center, IRAN University of Medical Sciences*

**Background:** Endometriosis is a chronic inflammatory disease in women, which characterized by the growth of endometrial tissue outside the uterus. Angiogenesis is one of the most important mechanisms in the pathogenesis of endometriosis. The aim of this study was to evaluate the regulatory effects of vitamin $D_3$ on the VEGF expression in ectopic (EESCs) and eutopic (EuESCs) endometrial stromal cells, peritoneal fluid and blood mononuclear cells in patients with endometriosis compared to nonendometriotic controls.

**Methods:** Endometrial ectopic and eutopic stromal cells were isolated by enzymatic digestion from 20 endometriotic patients and 10 non endometriotic controls. Also, the peritoneal fluid and peripheral blood mononuclear cells were separated by ficoll method from 10 patients and 10 controls. The VEGF gene and protein expression levels were measured by Real-Time PCR and ELISA, respectively. In the following, the cells were treated with optimal concentration of $1,25(OH)_2D_3$ in three different times, and the regulatory effects of this vitamin were evaluated in gene and protein expression levels of VEGF in all mentioned cells. Subsequently, the effects of 1, $25(OH)_2D_3$ on proliferation, expression and production of VEGF in HUVEC cells was examined.

**Results:** EESCs showed significantly higher VEGF expression than EuESCs and control endometrial stromal cells ($P<0.0001$). In addition, peritoneal fluid mononuclear cells in patient group produced higher levels of VEGF protein compared to controls ($P<0.01$). Considerably, Vitamin $D_3$ reduced the expression levels of VEGF in EESCs and peritoneal fluid mononuclear cells ($P<0.001$, $P<0.01$, respectively). Also 1, $25(OH)_2D_3$ caused inhibition of proliferation and VEGF expression at the gene and protein level in HUVEC cells in vitro.

**Conclusion:** Our results showed that1, $25(OH)_2D_3$is an appropriate candidate for reduction of angiogenesis in endometriosis by regulating the production of angiogenesis- related inflammatory factors.

**Keywords:** Endometriosis, $1,25(OH)_2D_3$, Stromal cells, VEGF

# Research and Development

# Oral Presentation

ICIA2018\Research and Development\Oral\5320
**5320**

# Apigenin: a flavonoid to manage the expression of P53 gene in gastric and bladder cancer

Fereshteh Abdolmaleki[1], Effat Farzanehnia[2]

*1. Student of Master in medical biotechnology School of Paramedical Sciences, Qazvin University of Medical Sciences*
*2. Student of Master in medical biotechnology School of Paramedical Sciences, Qazvin University of Medical Sciences*

**Background:** This study is aimed to evaluate the effect of Apigenin on the expression of P53 in gastric and bladder cancers (MKN45 and EJ138 cell lines)

**Methods:** The cell lines were cultured in enriched medium with FBS and kept in $Co_2$incubator to proliferate. The cells were treated with different concentration of apigenin (0 to 100µM)) and exposed to MTT solution to determine their cell viability. After detecting the IC50 of Apigenin, the cell was treated with the chosen concentration of Apigenin(0 to 60µM)and their expression in both levels of mRNA and protein were studied through real-time qPCR and Western blotting technique respectively, also MSP technique for DNA methylation and apoptosis assay were done forbetter understanding the action of the gene. All experiments were done triplicate and for three periods of times (24, 48, and 72 h).

**Result:** MTT assay showed that there is no cytotoxic effect on cells up to 30µM of Apigenin, The result of real-time qPCR and western blotting presented that the expression of P53 has been developed after exposing with apigenin dose and time dependently so that in 60 µM of apigenin and after 72 h we had the most expression of P53 in both level of mRNA and protein. Also, the result of MSP approved our hypothesis base on the methylation of P53 promoter and the result of apoptosis assay by Hoechst 33258 kit demonstrated that apigenin could promote the apoptosis of treated cells.

**Conclusion:** In both gastric and bladder cancer (MKN45 and EJ138 cell lines) P53 gene was methylated so its expression has been reduced. Our study displayed that apigenin could increase P53 expression to induce apoptosis and suppression of cancer cell. This flavonoid has not been done in this both cell lines and we hoped that it could be used as a breakthrough in cancer therapy.

**Keywords:** Apigenin, P53 gene, gastric cancer, bladder cancer

ICIA2018\Research and Development\Oral\6310
**6310**

**Investigation and Suggestion of Allergenicity Reduction from Osmotin/thaumatin-like superfamily**

Esmaeil Ebrahimie[1,3,4,5] ,Ali Niazi[1] ,Zarrin Minuchehr[2] ,Bijan Bambai[2] ,Najaf Allahyari Fard[2] ,Nassim rahmani[1]

1. *Institute of Biotechnology, Shiraz University, Shiraz, Iran*
2. *National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran*
3. *School of Medicine, The University of Adelaide, Adelaide, Australia*
4. *Division of Information Technology, Engineering and the Environment, School of Information Technology and Mathematical Sciences, University of South Australia, Adelaide, Australia*
5. *School of Biological Sciences, Faculty of Science and Engineering, Flinders University, Adelaide, Australia*

**Background:** Allergy is a widespread disorder that can lead to a systemic anaphylactic shock. Underlying asthma has been associated with increased risk of severe reactions and even death caused by food allergy. The prevalence and impact of allergic diseases are increasing worldwide. Osmotin/thaumatin-like superfamily is one of the most important plant allergen superfamily that is found in apple, sweet cherry, grape, kiwi and etc. In this research, we tried to suggest the solution for reducing or removing Allergenicity of this great group.

**Methods:** we collected all plant allergens from the allergen databases and prepared Osmotin/thaumatin-like superfamily, and then the pattern was obtained by using PROSITE. In addition, we find all submitted epitopes of Osmotin/thaumatin-like superfamily members by using SDAP and BIOPEP databases. Afterward with comparing the pattern and epitopes sequences; we selected similar amino acids and changed two of them with amino acids belonging to the same families to omit the Allergenicity and provide changing in epitope sequence.

**Results:** K83 amino acid from allergen epitope changed to H83, the only amino acid which could be replaced, and G84 amino acid from allergen epitope changed to D84, E84, S84, T84, and Y84. Before and after mutations, positions of allergen epitopes binding with heavy and light chains of IgE were analyzed and ΔH (the change in enthalpy) for these reactions wascalculated.

**Conclusion:** We determined that replacing of Lys with His and Gly with Thr, reduce the possibility of docking between allergen epitope and IgE, therefore reduction of allergenicity was expected.

**Keywords:** Plant allergen, Allergenicity, Osmotin/thaumatin-like, IgE

ICIA2018\Research and Development\Oral\7462
**7462**

# Effects of extremely low frequency electromagnetic fields with various intensities on immune system of rats

Mahsa Sobhani[1,2], Mohammad Mahdi Eftekharian [1,3],Ghasem Solgi[1,4],Safoora Nikzad[5], Alireza Zamani[1,4]

1. *Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
2. *Student Research Committee, Hamadan University of Medical Sciences, Hamadan, Iran*
3. *Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran*
4. *Molecular Immunology Research Group, Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
5. *Department of Medical Physics, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*

**Background:** Some epidemiologic studies have shown that extremely low frequency electromagnetic fields (ELF-EMF) affect human health as well as immune system cells and organs. Therefore, it will be reasonable to test whether ELF-EMF can change the level of cytokines like interferon-gamma (IFN-γ), interleukin-4 (IL-4) and expression of T-box transcription factor (T-bet) and GATA binding protein 3 (GATA-3) that regulates TH1/TH2 balance. Aim of this study was to evaluate the effects of ELF-EMF with various intensities on immune system function.

**Methods:** The experiments were performed on eighty two-month-old-male wistar (202.5±7.5gram) rats, which were divided to 5 groups and subjected to 50 Hz ELF-EMF with the flux densities of 1, 100, 500, and 2000 μT, 2h/day for two months. The controls were placed in identical chamber without ELF-EMF. In order to activate the immune system, human serum albumin (HSA) was injected by intraperitoneal route to the all groups on days 31, 44, and 58. The relative expression of T-bet and GATA-3 in spleen and thymus were evaluated with RT-qPCR. The serum level of IFN-γ and IL-4 were measured by enzyme-linked immunosorbent assay (ELISA).

**Results:** Under our experimental conditions, there was no significant differences between the mean mass of rats and spleens, whereas, the weights of thymuses were significantly decreased in ELF-EMF exposed group compared with controls ($p<0.05$).Our experiments showed that ELF-EMF significantly decrease the expression of T-bet and GATA-3 in spleen ($p<0.05$), whereas, there were no significant changes on genes expression in thymuses There were significantly decrease in concentrations of IFN-γ and IL-4 before stimulation of the immune system ($p<0.01$, $p<0.001$, respectively), but the serum levels of this cytokines were not significantly changed after stimulation.

**Conclusion:** It can be concluded that ELF-EMF exposure could altered the immune system function in the way suppression of immune system response.

**Keywords:** Cytokine, Extremely Low Frequency Electromagnetic Fields, Spleen, Thymus, T-Lymphocytes.

ICIA2018\Research and Development\Oral\7738
**7738**

# Natural killer cells expansion for adoptive immunotherapy: comparison of two isolation methods, three cytokines, IL-2, IL-15, or IL-18 and impact on NK cytotoxicity

Anahita Najafalizadeh[1], Nafiseh Esmaeil[1], Farzaneh Peighambarzadeh[1], Fariborz Mokarian[1], Abbas Rezaei[1], Mazdak Ganjalikhani Hakemi[1]

1. *Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
2. *Cancer Prevention Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background:** NK cells are progressively considered as medical tools for cancer immunotherapy. The development of applicable methods to generate a large number of functional NK cells is a crucial step to maximize the potential of this approach.

**Methods:** In this article, NK cells were isolated from PBMCs using 2 different methods. One method with MagniSort Human NK cell Enrichment kit, and the second method isolation with antibodies and complement (cytotoxic method). Purified NK cells were activated in vitro by IL-2 and IL-15 for 14 days and we used IL-18 on the day 14 for another 48 hours to increase the cytotoxicity. Finally, the HL-60 (Human promyelocytic leukemia cells) cell line was used as a target to assess NK cytotoxic activity.

**Result:** The purity of NK cells was 86% and 92% by cytotoxic method and MACS method, respectively. NK cells expanded 60-100 folds in day 14. The expanded NK cells were highly cytotoxic (90%) and they were more than 80% viable. Interestingly, the cytotoxic activity of NK cells was decreased in presence of IL-18.

**Conclusion:** Here we present a simple approach that enables the isolation of NK cells without any beads and magnets using mouse complement with anti-CD3 and anti-CD19 and compared it with the routine isolation method, using magnetic cell sorting. This simplified and efficient method for NK cells isolation and activation could be used in future clinical trials.

**Keywords:** Immunotherapy, NK cells, AML, Interleukin 2, Interleukin 15, Interleukin 18

ICIA2018\Research and Development\Oral\8152
**8152**

# High Yield Magnetosome Production by the Magnetospirillum Gryphiswaldense MSR-1 After Optimizing The Oxygen, Iron, and Carbon Sources and Concentrations in Culture Medium

Leila Hatami Giklou Jajan[1], Masoud Ghorbani[2], Seyed Fazlollah Mousavi, Seyed Nezamodin Hosseini, Behzad Ghareyazie, Behrooz Vaziri, Mohsen Abolhassani

*1. Pasteur Institute of Iran*
*2. Pasteur Institute of Iran, Research and Production Complex, Department of Viral Vaccines, Kilometer 25 Autobahn Karaj, Alborz, Iran*

**Background:** Magnetosome production yield of the Magnetospirillum gryphiswaldense strain MSR-1 was significantly increased after optimization of the oxygen, iron, carbon and nitrogen sources and concentrations on current culture medium without the need to the genetic manipulation.
**Results:** The optimum concentration of Sodium-L-lactate (25Mm), NaNO3 (40 Mm), ferric citrate (200 µM) and dissolved oxygen (2-5%) in the culture medium resulted a higher magnetosome yield than the current culture medium. An approximately four times higher magnetosome yield of 186.67 mg/liter was reached in batch cultures after optimization of the growth medium along with a significant increase of bacterial culture. The iron uptake was also increased significantly after optimization of the medium. A more than 3-fold magnetic response of bacteria to the magnetic field was significantly higher than the other test groups. Based on these findings, it was concluded that alteration in growth medium and optimizing the parameters could increase the magnetosome production yield without any gene manipulations within the bacteria genome. This could be of great importance in the production of industrial magnetosome products.
**Conclusion:** This optimization technic cannot only reduce the culture time period but can reduce the cost of production. Refinement of this method will enable the further increase of magnetosome yield.

ICIA2018\Research and Development\Oral\8298
**8298**

# Comparison of the Effects of Multimeric and Natural Soluble CD40L on the Antibody Production from B cell Line

Bahare Shokoohian[1], Fatemeh Yari[1], Mahshoid Mohammadipour[1], Zohreh Sharifi[1]

*1. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran*

**Background:** CD40L is a member of TNF family that plays an important role in innate and adaptive immunity. Since this protein plays its role through clustering the receptors, CD40, and also the natural soluble form of CD40L is not stable, it seems that multimeric form of CD40L can have a stronger effect than the natural trimeric form in activation of B lymphocytes and IgG production.

**Methods:** dodecameric (four-trimeric) form of CD40L was designed in silico by replacing the extra-cellular domain of CD40L with lectin domain of surfactant protein-D (SP-D). The fusion protein (SPD-CD40L) was expressed by HEK293 cell line. SPD-CD40L and natural soluble CD40L from platelet concentrate were purified by affinity chromatography. Specificity and molecular weight of both proteins were determined by ELISA and western-blot. Daudi cells were encountered natural and dodecameric CD40L, amounts of produced IgG were investigated by sandwich ELISA. The data were analyzed by the statistical tests of ANOVA, Tukey HSD and Wilcoxon.

**Results:** The specificity of the recombinant CD40L was confirmed. IgG production from Daudi cells increased due to exposure with both natural and recombinant CD40L. This increase was higher in exposure to recombinant CD40L than the natural protein and the difference in IgG increase after exposure to natural and recombinant CD40L was considered statistically significant.

**Conclusion:** recombinant dodecameric soluble CD40L protein (SPD-CD40L) can mimic the orientation of membrane-bound CD40L and thus create a stronger message than the natural soluble trimeric molecule in B-lymphocytes because of the spatial orientation of the molecule.

**Keywords:** CD40L, surfactant protein-D, IgG, B lymphocyte cell line

ICIA2018\Research and Development\Oral\9856
**9856**

**Development of a novels TRAIL-conjugate with improved half-life**

Reza Falak[1,2], SepidehMohseni[1], Mohammad DavoodzadehGholami[1], Elaheh Safari [1,2], Gholamalikardar[3]

1. *Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran*
2. *Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*
3. *Asthma, Allergy and Immunology Research Center, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** TRAIL is a potent inducer of cell death and may selectively eradicate a wide variety of human cancer cells without damaging normal ones. However, recombinant TRAIL has short half-life, necessitating its repetitive injection for maintenance of effective therapeutic concentrations, thus limiting its clinical applicability.
**Methods:** In this study, we designed a TRAIL fused with an albumin binding peptide (ABP) and evaluated its serum half-life. TRAIL and ABP-TRAIL were cloned in pET28a (+) and transformed into bacterial host. To compare the serum half-lives of the TRAIL and ABP-TRAIL in blood circulation, samples were collected after administration of a single dose in Balb/c mice and TRAIL was measured at periodic intervals by ELISA.
**Results:** ABP-TRAIL not only retained similar bioactivity as TRAIL in vitro, it also conferred 3.3 fold prolonged half-life in mice.
**Conclusion:** It seems that ABP-TRAIL is a suitable substitute for TRAIL, of course, if it can pass trial steps.

**Keywords:** ABP, ABP-TRAIL, Albumin, Half-life, TRAIL.

ICIA2018\Research and Development\Oral\9890
**9890**

# Improvement of CM11 a Antimicrobial Peptide Using D-diastereomer Amino Acid Substitution

Somayyeh Ghadimi, Kazem Ahmadi, Mahdi Fasihi-ramandi

*Molecular biology research center, system biology and poisonings institute, Baqiyatallah University of medical sciences*

**Background:** In order to improve the antibacterial activity of CM11, a cecropinA-melittin hybrid, we have substituted some of L-amino acids with their D-counterparts in the CM11 sequence.

**Methods:** Here structure – activity relationship studies of these anti-microbial peptides (AMPs), by means of in silico analyses of AMPs structures as well as in vitro evaluation of hemolytic and cytotoxicity assays, antimicrobial activity and cell selectivity were performed.

**Results:** Our results showed that different structural characters of L- and D-CM11 affected its biological behavior and reduced the hemolysis and cytotoxicity of D-CM11 in compare with L-CM11. Furthermore, D-CM11 had an improved antimicrobial activity and therapeutic index (TI) towards some of the gram-negative pathogenic bacteria such as S. sonnei, E .coli, S. marcescens, A. baumannii, P. vulgaris. P. aeruginosa, B. melitensis, B. abortus, E. cloacae that isolated from clinical samples in microdilution assays. These observations indicate that the antimicrobial activity of this amphipathic peptide (D-CM11) was not chiral specific.

**Conclusion:** Besides increasing our knowledge on the structure-function of CM11 and its D-enantiomer analogue, this study revealed that D amino acid substitution could preserve strong anti-bacterial activity of D-CM11 mostly due to direct peptide–membranes electrostatic interactions, without giving a toxic effect towards eukaryotic cells.

**Keywords:** Antimicrobial peptide, CM11, Cytotoxicity, D-amino acid, Diastereomer.

ICIA2018\Research and Development\Oral\9929
**9929**

# Production of functional recombinant human GM-CSF (rhGM-CSF) under specific promoter in Escherichia coli

Mahmoud Vahidi[1], Bahram Kazemi[2,3], Mahmood Bozorgmehr[4] , Mojgan Bandehpour[1,2,3]

*1. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Department of Biotechnology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*4. Oncopathology Research Center, Iran University of Medical Sciences,Tehran, Iran*

**Background:** Granulocyte-macrophage colony-stimulating factor (GM-CSF) is an important hematopoietic growth factor that stimulates multipotent progenitor cells and it also stimulates the differentiation of myeloid leukemic cells. Recently, some of the cytokines as other recombinant proteins could be produced using the recombinant DNA technology. Current study is aimed to clone and production of the Human GM-CSF under specific promoter in Escherichia coli and assessed its biological functions.

**Methods:** The hGM-csf gene construct was artificially synthesized; subsequently it was subcloned into the pcDNA3.1 (+) vector in HindIII restriction enzyme sites. Recombinant DNA was transferred and expressed in BL21 cells. rhGM-CSF protein was evaluated by SDS-PAGE and Western blotting. It was purified by Ni-NTA affinity chromatography. The purified protein concentration and also confirmation were determined by ELISA. MTT assay was applied to evaluate the biological activity of rhGM-CSF on the Erythroleukemic cell line proliferation.

**Results:** The hGM-csf gene was successfully cloned and transformed into expression E. coli cells. As a result, a specific band was observed both on the SDS-PAGE and nitrocellulose membrane after Western blotting. The purified protein concentration was equal to 100 pg/ml. MTT assay showed that exposed cells with rhGM-CSF were proliferated in a dose dependent manner.

**Conclusion:** The GM-CSF gene with specific promoter was successfully cloned in the prokaryotic system and transcription was carried out by T7 RNA polymerase. Therefore, mass production of GM-CSF can be a great help in clinical trials and research studies. Additionally, prokaryotic system, which used in current work, is less costly and less time-consuming.

**Keywords:** Human GM-CSF, Cloning, Prokaryotic system, MTT assay

ICIA2018\Research and Development\Oral\11094
**11094**

# Transfection of human beta-globin gene into the AAVS1 locus of K562 cell line using CRISPR/Cas9 technology

Elahe Alikhani, Mahin Nikogoftar, Amir Ali Hamidieh, Mehdi Shamsara

1. *Iran Blood Transfusion Organization , Tehran, Iran*
2. *National Institute of Genetic Engineering and Biotechnology, Tehran, Iran*
3. *Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Beta thalassemia is a kind of hereditary illness, which is due to an impairment in production of globin chain. There is a need to find a suitable treatment for this disease. CRISPR-Cas9 is a new promising genome editing technology having high efficiency in gene correction.

**Method:** In this study, we aimed to insert the human beta-globin gene specifically into the AAVS1 locus using AAVS1 Transgene knock-in vector kit (Origen). At first, the gene was amplified with PCR and cloned into the pTG19-T. Sothen subcloned into the pAAVS1-puro-DNR. For the second part of our study, the K562 cells were cultured. For determining the minimal fatal concentration of puromycin, the cells were exposed to the different dose of the antibiotic. We transfected this cells with both vectors (Pcas-Guide-AAVS1 and the recombinant PAAVS1-Puro-DNR) (Bio-Rad )and PE-GFPas control. The transfected cells were treated with Puromycin for two weeks.For evaluating the insertion; we extract the DNA from cells and confirmed the insertion of the gene by PCR. In the end, we evaluated the expression of inserted segment by RT-PCR.

**Results:** The Beta-globin gene was cloned into the PAAVS1-Puro-DNR.We confirmed our results by both digestion and sequencing. For determining the minimal fatal dose of puromycin, we exposed the cells with the different dose of antibiotics. The appropriate was 1 µg/mL. We transfected the cells with different protocols of electroporation. For evaluating the insertion, we amplified 700 bp segment with PCR from transfected cells. We confirmed the expression of inserted segment into the cells by amplified 200 bp with RT-PCR.

**Conclusion:** Our results showed that K562 is appropriate for studies of blood disease especially beta thalassemia because they are derived from blood cells and do not express the beta-globin gene naturally. One of the biggest problems of these cells is transfection and manipulating of them because of being suspended.

**Keywords:** Beta thalassemia, CRISPR-Cas9, K562 cell

# Stem Cells Based Immunotherapy

# Oral Presentation

ICIA2018\Stem Cells Based Immunotherapy\Oral\7727
**7727**

# Effects of Adipose-derived mesenchymal stem cells injection on immune responses to Leishmania major in BALB/c mice

Elham Zangane[1], Sara Soudi[1], Ahmad Zavaran Hosseini[1]

*1. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

**Background:** Mesenchymal stem cells have immunomodulatory property. MSCs response to infection through migration to infection site and modulation of immune cells that terminate to augmentation or suppression of inflammation. Recently MSCs are used in cell therapy of infection disease. In the correct study, we considered AD-MSCs immunomodulatory effect on L.major infection in BALB/c mice.

**Methods:** BALB/c mice were footpad injected by L.major, one group was received AD-MSCs at different interval post infection intravenously (i.v), second group received DMEM in i.v routs. Three time post challenge three mice of each group were sacrificed, spleen and lymph node cells were under secondary PHA/LPS and no-Antigen stimulation for IFN-γ, IL-4, TNF-α, IL-10 and nitric oxide (NO) measurement. Spleen parasite burden were determined by limiting dilution.

***Results:*** Intravenous injection of AD-MSCs to L.major infected BAB/c mice have stimulated increase in IFN-γ/IL-4 and TNF-α/IL-10 ratio in spleen and lymph node of infected mice at all study times. In addition, more NO production were detected in splenocyte of this group, and the significant less spleen parasite load were observed. As expected non-MSCs treated mice were demonstrated lower IFN-γ/IL-4 and TNF-α/IL-10 ratio and higher spleen parasite burden.

**Conclusion:** Immunomodulatory properties of AD-MSCs can direct immune response to the benefit of IFN-γ/IL-4 increase and better control of parasite burden in lymph node tissues against L.major infection. Although increase in TNF-α/IL-10 ratio and NO production did not happened at protection level, the results showed the AD-MSCs power in their regulation, finally it seems that AD-MSCs do not have complete protective proportion alone against Leishmaniasis and needs some alterations considered on a candidate in immunotherapy methods.

**Keyword:** Mesenchymal stem cells, Leishmania major, BALB/c

ICIA2018\Stem Cells Based Immunotherapy\Oral\7808
**7808**

# Entosis –like phenomenon and abrogation of MHC class I molecules (H-2D$^b$) in mouse primary bone marrow mesenchymal stem cells by CRISPR/Cas9 approach

AmrollahMostafazadeh[1,2*], BehnamKalali[2], Karin Mink[2], Martin Skerhut[2], Ramona Nitiu[2], RaphaelaSemper[2], Ahmed Sedegh[2],ThureAdler[3], Markus Gerhard[2]

1. *Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran*
2. *Institute for Medical Microbiology, Immunology and Heigen, Munich, Germany*
3. *Institute for Stem Cell Research, Helmholtz Center Munich, Germany*

**Background:** The glycoproteins encoded by the major histocompatibility complex (MHC) are a major barrier in allogenic bone marrow transplantation. In this study, mouse mesenchymal stem cells (MSCs) were used for the first time to examine the application of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9) approach in MHC de-mismatching.

**Methods:** MSCs were isolated from femur bone marrow of C57bl/6 mouse, cultured in RPMI-1640-10%FBS, and characterized by immunophenotyping using anti-stem cell antigen – I (Sca-I) and -CD45 antibody, and then transfected with px330 plasmids harbouring the Cas9- and H-2D$^b$specific guide RNA- encoding genes or with empty px330 plasmids or remained as untransfected cells. Then GFP (reporter gene) positive cells were sorted by FACS. After 8 days of incubation at 37$^\circ$C in $CO_2$ incubator, the sorted cells were stained with anti mouse MHC-I (H2D$^b$molecules) FITC conjugated antibody, and then analyzed by flowcytometry.

**Results:** 97.5% of the MSCs transfected with px330 empty plasmid, and 98.2% of untransfected cells were H2D$^b$ antigen positive. This value for px330-H2D$^b$sg RNA plasmids transfected cells was 31.5%. It means that the efficacy of CRISPR/Cas9 technique in abrogation of MHC –I gene expression was 68%. Moreover, during the bone marrow cells culture we provided some morphological evidence indicating that the Sca-I$^+$ CD45$^-$ stem cells can be invaded by several small bone marrow cells (more than 25 cells), implicating anentosis-like phenomenon.

**Conclusion:** CRISPR/Cas9 approach is an efficient tool in MHC abrogation. Independent to a complex cytokines and growth factors and necessity to a smaller panel of antibody for immunephenotyping, MCSs can be considered as an affordable source for MHC engineering study by CRISPR/Cas9 approach. Moreover this study for the first time provided some evidence for invasion of many cells to one mesenchymal stem cell.

**Keywords:** CRISPR/Cas9, mesenchymal stem cell, Major histocompatibility complex, Entosis

ICIA2018\Stem Cells Based Immunotherapy\Oral\8194
**8194**

# The in-vitro Immunomodulatory Effects of Exosomes Derived from Tumor Cell Line or Mesenchymal Stem Cell on the Functions of Splenocytes Cells isolated from a Mouse Model of Experimental Autoimmune Encephalomyelitis

AnwarFathollahi[1], Farshid Yeganeh[1], Mostafa Haji Molla Hoseini[1], Seyed Mahmoud Hashemi[1]

*1. Department of Medical Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Immunomodulation is a shared feature of mesenchymal stem cells (MSCs) and tumors. Exosomes are 40–100nm extracellular vesicles released from cells. Previous studies showed that MSCs and tumors utilize the exosomes to modulate immune responses. This preliminary study was performed to find out that whether exosomes derived from MSCs or 4T1 tumor cell line enable to modulate cytokine productions and gene expressions corresponding to T helper1 (Th1) and Th17, in an Experimental Autoimmune Encephalomyelitis (EAE) mouse model.

**Methods:** The splenocytes isolated from EAE mice re-stimulated with disease-inducing peptide (MOG35-55, 150 µ /ml), in the presence or absence of exosomes from either 4T1 (15 µg/ml) or MSCs (15 µg/ml). Forty-eight hours later, the cells were harvested, and the relative expression levels of master regulators of T cell sub-types, including T-bet, GATA-3, RORγt, and FOXP3 were determined using Real-Time RT-PCR assay. Additionally, the alteration of ELF4 mRNA levels was evaluated. The levels of IL-10, IFN-γ, TGF-β, and IL-17 in the supernatants of splenocytes culture were measured by ELISA 72 hours after the same treatments.

**Results:** The concentrations of IL-17 and IFN-γ in the supernatants of splenocytes culture stimulated with MOG alone, MOG+4T1 exosomes or MOG+MSC were not different (P> 0.05). However, both Exosomes from 4T1 and MSCs increased the levels of RORγt expression (1.633and 1.365fold, respectively). Exosomes treatment enhanced the expression of FOXP3, nonetheless, the production of IL-10 and TGF-β were increased only in splenocytes treated with 4T1 exosome (P=0.02 and P= 0.021 respectively). Comparison between cells that treated with 4T1 or MSCs exosome revealed that 4T1 exosome up-regulate ELF4 expression, (1.621 fold), a transcription factor which prevents Th17 differentiation.

**Conclusion:** MSCs and 4T1 exosomes modulate Th1/Th17 responses through FOXP3 up-regulation, however, 4T1exosomehas superiority for immunomodulation due to enhancement of ELF-4 transcription and increases IL-10 and TGF-β production.

**Keywords:** 4T1 cell line, Exosome, Immunomodulation, Mesenchymal stem cell

ICIA2018\Stem Cells Based Immunotherapy\Oral\8276
**8276**

# Histological study of Stem cell-based therapies to promote angiogenesis in ischemic vessel disease by tissue engineering method

Amani Saeede[1], Shahrooz Rasoul[1], Mortaz Esmaeil[2,3,4], Hobbenaghi Rahim[5], Mohammadi Rahim[6], Khoshfetrat Ali Baradar[7]

*1. Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University,Urmia, Iran*
*2. Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran3. Department of Immunology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*4. Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands*
*5. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*
*6. Department of Surgery and Diagnostic Imaging, Faculty of Veterinary Medicine, Urmia7University,Tabriz, Iran*
*7. Department of Chemical Engineering, Sahand University of Technology, Tabriz, 14 51335-1996, Iran*

**Background:** Stem cell therapy is novel approach to control or treatment of tissue ischemia associated with peripheral arterial disease. Progenitor cells derived from bone marrow or pluripotent stem cells have shown therapeutic value for restoring tissue function by boosting angiogenesis. The most common technique for stem cells is an injection of cells into specific areas. Therapeutic angiogenesis using bioengineered tissues composed of hydrogen scaffold and hematopoietic stem cell like mast cell and mesenchymal cell were adopted to assess their ability to induce vascular network formation in transected femoral artery location and improve functional recovery in ischemic hind limb in rat.

**Methods:** Thirty male white Wistar rats weighing approximately 200-250g were divided into three experimental groups (n = 6), randomly: In ischemia group (Ischemia) the femoral artery and was transected and hind limb of animals became ischemic, in hydrogel such as group (Hydro) in the ischemic animals the transected location was immersed with 50 µL hydrogel solution and in cell transplanted group (Hydro/Mast cells), in the ischemic animals the transected location was immersed with 50 µL Hydrogel mixed with 50µL PBS containing $10\times 10^6$ mast cells combination.

**Results:** Analyses of capillary density and histomorphometrical analysis were performed on day 14 after surgery. Mean number of blood vessels in groups with MC, MSc and mixture of them indicated a significant difference compared to other experimental groups ($P<0.05$). The mean number of medium and large blood vessels in HYDRO/MC group was significantly more than placebo groups ($P<0.05$). Immunohistochemistry indicated more positive immunoreactivity to CD34 protein in Hydro/Mast cells.

**Conclusion:** Our finding show, Infected locally Mast cells and Mesenchymal cells are a Novel approach for therapeutic angiogenesis that may be able to control ischemic vessel disease and provide improved long-term neovascularization in cases of peripheral arterial diseases.

**Key words:** Mast cells, Mesenchymal cell, Histology, Tissue engineering, Angiogenesis, Rat

ICIA2018\Stem Cells Based Immunotherapy\Oral\8440
**8440**

# The potential benefits of activated platelet-rich plasma on neuroprotection of Human Umbilical Cord Blood Mesenchymal Stem Cells in Mouse Model of Experimental Autoimmune Encephalomyelitis

Maryam Kheirandish[1], Ghazaleh Bordbari[2],Sharareh Bordbari[1], Afsaneh Jahangirian[1]

*1. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran*
*2. Dept of Immunology, Faculty of Medical Sciences, Esfahan University*

**Background:** Current clinical research supports the immunomodulatory and neuroprotective effects of administered Mesenchymal Stem Cells (MSCs) in Multiple Sclerosis. The therapeutic efficacy of expanded hUCB-MSCs in aPRP was evaluated in EAE model in comparison with ordinary cultured hUCB-MSCs.

**Methods:** hUCB-MSCs were collected from the umbilical cord blood. MSCs were cultured in DMEM-low glucose with 10% FBS or 10% aPRP. After inducing EAE in mice, the hUCB-MSCs were transplanted and disease progress was evaluated. Then, the mice were sacrificed, and histological analyses of CNS tissue were done.

**Results:** The comparison of weight and disease severity between groups showed that there is significant difference between experimental group which received cultured hUCB-MSCs in medium containing aPRP and other groups ($P < 0.05$).

**Conclusions:** Our findings demonstrated that aPRP supplemented medium not only improves the proliferation but also maintains functional activity of UCBMSCs and it can be a suitable substitution of FBS (Fetal Bovine Serum) in clinical purposes.

**Keywords:** Experimental allergic encephalomyelitis, Activated platelet-rich plasma (aPRP), Human Umbilical Cord Blood-derived Mesenchymal Stem Cells (hUCB-MSCs), Multiple Sclerosis.

ICIA2018\Stem Cells Based Immunotherapy\Oral\8585
**8585**

# Induction of cytotoxic T lymphocyte responses using dendritic cells transfected with total mRNA of cancer stem cells isolated from patients with gastric adenocarcinoma

Mehran Gholamin[1], Vahid Bagheri[2], Mohammad Reza Abbaszadegan[3]

*1. Department of Laboratory Sciences, School of Paramedical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran*
*2. Human Genetic Division, Immunology Research Center, Bu-Ali Research Institute, Mashhad*
*3. Medical Genetics Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** Cancer stem cells (CSCs) are responsible for tumor initiation, invasion, metastasis, relapse, and resistance to anticancer therapies. Therefore, targeting CSCs, especially using immunotherapy is very critical for cancer treatment. The present report is an evaluation of the immunotherapeutic potential of autologous dendritic cells (DCs) pulsed with total mRNA from gastric CSCs (GCSCs) in vitro for vaccination in GC.

**Methods:** Tumor samples were enzymatically dissociated. Cells were cultivated in serum-free media under low-adherent conditions. Sphere-forming cells were evaluated by flow cytometry using CD44 and CD54 antibodies. The mRNA levels of CD44 and its isoforms and stemness-related genes in sphere-forming cells were quantitated by comparative real-time PCR. To assess the tumorigenicity, sphere-forming cells injected into nude mice. Monocytes-derived DCs were transfected with autologous total mRNA from GCSCs. After co-culturing DCs with peripheral blood lymphocytes, expression of INF-γ gene and anti-GCSCs activity were investigated in primed lymphocytes.

**Results:** Primary spheres were observed in cell suspension obtained from four specimens from 36 GC cases. GCSCs expressed surface markers of CD44 and CD54. RT-PCR results indicated that genes of CD44, CD44-v3, -v6 and -v8-10 and stemness factors OCT4, SOX2, SALL4, and Cripto-1 were upregulated in GCSCs. GCSCs were also able to generate tumors in mice. mRNA level of IFN-γ gene increased in stimulated lymphocytes with DCs transfected with total mRNA of GCSCs (6-9 folds). Comparing cytotoxicity assay of primed lymphocytes with antigens of GCSCs isolated from four patients with normal tissue antigens and mock DCs, there was a significant difference in lysis of GCSCs.

**Conclusion:** The sphere-forming ability is beneficial for the proliferation and enrichment of GCSCs. Considering adverse autoimmune events or other side effects, DCs pulsed with total mRNA from sphere-forming cells that lyse GCSCs by primed lymphocytes in vitro may be utilized as a promising therapeutic vaccination in GC patients in future.

**Keywords:** gastric cancer, cancer stem cells, sphere-forming cells, dendritic cells

ICIA2018\Stem Cells Based Immunotherapy\Oral\9781
**9781**

# Assessment of phenotype and function of Tregs in an allograft islets and fat derived mesenchymal stem cells composite transplant for experimental autoimmune type 1 diabetes

Bahare Niknam[1,2], Jamal Mohammadi Ayenehdeh[1,3] , Nader Tajik[1], Seyed Mahmoud Hashemi[4]

1. *Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran*
2. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
3. *Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
4. *Department Medical Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Islet transplantation is one of the most promising therapeutic approaches in Type 1 Diabetes. In order to improve the viability and function of islet transplantation, it has been proposed to associate pancreatic islets with Adipose Tissue-derived Mesenchymal Stem Cells (AT-MSCs).

**Methods:** Allograft islets from BALB/c mice co-embedded with syngenic AT-MSCs from abdominal fat tissue of C57BL/6 in hydrogelic composite and delivered in to the peritoneal cavity of Streptozotocin (STZ) induced diabetic C57BL/6 mice. Five groups consist of Control, Hydrogel alone, Gel+MSC, Gel+Islet and Gel+MSC+Islet, delivered into the peritoneal cavity. 32 days after transplantation, Mononuclear cells from the Mesenteric lymph nodes (MLNs) and spleen were analysis For Treg and intracellular cytokine assey by flowcytometry.

**Results:** Analyses showed that AT-MSCs co-transplanted with allograft significantly increased Treg($P<0.05$) in MLNs but there is no significant variation among the groups in spleen. According to the MFI for IL-10 and TGF-β1 in MLNs analysis, the IL-10 and TGF-β1 significantly increased in Gel+MSC and Gel+MSC+Islet groups, in comparison with the other corresponding groups ($P<0.05$). However, in spleen, TGF-β1 significantly increased in Gel+MSC and Gel+MSC+Islet groups but variation in IL-10 was not significant.

**Conclusion:** these result shows that AT-MSCs Can promote islet survival and function and can induced functional Treg cells. Moreover, local MSCs transplantation also exert immunomodulatory effects on infiltrating and resident immune cells.

**Keywords:** Type 1 Diabetes, Mesenchymal stem cells, Treg

ICIA2018\Stem Cells Based Immunotherapy\Oral\10025
**10025**

# Preliminary Results of a Phase I Trial in Patients with Primary Malignant Gliomas using Transfected Mesenchymal Stem Cell with Herpes Simplex-Thymidine Kinase/Ganciclovir Complex

Saeed Oraee Yazdani, Ali Reza Zali

*Functional Neurosurgery Research Center, Shohada Tajrish Neurosurgical Comprehensive Center of Exellence, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** The management of patients with glioblastoma remains challenging. Despite aggressive therapies, median survival for malignant gliomas is less than 15 months. Mesenchymal stem cells (MSCs) possess tumor-tropic properties and consequently have been used to deliver therapeutic agents for cancer treatment.

**Methods:** In this study, six patients with newly diagnosed malignant glioma received autologous bone marrow MSC transfected by Herpes Simplex-Thymidine Kinase (HSV-tk) atvector particles through tumor bed injection 2 weeks after surgery and after confirmation of the pathology. They received ganciclovir as prodrug 14 days after tumor resection. All patients were treated by chemoradiation through or after completion of medication associated gene therapy. They have been following up every six months after intervention with history taking, filling questionnaire, physical examination and imaging. The first case enrolled in the study 15 months ago.

**Results:** The average time of follow up for patients are 12 months. One patient unfortunately passed away due to deep vein thrombosis and consequent pulmonary emboli that was unrelated to intervention. The rest of patients are living without any evidence of radiological or clinical recurrence. Local or systemic toxicity or increased brain edema did not occur in this study.

**Conclusion:** These data show that the MSC/HSV-tk/GC complex at the dose used in this study is safe. Long- term follow up and comparing with control group are needed to assess the efficacy of this combination for glioblastoma multiform treatment.

# Tolerance and Autoimmunity

# Oral Presentation

ICIA2018\Tolerance and Autoimmunity\Oral\7401
**7401**

# Chitin Micro Particles Induce Regulated $T_H1$ Response via IL-10 /IFN-γ Axis

Fatemeh Mami[1], Farshid Yeganeh[1], Elnaz Farahani[2], Ali Anisian[3], Mostafa Haji MollaHoseini[1]

*1. Department of Immunology, Faculty of Medicine, ShahidBeheshti University of Medical Science, Tehran, Iran*
*2. Department of Biology, Genetics, Cellular and Molecular group, School of Basic Science, Science and Research Branch of Islamic Azad University, Tehran, Iran*
*3. Department of Veternary Pathology, Islamic Azad University, Abhar branch, Abha , Iran*

**Background:** Our previous studies have shown marked protection among chitin microparticle treated *Leishmania* mice via regulated IL-10/IFN-γ response. Since new contrasting studies are reported on the induction of IL-10 and IFN-γ via Chitin Microparticles (CMPs) during immune stimulation, the present study, once more, examined the inconsistent responses to regarding immunologic response of chitin.
**Methods:** To verify whether CMPs could indeed up-regulate IL-10 /IFN-γ axis, spleen cells isolated from the MOG35-55-induced experimental allergic encephalomyelitis mice were cultured in the presence of MOG peptide and/or CMPs. The effects of CMPs on IFN-γ, IL-10, and IL-17 production were measured using ELISA. Moreover, T-bet, GATA-3, and RORγt expressions (real-time PCR) were investigated.
**Results:** MOG alone stimulated the production of IFN-γ but not IL-10. MOG/chitin stimulation resulted in a significant increase of IFN-γ and IL-10 levels. Additionally, the expression of T-bet, but not GATA-3, was increased in the CMP-treated spleen cells.
**Conclusions:** CMP induces GATA-3 independent IL-10 production and promotes T-bet dependent IFN- γ regulation. These results, together with our previous data, may contribute to clarify the adjuvant effect, which has been attributed to CMPs.

**Keywords:** Chitin; EAE; Immunomodulation

ICIA2018\Tolerance and Autoimmunity\Oral\7445
**7445**

# Lymph node stromal cells control B cell response directed against self-antigen

Reza Nadafi[1], Rogier M. Reijmers[1], Martje Erkelens[1], Eelco D Keuning[1], Lisa G M van Baarsen[2,3], Reina E Mebius[1]

*1. Department of Molecular Cell Biology and Immunology, VU Medical Center, Amsterdam, Netherlands*
*2. Amsterdam Rheumatology & immunology Center (ARC), Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands*
*3. Department of Experimental Immunology, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands*

**Background:** Lymph nodes play a critical role in the induction of immune responses by promoting the interaction between antigen-presenting cells and antigen-reactive lymphocytes. Previous studies have reported that this interaction is additionally controlled by lymph node stromal cells (LNSCs). There is much evidence to support the hypothesis that LNSCs can mediate deletional tolerance of autoreactive CD8+ T cells, through presentation of self-antigens in major histocompatibility complex I (MHC class I). However, the properties of LNSCs in controlling autoreactive CD4+ T cells through presentation of self-antigens in MHC class II has only been partially addressed by showing the LNSCs dependent maintenance of Tregulatory cells.Here we further determine whether LNSCs can control T follicular helper cells (Tfh) as they play a critical role in the development of humoral immunity by controlling the formation and cellular reactions that occur in germinal centres B cells.

**Methods:** Here we show that LNSCs control the formation of Tfh cells directed against self-antigen in an antigen dependent manner both in-vitro, by co-culture of LNSCs and CD4 T cells, and in-vivo by lymph node transplantation.

**Results:** In addition, controlling the Tfh cells directed against self-antigen significantly reduced B cells responsefor the same self-antigen. The data provides a mechanism by which LNSCs are central to prevent autoantibody production by B cells through the presentation of self- antigens in MHC-class II.

**Conclusion:** These findings have implications for opportunities to modulate humoral immunity at different stages of autoimmunity.

**Keywords:** Lymph node stromal cells, T follicular helper, T regulator, B cells, Tolerance, Autoimmunity

ICIA2018\Tolerance and Autoimmunity\Oral\7630
7630

# Up-regulation of co-inhibitory genes (PD-1 and Tim-3) by AD-MSCs: implication for immunotherapy of EAE

Adel Mohammadzadeh[1]

*1. Department of Immunology and Genetics, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran.*

**Background:** Experimental autoimmune encephalomyelitis (EAE) is characterized by central nerve system inflammation that causes axonal myelin shite damage by autoreactive T cells particularly Th1 and Th17. Impaired hemostasis of immunesystem is the One of the key characteristics of T cells dysfunctinality in this context.

Adipose derived –Mesenchymal stem cells (AD-MSCs) are unique stem cells, as they showed several immunomodulatory features, which have been representing a promising cell-based therapy for autoimmune diseases. Although several mechanisms are shown to be engaged in the process of Immunomodulatory function by MSCs including production of anti-inflammatory cytokines such as TGF-β, it is notable that the main mechanisms are not elucidated yet.

**Method:** after 72h co-culture of AD-MSCs and PBMC from the spleen of C57BL6 mice, to investigate the mechanisms through which MSCs might modulate T cells, the relative expression of the important co-inhibitory genes i.e.PD-1(Program cell Death-1) and Tim-3(T-cell immunoglobulin and mucin-domain containing-3) were analyzed on peripheral blood mononuclear cells (PBMCs), by using quantitative real time-PCR (qRT-PCR).

**Results and conclusion:** Our results demonstratethat MSCs contribute to T-cell down-regulation in the context of gene modulation of both PD-1(pv= 0.0012) and Tim-3(pv= 0.027) pathways and suggestthat the understanding of the roles and interactions between MSCs and Immune cells is highly relevant for the development of immunomodulatorydrugs and the discovery of biomarkers predictive of therapeuticresponse.

**Keywords:** EAE, PD-1, Tim-3, MSCs

ICIA2018\Tolerance and Autoimmunity\Oral\7799
7799

**One adjuvant with two different functions: Acylated and deacylated quillaja saponin-21 adjuvants have opposite roles when utilized for immunization of C57BL/6 mice model with $MOG_{35-55}$ peptide**

Amir Reza Heidari[1], Maryam Rastin[2], Reza Nosratabadi[3], Mahmoud Mahmoudi[2]

*1. Department of Immunology and Allergy, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*2. Immunology Research Center, Department of Immunology and Allergy, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*3. Department of Immunology, Immunology of Infectious Diseases Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

**Background:** A major of patients with multiple sclerosis (MS) suffer from central neuropathic pain (CNP). Currently, due to interference with motor disability, there are rare experimental autoimmune encephalomyelitis (EAE) models for assessing pain behaviors over the disease course. To address this issue, complete Freund's adjuvant (CFA) was replaced with an acylated triterpene glycoside saponin adjuvant named quillaja saponin-21 (QS-21) in EAE mouse model for CNP development. Deacylated form of QS-21 named QT-0101 has been suggested tohave an immunomodulatory effect. Thus, QT-0101 was also used as vaccine adjuvant to understand if it can modulate immune system against myelin oligodendrocyte glycoprotein ($MOG_{35-55}$) antigen.

**Methods:** Thirty two, 5-7 weeks old female C57BL/6 mice were divided into four groups. Except for negative control (PBS) group, other three groups received $MOG_{35-55}$emulsifiedwith CFA, QS-21 and QT-0101 adjuvants, respectively.Thermal hyperalgesiaas a CNP clinical manifestation throughhot plate test and clinical signs were assessed for two month post immunization (p.i). On day 21 and day 60 p.imice were sacrificed and $TCD4^+$, $TCD8^+$, $IL\text{-}17^+$, $IL\text{-}4^+$ and $CD25^+/FoxP3^+$ cells in total splenocytes population by flow cytometry technique were evaluated.

**Results:** Unlike MOG+QT-0101 group, EAE was established in MOG+QS-21 and MOG+CFA groups as mild relapsing-remitting and monophasic models, respectively. Thermal hyperalgesiadeveloped in the bilateral hindpaws on the onset of clinical symptoms in MOG+CFA and MOG+QS-21 groups and it was maintained until study completionin MOG+QS-21 group. $TCD4^+$, $TCD8^+$ and $IL\text{-}17^+$cells population in MOG+QS-21 and MOG+CFA groups,$IL\text{-}4^+$and $CD25^+/Foxp3^+$ cells population in MOG+QT-0101 group increased significantly (P0.05) compared to PBS group.

**Conclusion:** QS-21 could be a suitable adjuvant inCNP model establishment for future therapeutic researches in MS disease, while QT-0101 seems to havepotential for vaccine adjuvant with immunomodulatory property.

**Keywords:** Multiple sclerosis, Experimental autoimmune encephalomyelitis, Central neuropathic pain, Quillaja saponin-21, QT-0101

ICIA2018\Tolerance and Autoimmunity\Oral\7827
**7827**

# MiR-10a increase expression of RORγt and induce proinflammatory T helper 17 cells

Zohreh Bolandi[1, 2], Hossein Ghanbarian[1, 2], Sara Soudi[3], Seyed Mahmoud Hashemi[4*]

*1. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
*4. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** MicroRNAs (miRNAs) are endogenous, short non-coding RNA molecules (18–24 nucleotides) that regulate target gene expression by inhibiting translation and increasing mRNA degradation. Recently, miRNAs have been revealed to play a major role in T cell activation, proliferation and differentiation, therefore, by promoting the development of inflammatory T cells including the T helper 17, miRNAs can involve in autoimmune disorders. The aim of this study was to investigate the role of miR-10a in the regulation of RORγt expression, the key transcription factor in the generation of Th-17 cells, in mouse splenocytes population.

**Methods:** Splenocytes were isolated from 6-8 weeks old C57Bl/6 female mice by mincing the spleen with a syringe plunger followed by lysis of erythrocytes with 2mL of lysis buffer. Splenocytes were stimulated with anti-CD3/CD28mAbs. Next day, splenocytes were transfected with miR-10a mimic using Lipofectamine 2000 according to the manufacturer's protocol. After 48 hrs, cells were harvested and washed twice with PBS. RNA was extracted and Real-time PCR was performed to quantify the relative expression levels of miR-10a, RORγt, and IL-17 in transfected cells compared to nontransfected control.

**Results:**To overexpress of miR-10a in mousesplenocytes, miR-10a mimic was transfected via Lipofectamine 2000 into the splenocytes. Total RNA was extracted and Real-time PCR assay showed that miR-10a was significantly overexpressed in transfected cells and following the overexpression of the miRNA, the expression of RORγt and IL-17 increased in mouse splenocytes.

**Conclusion:** Here, we indicated that miR-10a could induce RORγt transcription factor expression that have an important role in Th-17 differentiation. It can be concluded that the overexpression of miR-10a leads to T cell differentiation into Th-17 cells and producing the IL-17, an important proinflammatory cytokine that has an important role in the induction of various autoimmune diseases.

**Keywords:** Splenocytes, miR-10a, Th-17, RORγt.

ICIA2018\Tolerance and Autoimmunity\Oral\8236
**8236**

# Induction of Regulatory T cell by Monocyte Derived DC after Differentiation with Tolerogenic Probiotics in SLE Patients

Seyed Alireza Esmaeili[1,2,3], Mahmoud Mahmoudi[1,2], Fatemeh Mardani[1,2,3], Sahar Khorasani[1,2,3], Zohreh Vahidi[1,2,3], Nafiseh Tabasi[1], Mahdieh khazaee[1] , Maryam Rastin[1,2]

1. *Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
2. *Immunology Department, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
3. *Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** Systemic lupus erythematosus (SLE) is a main autoimmune disease that uncontrolled inflammation lead to tissue damage, therefore control of excessive inflammation is necessary. Dendritic cells (DC) are determinative cell to shape direction of regulatory or inflammatory immune response. Inhibitory DC shift naïve T cell toward the Regulatory T cell (Treg) that could decrease inflammation by production of regulatory cytokines, also help to keep immune hemostasis and return of inflammatory T cells population balance. The aim of this study was induction of the regulatory T cell in co-culture with regulatory DC after producing trough tolerogenic probiotics in in-vitro treatment.

**Methods:** Monocytes of SLE patient cultured with GM-CSF and IL-4 cytokines for 5 days, then immature DC cultured with Lactobacillus delbrueckii and lactobacillus rhamnosus along with same concentration of both cytokines for two days in order to maturation of DC. The Phenotypes of generated DC examined by Flowcytometry and Real-time PCR. Naive CD4+ T cell isolated through negative isolation kit. Generated regulatory DC and Naive CD4+ Tcell were cultured in RPMI media for 5 days to induction of Treg cells and finally properties of regulatory T cell evaluated by Flowcytometry and Real-Time PCR methods.

**Results:** We observed the generated regulatory DC showed reduction in expression of the surface co-stimulatory molecules and elevation of anti-inflammatory cytokines and enzyme genes. Following, we could show significant increase of CD4+CD25+CD127- FOXP3+ cell populations and increase of FOXP3+ transcription factor and TGF-β cytokine but not IL10.

**Conclusion:** Our finding revealed that probiotic-induced regulatory DC could induce regulatory T cells and related inhibitory cytokine. Therefore, in vivo production of Treg cells trough regulatory DCs could be beneficial strategy for prevention or treatment of SLE disorders.

**Keywords:**Systemic Lupus Erythematosus (SLE), Probiotics, Dendritic Cell (DC), Lactobacillus, Regulatory T Cells (Treg)

ICIA2018\Tolerance and Autoimmunity\Oral\8264
**8264**

# MicroRNA-92a drives Th1 responses in the experimental autoimmune encephalomyelitis

Nahid Rezaei[1], Farideh Talebi[2], Samira Ghorbani[2], Abbas Rezaei[1], Farshid Noorbakhsh[2*], Mazdak Ganjalikhani Hakemi[1]

1. *Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
2. *Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** IFN-γ–producing Th1 cells are the main players in several autoimmune diseases, including multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE). Despite the fact that, Th1 fate is determined by specific transcription factors, the role of microRNAs is dispensable. MicroRNAs dysregulation have been linked to the progress of a number of autoimmune disease including MS. However, little is known about the role of miR-92a, one of the most upregulated miRNAs in MS, in the development of autoimmunity. Based on the remarkable change in CNS of patients with MS, miR-92a was selected for subsequent experiments to ascertain the possible association between the overexpression of miR-92a and immunopathogenesis of MS.

**Methods:** The expression level of miR-92a was assessed in the CNS tissues and splenocytes from mice with EAE using real-time RT-PCR. Then, the possibility that miR-92a is involved in the Th1 polarization was investigated by flow cytometric analysis. Additionally, the expression level of related targets was explored.

**Results:** Enhanced miR-92a expression in mouse samples with EAE at the peak of disease was accompanied with reduced expression level of TSC1 and DUSP10. Over expression of miR-92a in splenocytes led to more Th1 differentiation than that in cells transfected with negative control.

**Conclusion:** Our data discovered that upregulation of miR-92a by ex vivo transfection leads to elevated Th1 differentiation due to silencing of TSC1 and dusp10. Hence, regulation of miR-92a expression, which in turn modulates Th1 responses, may be a potential new therapeutic target in MS.

**Keywords:** EAE, miR-92a, Th1

ICIA2018\Tolerance and Autoimmunity\Oral\8273
**8273**

# The Effect of Lactobacillus Probiotics on Frequency of Th1 and Th17 Cells in Mouse Model of SLE Induced by Pristane

Fatemeh Mardani[1, 2], Seyed Alireza Esmaeili[1, 2], Sahar Khorasani[1, 2], Fahimeh Lavi[1, 2], Nafiseh Tabasi[3], Mahmoud Mahmoudi[1], Maryam Rastin[1]

*1. Immunology Research Center, BuAli Research Institute, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*2. Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran*
*3. Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** Systemic lupus erythematosus (SLE) is an important autoimmune disease and uncontrolled inflammation engaged multiple organs. Th17 cells are the major inducer of inflammation in SLE and IL-17 cytokine is the main Th17 cell mediator that responsible in recruitment and activation of inflammatory immune cells therefore, suppressing these cells is a major therapeutic strategy in SLE. Tolergenic probiotics are able to modulate the activated immune system and decrease the level of inflammation in SLE and other autoimmune diseases. The aim of this project is evaluation the effects of lactobacillus rhamnosus and delbrueckii on Th17 and their mediators in Pristane induced Balb/C mice.

**Methods:** SLE induced in mice by injection of Pristane to establishment of disease. Animals divided into two groups including pretreatment and treatment. probiotics and prednisolone receiving groups feeds daily for sixth month, then the level of Anti-nuclear (ANA) , Anti – dsDNA and anti RNP antibodies measured by immunofluorescent and ELISA methods in mice sera. After six months the mice killed and Frequency of Th1 and Th17 cells evaluated by flow cytometry method as well as The expression of RORγt and IL-17 measured by real-time PCR. Also INF-γ, IL-17 and IL-10 cytokines measured by ELISA techniques.

**Results:** We observed reduction the level of serum ANA, Anti-dsDNA and Anti-RNP and also delay onset SLE disease along with decrease of mass of lipogranuloma in probiotic and prednisolone receiving groups in bothpretreatment and treatment mice. Treatment with Probiotics and prednisolone decreased the frequency of both Th1 and Th17 cells as well as the amount of INF-γ, IL-17 cytokines and RORγt transcript while no change in the level of IL-10 has seen.

**Conclusion:** The results of current study showed that feeding with lactobacillus rhamnosus and delbrueckii can reduce the frequency of Th1/Th17 cells and suppress production of IFN gamma and IL-17 in mouse model of SLE, and therefor using probiotics may be an alternative way for alleviating the inflammation in SLE and possibly other TH1/Th17 mediated immune disorders.

**Keywords:** SLE, Lactobacillus, Tolerogenic Probiotics, Th1/Th17, Animal Model

ICIA2018\Tolerance and Autoimmunity\Oral\8597
**8597**

# Neat-1 LncRNA regulates inflammation and T cell differentiation in an animal model ofmultiple sclerosis

Farimah Masoumi[1], Samira Ghorbani[1], Farideh Talebi[1], Farshid Noorbakhsh*[1]

*1. Immunology Department, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Despite growing evidence that Long noncoding RNAs (lncRNAs) can widely take part in autoimmune diseases, ourknowledge of Multiple Sclerosis-related lncRNAs remains limited. Herein, we investigated the potential role of Neat-1lncRNA, in the context of autoimmune neuroinflammation.

**Methods:** The expression level of Neat-1 was measured in the CNS tissues from mice with experimental autoimmune encephalomyelitis (EAE). Expression analyses were also performed in mitogen and antigen-stimulated splenocytes, as well as activated macrophages using real-time RT-PCR. To examine the role of Neat-1 in macrophages polarization, Neat-1 siRNA was transfected into primary macrophages followed by M1/M2 macrophage polarization. Then M1/M2 genes expression were evaluated. Also, the role of the Neat-1in T cell differentiation was investigated bytransfection of CD4+ T cells with Neat-1 siRNA, followed by flow cytometric analysis of intracellular cytokines. Moreover, effect of Neat-1 downregulation on T cells proliferation was investigated using CFSE staining.

**Results:** Expression of Neat-1 was significantly increased in the in the spinal cords of EAE mice at days 15 and 25 post disease induction. Splenocytes stimulated with MOG peptide or anti-CD3/anti-CD28 antibodies showed significant upregulationof Neat-1, whereas expression of Neat-1 in stimulated bone marrow-derived macrophages was reduced. Neat-1 down regulation increased proinflammatory gene expression in macrophages and polarization toward M1 phenotype. Also, Neat-1 downregulation in activatedlymphocytes shifted the pattern of T cell differentiation towards Th1 cells. However, T cell proliferation remained unchanged following Neat-1 downregulation.

**Conclusion:** Our data highlight the anti-inflammatory actions of Neat-1 in the context of autoimmune neuroinflammation. Neat-1 influence differentiation ofT cells and activation of macrophages, providing potential therapeutic options forcontrolling inflammation in MS.

**Keywords:** LncRNA, Neuroinflammation, Multiple sclerosis, T celldifferentiation

# Transplantation

# Oral Presentation

ICIA2018\Transplantation\Oral\5252

**5252**

# Down-regulation of inflammatory signaling pathways despite up-regulation of Toll-like receptors; the effects of corticosteroid therapy in brain-dead kidney donors, a double-blind, randomized, controlled trial

Reza Jafari[1], Reza Aflatoonian[2], Reza Falak[1], Gholamreza Pourmand[3], SanazDehghani[3], Mojgan Mortazavi[4], Adeleh Adelipour[3], Abbas Rezaei[5], Nader Tajik [1]

1. *Immunology Research Center (IRC), Iran University of Medical Sciences, Tehran, Iran*
2. *Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran*
3. *Urology Research Center, Tehran University of Medical Sciences, Tehran, Iran*
4. *Isfahan Kidney disease research center, Isfahan University of Medical Sciences*
5. *Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background:** The brain death of a potential organ donor induces a systemic inflammatory response, resulting in inferior organ quality and function. Our study aimed to evaluate the effects of methylprednisolone (MPN) therapy on pattern recognition receptor (PRR) signaling in potential brain-dead (BD) kidney donors.

**Methods:** To evaluate the effects of MPN therapy on PRR signaling in BD kidney donors we performed a prospective randomized treatment-versus-control study. Fifty-one potential kidney donors were randomly divided into three groups: brain-dead donors (BDDs) who received 15mg/kg/d of methylprednisolone (group T1, n=17), BDDs who received 15mg/kg/d of MPN at the time of filling consent for kidney donation and 100mg/2h until kidney harvest (group T2, n=17), and normal donors as controls n=17. Gene expression for Toll-like receptors (TLRs) 1-9 and their signaling pathway molecules including MYD88, TRIF, NF-KB1, IRAK, IRF3, and IRF7, as well as the inflammatory cytokines RANTES, IL-1β, TNF-α, IL-6, CXCL8, IL-18, IFN-α, and IFN-β was determined by PCR array. Due to the crucial role of TLRs 2 and 4 in pattern recognition, surface expression of these molecules was analyzed by flow cytometry. Plasma levels of inflammatory cytokines were measured by immunoassay. Finally, serum creatinine and cystatin C were measured in 100 kidney recipients one week and one, three, and six months after transplant.

**Results:** Polymerase chain reaction (PCR) array gene expression revealed greater expression of TLRs and signaling molecules in group T1 than in the controls. Surface expression of TLRs 2 and 4 were significantly greater in group T2 than in group T1 ($P<0.05$). Plasma concentrations of inflammatory cytokines were significantly greater in group T1 than in controls ($P<0.05$). The recipients that received kidneys from group T1 had significantly higher levels of creatinine and cystatin C than the recipients of kidneys from both group T1 and controls ($P<0.05$).

**Conclusion:** Administration of MPN to BDDs at specified periods until kidney harvest resulted in less systemic inflammation in the BDDs and improved renal function in kidney graft recipients compared with common MPN therapy.

**Keywords:** Innate immunity, Inflammation, Brain-dead donors, Toll-like receptors

ICIA2018\Transplantation\Oral\7396
**7396**

# Increased expression of TGF-β, SMAD3 and miR-21 in renal transplant recipients that developed allograft dysfunction: a cohort study

Farshad Foroughi [1], Pedram Ahmadpoor[2], MortezaHosseinzadeh[3], SanazKeshavarzshahbaz[4,5] , MehriBarabadi[4], Mir Saeed Yekaninejad[6], Mina Ghorbanpour[6], Ehsan Soltaninejad[4] , Farshid noorbakhsh[4], Aliakbar Amirzargar[4,7]

1. *Department of Immunology, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran*
2. *Department of Nephrology, Shahid Labbafinejad Medical Center and Chronic Kidney Disease Research Center, Shahid Beheshti*
3. *University of Medical Sciences, Tehran, Iran*
4. *Department of Immunology, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran*
5. *Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
6. *Department of Immunology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
7. *Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
8. *Molecular immunology research center, Tehran university of medical sciences, Tehran, Iran*

**Background:** Chronic allograft nephropathy is the most important factor in long term survival of kidney transplantation. Leukocyte infiltration into the graft and profibrotic gene expression have pivotal effects on kidney transplantation outcome. The present study sought to determine the expression of sequential TGFβ, SMAD3, miR-21 and miR-29 gene expression in human renal allograft.

**Methods:** Blood samples from 52 consecutive renal transplant patients were evaluated at the time of transplantation and at two times (90 and 180 days) after transplantation to analyze the gene expression of TGFβ, SMAD3, miR-21 and miR-29 by real time PCR. A total of 30 biopsies, including protocol biopsy (n=24) and cause biopsy (n= 6), were investigated according to the Banff criteria.

**Results:** The gene expression of TGF β, SMAD3 and miR-21 were found to be significantly higher in graft dysfunction than well-functioning grafts ($p<0.001$, $p=0.02$, $p<0.001$), while the expression of miR-29was lower significantly in graft dysfunction ($p<0.001$). Receiver Operating Characteristic (ROC) Curve Analysis showed that the calculated AUC was 0.77 and 0.83 at the third month for TGFβ and miR-21 respectively ($P=0.001$, $p<0.001$). Multiple logistic regression analysis showed that an increase in TGF β and miR-21 expression leads to higher risk of graft dysfunction (OR=1.96, OR=4.19).

**Conclusion:** During renal transplantation, TGF β, Smad3 and miR-21 gene expression increased in graft dysfunction subjects. Performing such measurements by using non-invasive techniques that include real-time PCR, a comparison of their results with the pathology findings and the patient's clinical condition may assist with the discovery of appropriate cellular and molecular markers for patients with kidney transplants. These findings may prospectively predict allograft dysfunction and help elucidate the underlying pathogenic mechanisms.

**Keywords:** kidney transplantation, graft dysfunction, miRNA, TGF β

ICIA2018\Transplantation\Oral\7503
**7503**

## Urinary Kidney injury molecule-1 (KIM-1) mRNA analysis in kidney transplant recipients

Sanaz Keshavarz Shahbaz[1,2*], Mehri Barabadi[1], Farshad Foroughi[3], Aliakbar Amirzargar[2,4]

1. *Department of Immunology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
2. *Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
3. *Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran*
4. *Molecular Immunology Research Center, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** The Occurrence of acute rejection episode as the outcome of renal transplantation is so difficult to predict, even with an allograft biopsy. Kidney injury molecule-1 (KIM-1), a transmembrane glycoprotein demonstrates the considerable expression in dedifferentiated cells within damaged regions of the proximal tubule but it does not express in normal renal tissue. Hence, we hypothesized that KIM-1 might serve as biomarkers for predicting early acute rejection after kidney transplantation.

**Methods:** In this study, 30 kidney transplant recipients were studied**.** Urine samplesat3times (1 day, 3 monthsand6months after transplantation) were taken.RNA extraction, cDNA synthesis and mRNA expression of KIM-1geneby RT-PCR were performed.

**Results:** The KIM-1 gene expression in urine samples at three and six months after transplant increased significantly in comparison with stable graft function patients($p = 0.01$).

**Conclusion:** These data suggests that urinary KIM-1 mRNA could be used as promising markers for prediction of early acute kidney allograft rejection.

**Keywords:** KIM-1, Renal Transplant recipient, acute renal allografts Rejection.

ICIA2018\Transplantation\Oral\9857
**9857**

# Natural killer cells exhibit an activated phenotype in peripheral blood mononuclear cells of renal allograft rejection recipients: a preliminary study

Azita Talebzadeh Bonakdar[1], Mohammad Mahdi Mohammadi[1], Afshin Namdar [2], Pedram Ahmadpoor [3], Davood Jafari [2], Arron Munggela Foma [4], Mir Saeed Yekaninejad [5], Ali Akbar Amirzargar [2,4]

1. *Department of Immunology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran*
2. *Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
3. *Chronic kidney disease research center, Labbafinejad Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
4. *Molecular Immunology Research Center, Tehran University of Medical Sciences, Tehran, Iran*
5. *Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** A growing body of evidence has revealed the role of innate immune cells in transplantation; however, the nature of natural killer (NK) cells involvement in rejection is still elusive. The purpose of the current study is to determine the impact of NK cell activities in acute and chronic renal transplant rejection.

**Methods:** This preliminary case-control study comprised 63 participants, of which 19 were patients with kidney allograft rejection (8 patients with acute rejection (AR) and 11 patients with chronic rejection (CR)) and 44 were the control groups, comprising 22 patients who received kidney transplant with well-functioning graft (WFG) and also 22 healthy subjects. In addition to the frequency of NK cells, the intracellular IFN-γ production and surface expression of CD107a as cytotoxic activity were measured using flow cytometry.

**Results:** Our results revealed a significant increase in CD107a expression (P=0.021) in patients with AR in comparison with WFG. Moreover, there was a significant rise in the production of IFN-γ in patients with CR when compared to WFG group (P=0.003). Finally, a decrease in the frequency of NK cells in rejection patients was observed compared with control groups; however, this reduction was not statistically significant.

**Conclusion:** These findings suggest that the increase in NK cell cytotoxicity is correlated with rejection in patients with kidney transplantation and might be consider as a predictive marker in prevalence of graft rejection.

**Keywords:** Kidney transplant rejection, Natural killer cell, CD107a, IFN-γ, Cytotoxicity.

# Vaccine

# Oral Presentation

ICIA2018\Vaccine\Oral\7540
**7540**

# Plant base production of virulence factors from Diarrheagenic Escherichia coli; An insight into its immunological evaluation in animal model

Fatemeh shojaei jeshvaghani[1] , Jafar Amani[2], Mahyat Jafari[1], Ali Hatef Salmanian[1]

*1. Department of Agricultural Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran*
*2. Applied Microbiology Research Center, Baqiyatallah Medical Science University, Tehran, Iran*

**Background:** The most important infectious agents responsible for human enteric disorders include several viruses and bacterial, such as Diarrheagenic Escherichia coli. Among this group, EHEC and ETEC cause the largest number of diarrheal cases. In humans, EHEC infections result in bloody or non-bloody diarrhea, which may be complicated by haemorrhagic colitis and haemolytic uraemic syndrome (HUS). Infection by ETEC is accompanied by a non-inflammatory watery diarrhea. We hypothesized that the chimeric recombinant forms of the antigenic moiety of these bacteria, which delivered via edible route, could reduce colonization of infectious agent.
**Methods:** In our study a synthetic gene composed of cfa, eae, stx and lt (SICL) attached by linkers was constructed and codon optimized for expression in plant host. The chimeric gene was cloned in the binary vector pBI1400 containing fatty acid elongase (FAE) promoter as a seed-specific promoter. The gene was then transferred to canola plants by agrobacterium mediated protocol. Three mice groups were used in the immunization program, one group fed by whole seed of transgenic canola plant, the second immunized with combination of oral and parenteral administration and the last group fed by nontransgenic canola plant as a control. Blood samples were taken from mice after last immunization for analysis of SICL specific IgG and IgA antibodies.
**Results:** The chimeric protein showed considerable percentage of total soluble protein in transgenic canola seed. Our studies demonstrate that oral feeding alone or combination with injection can equally develop SICL specific immune response. No antibody response was detected in the mice that were immunized with nontransgenic plant.
**Conclusion:** We demonstrated that the application of transgenic plants containing recombinant protein could act as an effective tool for the production of a vaccine candidate. Furthermore, the oral delivery of antigen could protect the animal model against bacterial challenge.

**Keywords:** Diarrheagenic Escherichia coli, transgenic plants, vaccine candidate, immune response.

ICIA2018\Vaccine\Oral\7658
**7658**

# Th1-biased immune responses elicited by Plasmodium falciparum thrombospondin related adhesive proteinand poly (I: C) adjuvant in BALB/c mice

Maryam Ameri Torzani[1,2], Akram Abouie Mehrizi[1*], Sedigheh Zakeri[1], Laleh Babaeekhou[2]

1. *Malaria and Vector research Group, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran*
2. *Department of Biology, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran*

**Background:** Plasmodium falciparum thrombospondin related adhesive protein (PfTRAP) is a leading malaria vaccine candidate antigen on sporozoite. However, the recombinant malaria vaccine candidate antigens are poor immunogenic and additional appropriate immune stimulants, such as an efficient adjuvant, are highly needed to enhance protective Th1 type immune responses. In this regard, polyinosinic: polycytidylic acid (poly (I: C)), was considered as the potential adjuvant and the immunogenicity of PfTRAP formulated with this adjuvant was evaluated in BALB/c mice.

**Methods:** The pftrap gene was codon optimized based on the E. coli codon usages and cloned in NdeI-XhoI sites of pET24a plasmid. The recombinant PfTRAP antigen was expressed in E. coli BL21 (DE3) and purified by using Ni-NTA agarose. Three test groups of BALB/c mice were immunized with rPfTRAP antigen alone or in combination with poly (I:C) or CFA/IFA adjuvants. The control groups received PBS1× or corresponding adjuvants without antigen. Immunization was carried out three times, 2 weeks intervals via subcutaneous rout. Anti-PfTRAP IgG and IgG subclasses were measured 10 days after each immunization.

**Results:** rPfTRAP antigen formulated with poly (I: C) elicited high levels of anti-rPfTRAP IgG antibodies comparable to CFA/IFA adjuvant after the second boost. In addition, rPfTRAP formulated with poly (I: C) elicited the higher ratio of IgG2a/IgG1, and IgG2b/IgG1 than the mice immunized with this antigen emulsified in CFA/IFA.

**Conclusion:** The present investigation revealed that the rPfTRAP delivery in poly (I: C) had potential to modulate Th1 immune responses against rPfTRAP antigen.

**Keywords:** Plasmodium falciparum, thrombospondin related adhesive protein, Adjuvant, malaria vaccine

ICIA2018\Vaccine\Oral\7661
**7661**

# Evaluation of immune responses to novel Plasmodium vivax circum sporozoite based vaccine candidates in combination with second generation adjuvants in C57BL/6 mice

Samaneh H. Shabani[1,2], Sedigheh Zakeri[1*], Akram A. Mehrizi[1], Yousef Mortazavi[2], Navid D. Djadid[1]

*1. Pasteur Institute, Iran*
*2. Zanjan University of Medical Sciences, Iran*

**Background:** The majority of malaria vaccine studies have mainly focused on Plasmodium falciparum, but Plasmodium vivax have been ignored. However, P. vivax is able to develop hypnozoites, which increases the morbidity caused by a single infection and sever malaria. Additionally, a vaccine targeting of P. vivax represents a necessary tool when considering the elimination/eradication goal. Many efforts have been conducted to design a vaccine based on circum sporozoite protein (CSP). Because of limited immunogenicity and less effectiveness of protein-based vaccines, these types of vaccines require adjuvant to induce a protective and long-lasting immune response. In this investigation, we evaluate the immunological potency of two newly designed PvCSP based vaccines in combination with a novel adjuvant system (AS).

**Methods:** Both CS127 and CS712 constructs include N- and C-terminal parts and a truncated region containing repeat sequences with different arrangement from both PvCSVK210 and PvCSVK247 subtypes. After expression, purification, desalt and concentration, constructs were formulated with novel AS (NLX, CpG and QS21). 6-8 weeks female C57BL/6 mice were immunized with 3 boosts with 2 week interval. Humoral responses include specific antibodies and subclasses against PvCSP, titration and avidity of antibodies, and cellular responses includes lymphocyte proliferation assay and cytokine profiles were evaluated with ELISA, MTT and cytokine assay, respectively.

**Results:** Our results show that both constructs are highly immunogenic in C57BL/6 mice. Both candidates in combination with AS induce high levels of antibody against PvCSP with high titration and avidity of Th1 related antibodies. Analysis of the induced T cells high-lighted different cytokine profile with significant secretion of IFN-γ and Th1 responses.

**Conclusion:** These results need further clinical investigation of these two candidates in primate models to reach the added value in both immunogenicity and protective efficacy.

**Keywords:** Plasmodium vivax, malaria; adjuvant system, circum sporozoite protein

ICIA2018\Vaccine\Oral\7856
**7856**

# A novel endogenous adjuvant for development of therapeutic vaccine against HIV-1 infection

**Alireza Milani[1], Azam Bolhassani[1]**

*1. Department of Hepatitis and AIDs, Pasteur Institute of Iran, Tehran, Iran*

**Background:** Vaccination is currently considered as an additional therapeutic approach to stimulate HIV-specific immune responses. In order to development of an effective vaccine against HIV-1 infection, novel vaccine strategies are required. Promising results of DNA-based, protein-based and heterologous prime/boost-based strategies showed their influence on eliciting both humoral and cellular immune responses. Recently, the endogenous adjuvants such as heat shock proteins (HSPs) have been suggested effectively to induce antigen-specific humoral and cellular immune responses.

**Methods:** The recombinant HIV-1 Nef, and Nef fused to Hsp27 DNA and also protein constructs were generated in eukaryotic and prokaryotic expression systems, respectively. Mice were immunized with these constructs based on three strategies of DNA/DNA, Protein/protein and DNA/Protein. To determine the induction of immune response, sera and splenocytes were analyzed for humoral and cellular responses, respectively.

**Results:** Analysis of the immune responses indicated that the Hsp27-Nef fusion protein significantly increased the Nef-specific T cell responses. Indeed, this regimen induced high levels of IgG2a and IFN-γ directed toward Th1 responses and also Granzyme B secretion compared to other immunization strategies. Moreover, the immunostimulatory properties of Freund's adjuvant were significantly less than Hsp27 in different immunization strategies.

**Conclusion:** These data demonstrated that the use of Hsp27 in protein-based strategy could improve HIV-1 Nef-specific B- and T-cell immune responses as a promising HIV-1 vaccine candidate in future.

**Keywords:** Therapeutic HIV vaccine, Nef, Endogenous adjuvant, Small heat shock protein 27

ICIA2018\Vaccine\Oral\8042
**8042**

# Characterization and evaluation of DNA plasmids coding for immunogenic salivary proteins of Phlebotomus sergentias vaccine candidates against Leishmania tropica in a BALB/c mice model

Elham Gholami[1,2], Tahereh Taheri[2], Negar seyed[2], Sima Habibzadeh[2], Yasaman Taslimi[2], Mohammad Ali Mazloomi[1], Shaden Kamhawi[3], Jesus G Valenzuela[3], Fabiano Oliveira[3], Sima Rafati[2]

1. *School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran*
2. *Department of Immunotherapy and Leishmania Vaccine Research, Pasteur Institute of Iran, Tehran, Iran (the Islamic Republic of)*
3. *Vector Molecular Biology Section, Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland, United States of America*

**Background:** In the leishmaniasis, phlebotomine (Ph.) sand flies transmit Leishmania to a mammalian host through depositing the parasite in the presence of their saliva in the skin during feeding. Cutaneous Leishmaniasis is caused by L. tropica and L. major in Iran, being mostly transferred to the host with Ph. sergenti and ph. papatasi, respectively. There is no human vaccine for this disease. According to the reports on animal experiments, a pre-exposure to sand fly saliva results in protection against leishmaniasis. Furthermore, it has been reported that Ph. papatasi salivary protein SP15 (PpSP15) provides protection against L. major infection. Here, we have analyzed the immune response to distinct salivary proteins of Ph. sergenti in the BALB/c mice model.

**Methods and Results:** Results of immunization experiments using 14 DNA plasmids coding for Ph. sergenti salivary proteins show that PsSP9 (14.05-kDa) induces protection against L. tropica infection. Forty-eight hours after Ph. sergenti salivary gland homogenate (SGH) injection, PsSP9 immunized mice exhibited a DTH response and a low antibody response against SGHs, and was associated with a high ratio of IFNɣ/IL5 expression levels in the draining Lymph node. These mice exhibited a small ear swelling as well as a low parasite load after L. tropica infection. IFN-ɣ induction after Ph.sergenti SGH exposure could be partially responsible for the provided protection in the mice immunized with PsSP9, via anti-Leishmania immunity priming and/or direct parasite killing.

**Conclusion:** Our work suggests that PsSP9, a member of the PpSP15 family, is a salivary protein that can provide protection against L. tropica infection and it also suggests that this family of proteins in Ph. sergenti, Ph. duboscqi and Ph. papatasi may have similar immunogenic and protective properties against different species of Leishmania. Our data imply the beneficial effect of applying arthropod saliva components in vaccination strategies for diseases caused by vector-borne pathogens.

**Keywords:** Phlebotomus sergenti, Salivary Gland antigen, Leishmania tropica, DTH, vector-borne diseases, vaccine

ICIA2018\Vaccine\Oral\8416
**8416**

# Investigation of the cellular and humoral immune responses to recombinant fragments of filamentous hemagglutinin and pertactin of Bordetella pertussis in BALB/c mice

Peyman Bakhshaei[1], Mohammad Hossein Kazemi[1*], Maryam Golara[1], Sara Abdolmaleki[1], Roya Khosravi Eghbal[1], Jalal Khoshnoodi[1], Mohammad Ali Judaki[1], Vahid Salimi[2], Masoumeh Douraghi[3], Mahmood Jeddi Tehrani[4], Fazel Shokri[1,4]

1. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
2. *Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
3. *Department of Microbiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
4. *Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*

**Background:** The immune response to acellular vaccines has been generally associated with a shift towards the Th2 profile. In the present study the cellular and humoral immune responses of BALB/c mice to recombinant fragments of filamentous hemagglutinin (FHA), pertactin (PRN) and native pertussis-toxin (PT) has been investigated through measuring IFN-γ, IL-4 and IL-17 cytokines and also specific antibody response to antigens following immunization with different formulations of the antigens.

**Methods:** Two and four overlapping recombinant fragments of PRN and FHA, respectively, were produced in E-coli and purified by affinity chromatography, while PT was purified from bacterial suspension of Bordetella pertussis. The purity and concentration of antigens were determined by SDS-PAGE and BCA assays, respectively. BALB/c mice were immunized twice with different combinations of antigens together with CpG or alum as adjuvant. Immunized mice were subsequently aerosol challenged with B. pertussis. Bacterial growth was assessed in Broncho-alveolar lavage (BAL) samples by CFU-assay. The levels of cytokines in supernatants of antigen-stimulated splenocytes and also the antigen-specific antibody titers in serum were measured by ELISA.

**Results:** The levels of IFN-γ, IL-4 and IL-17 cytokines in supernatants of all immunized mice were significantly high. The level of IFN-γ was higher in response to CpG formulated antigens. A substantial specific antibody response was observed to both PRN and FHA antigens in serum of all immunized mice, particularly those immunized with all antigens. No bacterial colony was observed in culture of BALs of vaccinated mice.

**Conclusion:** It seems that all combinations of our recombinant FHA and PRN antigens could effectively induce Th1, Th2 and Th17 immune responses as well as specific antibody response leading to bacterial clearance from lung of immunized mice. Formulation of the antigens with CpG induced substantially higher Th1 response.

**Keywords:** acellular pertussis vaccine, antibody response, cytokine response, filamentous hemagglutinin, pertactin, Bordetella pertussis

ICIA2018\Vaccine\Oral\8582
**8582**

# Generating recombinant Lactococcus lactis expressing SP15 of Phlebotomus papatasi As a live nonpathogenic vaccine

E. Davarpanah[1,2], N. Seyed[2], J. G. Valenzuela[3], S. Rafati[2], R. Safaralizadeh[1], T. Taheri[2]

1. *Department of Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran.*
2. *Department of Immunotherapy and Leishmania Vaccine Research, Pasteur Institute of Iran, Tehran, Iran.*
3. *Vector Molecular Biology Section, LMVR, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland, United States of America*

**Background:** Cutaneous leishmaniasis is a major public health problem that is transmitted by biting of Phlebotomus papatasi (P. papatasi) sandfly. There is no effective drug therapy and vaccination methods against leishmaniasis. Lactococcus lactis (L. lactis) is a nonpathogenic gram-positive bacteria normally used in dairy industry. We used L. lactisas a live host for expression of SP15 protein as a cell wall secretory component from P. papatasis aliva.

**Methods:** Two genes SP15-EGFP and EGFP were synthesized after codon optimization for heterologous gene expression in L. lactis. Both genes were cloned in a specific vector in downstream of signal peptides PrtP that is responsible for expression as cell wall secretory. The protein expression was confirmed by western blotting. Then, different groups of BALB/c mice were immunized with recombinant L. lactis. After challenging of immunized mice with L. major plus Salivary Gland Homogenate (SGH), footpad swelling, cellular and humoral immune response and also nitric oxide were measured. Furthermore, parasite load in lymph nodes (LN) performed using real-time PCR at different time points.

**Results:** DNA fragments were codon optimized based on L. lactis genome that has 35.24% GC content, synthesized and subcloned in pNZ8121 to express secretory SP15 protein on bacterial cell wall. Immunized mice with recombinant L. lactis-SP15+EGFP have shown less swelling in the infected footpad and parasite load in LNs in comparison with immunized mice with wild-type or non vaccinated groups ($P<0.05$).

**Discussion:** Recombinant L. Lactis expressing SP15 could act as potential vaccine candidate in order to combat and control against L. major infection in highly susceptible BALB/c mice.

**Keywords:** Phlebotomus papatasi, SP15, Leishmania major, Lactococcus lactis

ICIA2018\Vaccine\Oral\9998
**9998**

# Vaccination with synthetic peptides from outer membrane protein A increase antibody titers against Acinetobacter baumannii

Kobra Mehdinejadiani[1], Ali Hashemi[2], Mojgan Bandehpour[3], Seyed Amir Jalali[1], Hoda Rahmani[1], Shayesteh Mehdinejadiani[4], Vahedeh Hosseini[3], Nariman Mosaffa[1]

1. *Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences,Tehran,Iran*
2. *Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences,Tehran,Iran*
3. *Department of Biotechnology,School of Advanced Technologies in Medicine,Shahid Beheshti University of Medical Sciences, Tehran,Iran*
4. *Department of Anathomy,School of Medicine, Tehran University of Medical Sciences,Tehran,Iran*

Background: Acinetobacter baumannii is an opportunistic pathogen and known to cause healthcare-associated infections, especially in ICUs of hospitals. New methods to prevent and treat such infections are a critical need. We have previously reported that five peptides of outer membrane protein A are theoretically immunogenic using bioinformatics tools. In the present study, we report which peptides can induce strong immune responses in mice.

**Methods:** Mice were vaccinated with various peptides of OmpA plus aluminum hydroxide (Al (OH) 3) adjuvant. Impact of peptides on induction of antibody production was defined.

**Results:** The present results indicated that Anti-peptide IgG titers related two peptide were higher in comparison with other peptides and groups of control. In fact, immunization with immunogenic peptide vaccine elicits a robust antibody response.

**Conclusion:** As there are no reports on the immunogenic peptides of OmpA, results presented here, will help to select such peptides as vaccine candidate. These results inform continued development of the vaccine based on peptide against A. baumannii, and also are of general importance in that they induce strong immune responses. It may be an effective approach for preventing infection by A. baumanni.

**Keywords:** Acinetobacter baumannii, Vaccine, outer membrane protein A, Peptide

# Veterinary Immunology

# Oral Presentation

ICIA2018\Veterinary Immunology\Oral\7660
**7660**

# Bovine Enterotoxigenic Escherichia coli Antigen 43 as a potential vaccine for calf colibacillosis

Reyhaneh Ghorbanpour[1], Gholamreza Nikbakht Brujeni[1]

*1. Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

**Background:** Enterotoxigenic Escherichia coli (ETEC) is the most common bacterial cause of diarrhea in human and farm animals. In spite of the global importance of ETEC infections, there is no vaccine available for widespread protection. Recent studies are directed toward recognizing genomic conserved antigens of E.coli, including auto transporter proteins. Antigen 43 (Ag43) is an auto transporter protein that causes autoaggregation and biofilm formation, with a low frequency in commensal strains. It is among a few genes that distinguish ETEC pathotypes from nonpathogenic strains, which are also important in development of efficient vaccine.

**Methods:** In the current study, a passenger domain of Ag43 in a bovine ETEC strain was amplified and cloned into a pET-28a (+) plasmid. The recombinant Ag43 was purified via a Ni-NTA agarose column and verified by western blotting. Female BALB/c mice were immunized and their infant mice were challenged by the same strain to evaluate the protective effect.

**Results:** The results implied a significant increase of specific antibody levels due to vaccination of mice by recombinant Ag43 in compared with control group. In addition, the protective effect, by measuring daily percentage of survived/ challenged infant mice, was observed post challenge by $10^2$ cfu of homologous bacteria in compared with control group.

**Conclusion:** According to importance of introduction the novel non classical antigens as vaccine candidates against ETEC in farm animals, here it was indicated that the passenger domain of Ag43 protein can be potentially valuable target in development of bovine colibacillosis vaccine.

**Keywords:** Enterotoxigenic E. coli (ETEC), Autotransporter, Antigen 43, Vaccine

ICIA2018\Veterinary Immunology\Oral\7705
**7705**

# Production of monoclonal antibody (MAb) against p24 protein of bovine leukemia virus (BLV)

Sanaz Zarasvand Nia[1], Masoud Reza Seyfi Abad Shapouri[2], Sepide Salehipour Bavarsad[1], Zohre Najafi[1], Reza Pasandideh[3]

*1. DVM Graduated from Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran*
*2. Professor Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran*
*3. PhD Graduated of Animal Genetics and Breeding, Ramin Agriculture and Natural Resources University of Khuzestan, Mollasani, Iran*

**Background:** Bovine leukemia virus (BLV) is the cause of Enzootic bovine leukosis (EBL), the most common neoplastic disease of cattle. Most of the BLV infected cows do not show any clinical signs and as a carrier, have the potential to transfer the virus to other animals through infected lymphocytes, lifetime. BLV infections cause significant economic losses to the livestock industry and a considerable cost is spent for its eradication and control. Since, there is no vaccine or effective treatment against BLV infections, effective control and eradication could be possible by rapid identification of infected cows and culling or separating them.

**Methods:** Balb/c mice were immunized by the recombinant p24 antigen and the mouse showing the highest titer of anti-p24 antibodies by indirect ELISA was selected for donating spleen for fusion. Spleen cells were fused with SP2/0 myeloma cells using polyethylene glycol. Hybridoma cells were selected in HAT medium and screened by indirect ELISA. The positive clones were subjected to 2 times subcloning. In order to verify the immunogenicity of epitopes against which the monoclonal antibodies have been produced, the possibility for inhibition of monoclonal antibodies reaction by a polyclonal antibody against BLV (the positive control of a commercial ELISA kit) was assessed in a competitive ELISA.

**Results:** Based on our results, a mAb against p24 was produced and reacted succesfully with natural antigens.

**Conclusion:** With regards to the antigenicity of p24, the specific monoclonal antibodies produced against it may be suitable for developing a competitive ELISA.

**Keywords:** Monoclonal antibody, p24 protein, bovine leukemia virus

ICIA2018\Veterinary Immunology\Oral\8213
**8213**

# Determination of CD marker expression profiles of Theileria annulata schizont infected cell lines in different passage numbers using RT-PCR analysis

Gholamreza Habibi[1], Hossein Modirrousta[2] , Asghar Afshari[1]

*1. Department of Parasite vaccine research and production of Razi vaccine and serum research institute, Agriculture Research, Education and Extension Organization (AREEO), Karaj, Iran*
*2. Department of Wildlife research of Razi vaccine and serum research institute, Agriculture Research, Education and Extension Organization (AREEO), Karaj, Iran*

**Background:** Bovine theileriosis caused by Theileria annulata is an important haemoprotozoan vector-borne disease in Iran. The attenuated vaccine cell line was prepared for induction of immunity after 260 passages in cell culture media. Vaccine seed characterization was performed by molecular and biological methods and now we are going to determine the cell markers of Theileriosis vaccine local cell line. However, CD (cluster of differentiation) molecules are cell surface markers which are very useful for the identification and characterization of cells and the different subpopulations of leukocytes. They are usually recognized by specific antibodies by flow cytometry and immunohistochemistry. Here we emphasize on the application of RT-PCR for defining the cell markers of the bovine leukocyte vaccine cell line with high passage number in comparison with a newly prepared low passage cell line.

**Methods:** The vaccine cell line at high passage number and a new prepared cell line in low passage number were grown in cell culture medium and the harvested cells were subjected to RNA isolation and further RT-PCR by specific primers for a panel of bovine CD markers (CD4, 5, 11a, 14, 43, 45 and 79a).

**Results:** The PCR was set up for 7 CDs and was used for the two mentioned above cell lines. The comparison of two sets of CDs expression profiles showed the marker expression for the T cells, dendritic cells, monocytes and macrophage on both examined cell lines but the low passage cell line showed exclusively the CD79a marker for B cells.

**Conclusion:** The present work is the first study for determination of Theileriaannulata schizont infected cell line markers for cell identity test by RT-PCR and showed us the technique might be an alternative assay for flow cytometry.

**Keywords:** Theileria, RT-PCR, CD, cell line.

ICIA2018\Veterinary Immunology\Oral\8364
**8364**

# In vivo and In vitro Evaluation of Calf Lung Surfactant Extract on Pro-Inflammatory and Anti-Inflammatory Cytokine Changes

Zohre Eftekhari[1], Mohammad Reza Mokhber Dezfouli[2], Mahiar Jeloudari Mamaghani[3], Babak Beikzadeh[4]

1. *Department of Quality Control, Research and Production Complex, Pasteur Institute of Iran*
2. *Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran*
3. *Department of Drug Substance Production, Noargen Biopharmatech Company*
4. *Department of microbiology and Immunology, faculty of veterinary medicine, University of Tehran*

**Background:** Inflammatory reactions in the lung pathophysiologic conditions are a critical problem in the treatment process, which in some cases lead to death, particularly in neonate. Exogenous lung surfactant has been considered a candidate to cure inflammation in the lungs. Hence, the efficacy of this substance has been examined for changes in inflammatory and anti-inflammatory cytokine profile.

**Methods:** Calf lung surfactant extract (CLSE) was obtained by water- salt extraction of freshly slaughtered calves' isolated minced. Finally, obtained active pharmaceutical ingredients formulated by adding dipalmitoylphosphatidylcholine and palmitic acid as excipients and suspension was made by adding NaCl. The quality of finish product determined by hydrophilic interaction liquid chromatography coupled to electrospray ionization−tandem mass spectrometry. The New Zealand White Rabbits as appropriate animal model were treated with formulated CLSE, and then blood samples collected and the level and gene expression of IL-10, IL-6, IL-1β, IFN-γ and TGF-β were assessment before and after surfactant treatment for 30 days by ELISA and Real time PCR in isolated peripheral blood mononuclear cell, respectively.

**Results:** The results indicate that IFN-γ and TGF-β increased at 24, 48 and 72 h which were statistically significant compared to baseline. While, IL-6 and IL-1β in also started to decrease over time in response to surfactant which these changes were significant. The results showed that the gene expression of IL-6, IL-10 and IL-1β decreased during the time after exposure to the surfactant. The gene expression of IFN-γ and TGF-β increased due to surfactant therapy which reached its maximum expression after 7 days.

**Conclusion:** This study suggests that CLSE could contribute in reducing pathology effects of respiratory distress syndrome in neonates which can be used as auxiliary and protective drug in respiratory diseases.

**Keywords:** Exogenous surfactant, Inflammatory, Cytokine profile, Calf lung surfactants

ICIA2018\Veterinary Immunology\Oral\8464
**8464**

## Cellular immune responses of vaccinated chickens with fowl pox vaccine

Mohammad Azizi[1], Bahman Khalesi[2], EsmaeilAsli[1,3], Ali ReazaFarahani[4], Ali Mohammad Behroozikhah[5], Abbas Alamian[2]

1. *Department of Microbiology , Faculty of Sciences, Islamic Azad University, Karaj, Iran*
2. *Department of Poultry Vaccines Production, Razi Vaccine and Serum Research Institute, AREEO, Karaj, Iran*
3. *Immunology Research Group, Razi Vaccine and Serum Research Institute, AREEO, Karaj, Iran*
4. *Department of Immunology, Faculty of Medicine, Iran University of Medical sciences, Tehran, Iran*
5. *Department of Brucella Vaccines Production, Razi Vaccine and Serum Research Institute, AREEO, Karaj, Iran*

**Background:** Fowlpox is a disease of chickens with world-wide distribution. The disease hasan economic impact on the poultry industry. This study provides more information that will facilitate poultry industry to a better knowledge of the role of fowl-pox in the poultry immune system. This study was to evaluate the cellular immune responses of vaccinated chickens with fowl pox vaccine.

**Methods:** Three groups of certain specific pathogens free (SPF) chickens were (21day sold, n=40 per group) were used. One group served as then negative control (PBS) and the other two groups were inoculated with either the local FP vaccine or commercial vaccine. Inoculations of all chickens were done by wing-web puncture with a double needle. Blood samples of each group were collected at weeks 1, 2, 3, 4, 5, and 6 after post-vaccination. Peripheral blood mononuclear cells (PBMC) were isolated of each blood sample using Ficoll–Hypaque density gradient centrifugation. The percentages of $CD3^+$, $CD3^+CD4^+$, and $CD3^+CD8^+$T-lymphocytes were analyzed by flowcytometry.

**Results:** The results of this study showed that vaccinated chickens elevated one week later swelling formation ("take") at the site of vaccination. A maximum elevation of "takes" at day 7 post vaccination were observed. The groups of vaccinated chickens had higher T-cell proliferation responses than the control group. The percentages of $CD3^+$,$CD3^+CD4^+$, and $CD3^+CD8^+$T- lymphocytes were increased ($P<0.05$) in vaccinated chickens with commercial and local FP vaccines. There were no significant differences between both groups of vaccines.

**Conclusion:** This study revealed that the protective immunity may be associated with increased cellular immunity, which has been interpreted as enhancing the proliferation of T cells and increasing $CD4^+$to $CD8^+$ratios due to vaccination of the FP vaccine. This suggests that increased the induction of the immune responses by predominantly a Th1-type response.

**Keywords:** Fowlpox vaccine, Flowcytometry, Chickens, CD4/CD8T-Lymphocytes

ICIA2018\Veterinary Immunology\Oral\8644
**8644**

# Production, Purification and evaluation of Specific Immunoglobulin Yagainst Streptococcus iniae and Lactocuccus garvieae to prevent streptococcusis in Rainbow trout

Mojtaba Mirzaei[1], Firouz Ebrahimi[2], Narjes Hasan Fakhrabadi[3]

*1. Doctor of veterinary medicine student, department of pathobiology, school of veterinary medicine, university of Zabol, Zabol, I.R. Iran*
*2. Department of Biology research center, Faculty of Basic Sciences Imam Hosain University, Tehran - I.R. Iran*
*3. Doctor of veterinary medicine student, department of pathobiology, school of veterinary medicine, university of Zabol, Zabol, I.R. Iran*

**Background:** The use of IgY technology has been used to control many microbial infections. Streptococcus iniae and Lactococcus garvieae are two important pathogens in wild and breeding fish, especially salmon, in the world and in Iran, and have caused many economic losses. The purpose of this study was to produce, purify and evaluate IgY antibodies against these bacteria.

**Methods:** immunization of hens with prepared vaccine of these bacteria in different groups performed, IgG against each antigen in hen serums were detected, IgY was purified from the yolk using PEG 6000 and was dialyzed against distilled water. Product was evaluated by SDS PAGE method. IgY was then evaluated by indirect ELISA. To evaluate the effectiveness of antibodies to prevent pathogenicity of bacteria, the challenge test was carried out in rainbow trout in 6 groups. The first group received only bacteria, the second group received bacteria and IgY(IP rout), the third group received IgY for 7 days (PO rout), the fourth group received non-specific IgY and the bacterium (IP). The fifth received only IgY (IP) and no bacterium was prescribed for the sixth group, this test was performed for each bacterium and each group was evaluated in 2 replicates.

**Results:** The results of SDS PAGE showed that the produced product contains IgY, and the results of indirect ELISA confirmed that in a concentration of 38 ng of IgY in each well can detect antigens. The results of challenge showed that mortality rate in group1 was 8.3%, and in the group 3, was 58.3% and in group 4 was 83.3% However, in the positive control group, which received only bacteria, mortality was 83.3%.

**Conclusion:** use of IgY technology can be used to reduce pathogenicity of Streptococcusis in rainbow trout, so it is readily available and at low cost.

**Keywords:** Immunoglobulin Y technology, in direct ELISA, Streptococcusis, rainbow trout

ICIA2018\Veterinary Immunology\Oral\8682
**8682**

# Production of recombinant human polyclonal antibody Fab fragment antivenom specific for Iranian viper Echiscarinatus in Rosetta-g bacterium

MohammadHasanMotedayen[1],GholamrezaNikbakhtBrojeni[2],HoseinZolfagharian[1],MohammadMahdyRanjbar[3]

*1. Department of Immunization and Plasma Production, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran*
*2. Department of Microbiology and Immunology, School of veterinary Medicine, University of Tehran, Tehran, Iran*
*3. Department of virology, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran*

**Background:** Snake bite poisoning is a serious threat in many of tropical and subtropical countries including in Iran. The best acceptable treatment of envenomated humans is the use of antivenoms. However, present horse-derived antivenoms, despite their unique effects on the treatment of snake victims, they were not fully perfect hence, it needs more improvements.
**Methods:** In this study, recombinant human Fab fragment antivenom was produced in Rosetta-g bacteria, using a gene library, which was constructed in the previous study. The product Fab was purified in several steps, desalted and LPS-depleted, using ammonium sulfate solution, dialysis against PBS and Triton X-114 solution, respectively. Subsequently, the product was initially confirmed by the SDS-PAGE test and ELISA assay, respectively. Finally, neutralization potency of the product was investigated in laboratory Syrian mice.
**Results:** Results showed corresponding reduced bands to Fab fragment with the molecular weight of about 28 kDa, at a concentration of 3.1 mg/ml and significant differences of product antivenom with control groups ($P< 0.05$) in ELISA assay. The neutralization potency of present product against E. carinatus venom was found to be about $7LD_{50}$/ml (54.6 µg/ ml) when tested on mice.
**Conclusion:** Based on the results obtained in the present study, it seems that the product Fab fragment antivenom has the ability to neutralize the in vivo biological activity of the Iranian viper E. carinatus snakevenom. However, more studies require reaching a suitable concentration of antivenom fragment.

**Keywords:** Echiscarinatus, Fab fragment, gene library, antivenom, polyclonal

# Allergy and Immunotherapy of Allergic Disease

# Poster Discussion

ICIA2018\Allergy and immunotherapy\Poster Discussion\6331
**6331**

**Use of a Murine Model to Investigate Potential Allergens Associated to *Ailanthus Altissima* Pollen Allergy**

Fateme Mousavi[1], Youcef Shahali[2], Gholamali Kardar[3], Ahmad Majd[4, 5], Farrokh Ghahremaninejad[4], Zahra Pourpak[3].

*1. Space Biology and Environment center, Aerospace Research Institute, Ministry of Science Research and Technology, Tehran, Iran*
*2. Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension organization (AREEO), Karaj, Iran*
*3. Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran*
*4. Department of Plant Sciences, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran*
*5. Department of Biology, Faculty of Biological Sciences, Islamic Azad University, North Tehran Branch, Tehran, Iran*

**Background:** The murine models that have been *widely used in the study of allergy desensitized mice can produce IgE and IgG1 in response to the injection of antigen/adjuvant combination.* A.altissima pollen (AAP) has been recently reported as an emerging aeroallergen in Iran. So far, several AAP candidate allergens have been identified by the screening of allergen-specific IgE in human sera from AAP *sensitized patients. In the present study, a murine model has been used to investigate AAP specific IgE binding proteins by an immunopreoteomics approach.*
**Methods:** Fresh pollens were collected during the 2014 AAP season from male trees planted in an urban green space of Tehran, Iran. The pollen proteins were extracted in phosphate-buffered saline (PBS). Thirty male BALB/c mice were randomly divided into two groups of AP extract sensitized and sham that respectively received AAP PBS extract and a PBS control by intraperitoneal injections at regular intervals. The optimized AAP protein extracts were analyzed using 2D-gel electrophoresis and were subsequently confronted to pooled sera of sensitized mice.
**Results:** Two-D gel electrophoresis of AAP extract allowed the separation of 125 protein spots distributed in a wide range of pI and molecular masses. Two-DE immunoblotting using pooled sera of sensitized mice led to the detection of 14 IgE reactive spots with molecular masses ranging from 12 to 40-42 KDa, including one very acidic, three acidic, one neutral, eight basic and one very basic protein spots.
**Conclusion:** The results do not correlate with our previous analyses using human AAP-sensitized sera. These findings reflect some differences in the sIgE reactivity to allergenic proteins in animal models. Since the sensitization process in mice has not been naturally developed but rather induced trough the multiple injections of high doses of antigen in combination with *adjuvants.*

**Keywords**: Murine model, Allergy, Pollen, *Ailanthus altissima*

ICIA2018\Allergy and immunotherapy\Poster Discussion\7874
**7874**

# Design and Evaluation of Sal k 1 Peptide Based Vaccine and Comparison with Recombinant Allergen and Russian thistle Extract

Saeideh Tabesh[1,2], Fanuel Songwe[2,3], Mohammad Reza Fazlollahi[2], Mir Saeed Yekaninejad[4], Gholam Ali Kardar[2,3*], Seyed Alireza Razavi[1,*]

*1. Department of Immunology, School of Public Health, Tehran University of Medical Sciences*
*2. Immunology Asthma & Allergy Research Institute, Tehran University of Medical Sciences*
*3. Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences-International Campus (IC-TUMS)*
*4. Department of epidemiology and biostatics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** The Salsola kali pollen is one of the most important causes of allergic rhinitis in the desert and semi-desert area. Its main allergen, Sal K 1, is responsible for more than 90% of the sensitivity of the plant. Allergic rhinitis occupies a large part of the daily life of patients, also the economic consequence of this disease has the large burden on the community. Although the immunotherapy is the only treatment that can modulate the immune system, but given the low efficacy of this method, it is necessary to find a more effective and alternative therapeutic way by these molecular and bioinformatics tools.
**Methods:** In this study, Sal K 1-KLH vaccine was prepared on the basis of B-cell epitopes. In the next step, the specific IgG and IgE against this allergen or its recombinant protein, Sal K 1, were measured by ELISA in immunized BALB/c mice sera. Also, the inhibition of IgE by mouse IgG was evaluated using an inhibitory ELISA. The IgE reactivity designed vaccine was evaluated by dot blot test and compared with the recombinant protein and Salsola kali extract. Finally, proliferation of T lymphocyte was evaluated in cell culture of patients' PBMCs by MTT assay.
**Results:** Bioinformatics analysis revealed that peptide based vaccine had different conformational structure than its normal allergens; also had the characteristics of B-lymphocyte epitope. Vaccination with vaccine, produced high levels of protective IgG in mice, which could inhibit the binding of patients IgE to recombinant proteins. The results show designed vaccine, unlike the recombinant protein and extract, did not induce T-cell lymphocytes response also it showed decreased IgE reactivity.
**Conclusion:** It can be concluded that the designed vaccine will be safe for patients and can be considered as an effective therapeutic candidate in specific immunotherapy.

**Keywords:** Allergen, Allergen-specific immunotherapy, B epitope, Sal k 1.

ICIA2018\Allergy and immunotherapy\Poster Discussion\8095
**8095**

# Survey the Effect of Saffron Supplementation on The Antibody Titer to Heat-Shock Protein (HSP)70, hsCRP and Spirometry Test in Patients with Mild and Moderate Persistent Allergic Asthma

Marzie Zilaee[1, 2], Farhad Abolnezhadian[3], Seyed Ahmad Hosseini[*1, 2], Mohammad Ghasemi[4]

*1. Nutrition & Metabolic Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*
*2. Nutrition Department, Faculty of Paramedicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
*3. Clinical Immunology and Allergy, Allergy Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
*4. Immunology Department, Faculty of medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

**Background:** Asthma is a heterogeneous disease, which usually associated with chronic airway inflammation. The anti-heat shock protein (anti-HSP) 70 is a novel risk factor for asthma. The aim of the present study was to survey the effect of saffron supplementation on anti-HSP70, high-sensitivity C-reactive protein (hs-CRP) and spirometry test in patients with allergic asthma.
**Methods:** In our clinical trial study, subjects (N= 76, 30 women and 46 men, 18–65 years old) with mild and moderate allergic asthma were randomized into two groups: a group of patients who received two capsules of saffron (100mg/d) and a control group who received two capsules of placebo for 8 weeks. Anti- HSP70, hs-CRP, eosinophil and basophils and spirometry test were determined in patients before (week 0) and after (week 8) intervention. SPSS software (version 16.0; Inc, Chicago, IL) was used for data analysis.
**Results:** Results showed that saffron in comparison with placebo significantly reduced the hs-CRP (p=0.001), anti-HSP70 (p=0.000), eosinophils (p=0.005) and basophils (p=0.01) concentrations. In spirometry test, FEV1/FVC ratio increased in both saffron and placebo groups (p=0.01). Frequency of the shortness of breath and use of salbutamol spray in saffron group (no in placebo group) decreased significantly (p=0.00).
**Conclusion:** Results of the present study suggested that saffron supplementation in patients with allergic asthma decreased significantly anti-HSPs 70, hs-CRP and inflammatory cells and improved some clinical symptoms of asthma.

**Keywords:** Asthma, Anti-HSP70, Inflammation, Saffron.

ICIA2018\Allergy and immunotherapy\Poster Discussion\8620
**8620**

# Environmental Condition and Allergenic Airborne Pollen Counts of Chenopodiaceae/Amaranthaceae Families in Tehran, IRAN

Maryam Sharif Shoushtari[1,2], Ahmad Majd[3], Mostafa Moin[2,4], Fariborz Zandieh[5], Masoud Movahedi [2,4], Zahra Pourpak[2,4]

1. *Young Researchers and Elites club, Science and Research Branch, Islamic Azad University, Tehran, Iran*
2. *Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran*
3. *Department of Plant Biology, faculty of biology science, Islamic Azad University, North Tehran Branch, Iran*
4. *Department of Immunology and Allergy, Children Medical Center, Tehran University of Medical Sciences, Tehran, Iran 5. Bahrami Children Hospital, Tehran University of Medical Sciences, Department of Immunology, Asthma and Allergy, Tehran, Iran*

**Background:** Pollen grains of Chenopodiaceae/Amaranthaceae family members cause allergic respiratory system diseases in summers especially in areas with arid climates. The objective was to evaluate the association between Chenopodiaceae/Amaranthaceae pollen dynamics in the atmosphere of Tehran and relationships with meteorological factors for eight years.
**Methods:** The districts of Tehran were located in northern, southern, eastern, western and central regions. Airborne pollen data were collected from January 2010 to December 2017 by two air samplers; volumetric methods employing a Burkard grains/$m^3$ and gravitational method, Durham sampler grains/$m^2$, to identify and determine pollen concentration in the air and separated in Chenopodiaceae/Amaranthaceaefamily. Then, relationship between the meteorological parameters including the mean values of temperature, sunshine hours, relative humidity, wind speed and raining was investigated.
**Results:** Chenopodiaceae/Amaranthaceaepollen season lasted in Tehran from mid-May until the mid-November. For the five area of Tehran, consisting of 19 genera types were identified in the eight years. Pollen production per square meter was higher in the center and south and lower in the east areas of Tehran. Highest pollen counts were obtained during Mid-August and lowest in the June. The comparison of Chenopodiaceae/Amaranthaceae pollen counts with a Durham sampler showed the automatic counter a better correlation than Durham sampler but this sampler when pollen dispersal was high and pollen identify is better than automatic counter sampler. The correlations obtained between daily pollen counts and meteorological parameters showed that the airborne presence of this pollen type is associated positively with temperature and sunshine hours and negatively with rainfall and relative humidity.
**Conclusions:** The Chenopodiaceae/Amaranthaceae in weeds pollen calendar and its association with meteorological factors depend mainly on daily temperature, sunshine hours which may help draw the attention of physicians and allergic patients every days with high Chenopodiaceae/Amaranthaceae pollen counts. This is an important relationship to consider whit interpreting individual clinical trials.

**Keywords:** Chenopodiaceae/Amaranthaceae, Pollen counts, Pollen monitoring, Pollinosis, Tehran

ICIA2018\Allergy and immunotherapy\Poster Discussion\8627
**8627**

# CD63 and CD203c Are Strongly Expressed on The Basophils of Patients With Chronic Urticaria and Are Novel Biomarker for Diagnosis of This Disease

Erfan babaei[1], Rasoul Nasiri kalmarzi[2], Ali jalili[2], Javid Morad Abbassi[3]

*1. Department of Immunology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran*
*2. Cancer and Immunology Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran*
*3. Asthma and Allergy Center, Tehran Medical Sciences, Branch of Academic Center for Education, Culture and Research (ACECR), Tehran, Iran*

**Background:** Chronic urticaria (CU) is a skin disorder that considered appearing wheals with itch and pruritus anywhere on the body for more than 6 weeks. Auto reactivity of the CU-patients sera can be established in 30% to 50% of patients, depending on the population, through the autologous serum skin test (ASST). The ASST has only moderate specificity as a marker for functional autoantibodies against the high-affinity immunoglobulin E (IgE)-receptor FcεRIα. There is a need for a strong laboratory test for the diagnosis of CU, which can persistently and objectively support the anamnestic diagnosis. In the current study we aimed to examine the expression levels of both CD63 and CD203c on basophils of patients with CU.
**Methods:** The study included 45 patients diagnosed with CU, according to the EACCI/GA (2) LEN/EDF/WAO guidelines, Peripheral blood of patients (25 ASST+ CU and 20 ASST− CU) and 25 healthy subjects were tested for expression of activation markers including CD63 and CD203c.
**Results:** we observed that the number of basophils expressing CD63 and CD203 was significantly higher (both $p<0.05$) in CU-patients than the normal individuals. In addition, the levels of CD63/CD203+ in patient with positive ASST test were lower than negative ASST test group. However, it was not significant ($p>0.05$).
**Conclusion:** Based on this study the flowcytometric analysis for enumerating basophils expressing CD63 and CD203c is a suitable diagnostic tool for patients with CU. However, further studies enrolling more patients are necessary.

**Keywords:** Chronic Urticaria, CD63, CD203c, ASST

ICIA2018\Allergy and immunotherapy\Poster Discussion\8643
**8643**

# The Modulatory Effects of Estrogen and Progesterone on Expression of Th9-related Transcription Factors and Secretion of IL-9 from PBMCs of Post-Menopausal Asthmatic Patients

Leila Nejatbakhsh Samimi[1], Majid Khoshmirsafa[1], Morteza Fallahpour[2], Seyed Ali Javad Mousavi[3], Rasool Baharloo[1], Paria Jorbozehdar[1], Reza Falak[1]

*1. Department of Immunology, School of medicine, Iran University of Medical Sciences, Tehran, Iran*
*2. Department of Clinical Allergy and Immunology, Hazrate-Rasoole Akram General Hospital, Iran University of Medical Sciences, Tehran, Iran*
*3. Department of Internal Medicine, Hazrate-Rasoole Akram General Hospital, Iran University of Medical Sciences, Tehran, Iran*

**Background:** IL-9-secreting $CD4^+$T cells (Th9), defined as subset of T cells which play a crucial role in development of allergic asthma. The goal of the present study was to determine the effects of 17β-estradiol (E2) and progesterone (P4) in singular or in combination form on expression of IRF-4, PU.1 and BATF as effective transcription factors on differentiation of IL-9 producing cells in mild to moderate asthmatic patients versus non-asthmatic healthy controls.
**Methods:** Peripheral blood mononuclear cells (PBMCs) were isolated and cultivated in the presence or absence of phytohemagglutinin (PHA) in parallel to E2 or P4. In brief, $10^{-8}$ M of E2 or $10^{-6}$ M of P4, equivalent to serum levels observed during hormone replacement therapy (HRT), or a combination of both hormones were added on PBMCs in presence or absence of 1% PHA. Following mRNA isolation and cDNA synthesis, real-time PCR was used to evaluate the expression level of IRF-4, PU.1 and BATF. We also measured the concentration of IL-9 as the related cytokine in cell culture supernatants by ELISA.
**Results:** The median (IQR) IL-9 secretion was 3.2(2.1-5.7) and 1.77(1.3-3) in asthmatic patients and control group for E2 treatment, respectively (Pv=0.0087). In addition, the median (IQR) IL-9 secretion was 2.4(1.6-3) and 1.5(1.1-1.77) in asthmatic patients and control group for E2+P4 treatment, respectively (Pv=0.009). BATF expression in PBMCs was significantly increased in the presence of E2 or the combination of E2+P4 in asthmatic patients compared with the control group. Expression of PU.1 and IRF-4 were also increased; however, they were not statistically significant.
**Conclusion:** Treating PBMCs with estrogen alone or in combination of estrogen and progesterone (as an in vitro example of HRT) could derive differentiation of PBMCs toward IL-9 producing cells which might exacerbate the clinical symptoms of the allergic asthma.

**Keywords:** Allergic asthma, PBMCs, Estradiol, IL-9, Progesterone

ICIA2018\Allergy and immunotherapy\Poster Discussion\9895
**9895**

## Relationship between Migraine and Food Allergy

Safoora pordel[1], Shokrollah Farrokhi[1]

*1. Department of Immunology, Asthma and Allergy, The Persian Gulf Tropical Medicine Research Center, The Persian Gulf Biomedical Research Institute, Bushehr University of Medical Sciences, Bushehr, Iran*

**Background:** The factors triggering the onset of migraine attacks are not completely understood. Foods and food additives could induce migraine attacks. The aim of this study was to evaluate the relationship between food allergy and migraine attacks based on skin prick test (SPT) reactivity.
**Methods:** Fifty-one known patients with migraine disease were enrolled. The extracts of standardized food allergen were used in the skin test. Then, the patients with positive SPT were avoided for food allergen for three months. The quality of life (QOL) of the patients was assessed by using SF-26 questionnaire.
**Results:** A significant difference was observed between positive SPT reactivity to shellfish (P= 0.002) as well as tomato (P= 0.038) and migraine attacks. The most frequent food allergens were peach (45%), strawberry (41.1%) and egg yolk (39.2%), respectively. Moreover, after allergen avoidance, the number of migraine attacks was reduced and QOL was improved for the patients.
**Conclusion:** There was a significant relationship between migraine attacks and food allergy. Therefore, this study suggested that early diagnosis of food allergens and their avoidance may improve QOL of migraine patients.

**Keywords:** Food Allergy, Migraine, Skin prick Test

ICIA2018\Allergy and immunotherapy\Poster Discussion\9898
**9898**

## The Efficacy of Autologous Plasma Therapy in Patients with Chronic Urticaria

Safoora pordel[1], Shokrollah Farrokhi[1]

*1. Department of Immunology, Asthma and Allergy, The Persian Gulf Tropical Medicine Research Center, The Persian Gulf Biomedical Research Institute, Bushehr University of Medical Sciences, Bushehr, Iran*

**Background:** Chronic urticaria is an allergic skin disease characterized by severe pruritic wheal persisting at least for a period of 6 weeks. The aim of this study was to evaluate the efficacy of plasma therapy in treatment of idiopathic chronic urticaria.
**Methods:** In this cross sectional study, the quality of life (QOL) of 30 patients with idiopathic chronic urticaria (autoimmune and spontaneous) was evaluated before and after autologous plasma therapy by using standard questionnaires (DLQI and TSS).
**Results:** The findings showed that the means of DLQI and TSS scores in the patients after plasma therapy was significantly reduced. This indicates an improvement of the QOL in the patients. Moreover, it was found that the plasma therapy was significantly effective in both sexes.
**Conclusion:** The results of the present study suggest that autologous plasma therapy could be a suitable method for the treatment of patients with idiopathic chronic urticaria.

**Keywords:** Chronic urticaria, Plasma therapy, DLQI and TSS scores, Quality of life

ICIA2018\Allergy and immunotherapy\Poster Discussion\10002
**10002**

# Evaluating Immunomodulatory Effects of Vitamin D on IL-17 Expression in Peripheral Blood Mononuclear Cells of Patients with Asthma

Mohsenfarhang zargar[1], Saeedeh Erfanin[2], Maliheh Moradzadeh[3], Bahareh Razeghi Haghighi[1]

*1. Student of laboratory sciences, Student Research committee, Jahrom University of Medical Saciences, Jahrom, Iran*
*2. MSc Department of Advanced Medical Sciences and Technologies, School of Medicine, Jahrom University of Medical Sciences (JUMS), Jahrom, Iran*
*3. PhD Golestan Rheumatology Research Center, Golestan University of Medical Sciences, Gorgan, Iran*

**Background:** Asthma is a form of hypersensitivity type 1 in respiratory airway that many immunity factors are involved in its development and progression. Laboratory evidence indicates that the anti-inflammatory metabolites of vitamin, 25 D-dihydroxy vitamin D3 and similar compounds can be performed by pairing with vitamin D receptors and caused down regulation or translation of cytokines production such as IL2, IL12, TNF -alpha, INF-γ, agent stimulating macrophages, granulocyte colony-giver and IL-1B and IL-6. Therefore, given the importance of cellular immunity in order to increase the pathogenesis of the disease, the aim of present study is a direct effect of vitamin D on cell blood mononuclear mainly T lymphocytes in medium patients with asthma and control groups.
**Methods:** 10 cc heparinized peripheral blood was taken from 20 patients with asthma and 20 healthy controls. The isolated peripheral blood mononuclear cells were treated with $10^{-7}$ and $10^{-6}$ M of vitamin D and dexamethasone, for 24-hour culture. After RNA extracted from 1 million cells, cDNA synthesis and gene expression Real-time PCR was used for IL-17gene.
**Results:** The results of this study indicated the overexpression of IL-17 in patients with asthma compared to the control group, although this difference is not significant (P = 0.061). In this study, vitamin D3 and dexamethasone at different modes to no treatment group of patients with asthma were significantly different (P <0.05) but treatments $10^{-7}$ and$10^{-6}$ of vitamin D3 and dexamethasone did not show a significant difference (P> 0.05). Vitamin D3 and dexamethasone different modes compared to no treatment in healthy subjects, in none of the cases did not show significant differences (P> 0.05).
**Conclusion:** The effect of vitamin D treatment as well as on other routes should also be evaluated TBET gene to an effective step in understanding the molecular mechanisms of diseases in order to achieve the objectives described to be removed.

**Keywords:** IL-17, Vit D, Asthma

# Cancer Immunology

# Poster Discussion

ICIA2018\Cancer Immunology\Poster Discussion\7482
**7482**

# MiRNA-143 Replacement Therapy Harnesses the Proliferation and Migratrion of Colorectal Cancer ells *in vitro*

Leila Karimi[1] , Dariush Shanehbandi[1] , Behzad Mansoori[1] , Ali Mohammadi[1], Milad Asadi[1] , Behzad Baradaran[1]

*1. Immunology Research Center , Tabriz University of Medical Sciences , Tabriz , Iran*

**Background:** Colorectal cancer (CRC) is rated as the third and second most common cancer in men and women. CRC is the fourth most common cause of cancer related mortality worldwide and accounts for ~9% of all cancer cases. Abbarent expression of microRNAs (miRNAs) is correlated with numerous malignancies such as CRC. Restoration of tumor supressor miRNAs seems to be an effectual strategy for treatment of the relating malignancies. MiR-143 is a conspicuous example of tumor supressor miRNAs which its down-regulation has been reported in CRC. This miRNA is involved in negative regulation of metastasis-related genes including KRAS, c-Myc and MMP-9 as well as anti-apoptotic Bcl2.
**Methods:** In the present study, the lowered expression of miR-143 in SW-480 CRC cell line was confirmed by quantitative Real Time PCR (qRT-PCR). Subsequently, miR-143 replacement was performed by introducing the pCMV-miR-143 vector into the model cells. After the restoration of miR-143 expression, changes in proliferation and migration of the cells were assessed by MTT and scratch tests, respectively. Furthermore, relative expression of KRAS, c-Myc, MMP-9 and Bcl2 as putative targets of miR-143 were investigated by qRT-PCR. DAPI staining was utilized to detect apoptosis occurrence in the transfected cells. Moreover, the ratio of Bax to Bcl2 expression, as a potential indicator of the response to apoptosis induction, was compared between the study groups.
**Results:** MTT assay revealed a decrease in the viability of miR-143 transfected cells. In addition, a reduced migratory potential for the transfected cells was indicated by scratch test. DAPI staining confirmed the occurrence of apoptosis in the mentioned cells. According to qRT-PCR results, expression of Bcl2, KRAS, MMP-9 and c-Myc mRNAs was significantly decreased in the miR-143 grafted cells compared to the controls. Furthermore, Bax/Bcl2 ratio showed a significant increase in the miR-143 overexpressing cells.
**Conclusion:** MiR-143 replacement can be an effective strategy for the treatment of colorectal cancer and harnessing its invasive properties.

**Keywords:** miR-143, Colorectal cancer, Metastasis, microRNA replacement therapy, Apoptosis

ICIA2018\Cancer Immunology\Poster Discussion\7549
**7549**

# Association of IL-27 rs153109 and rs17855750 Polymorphisms with Risk and Response to Therapy in Acute Lymphoblastic Leukemia

Alireza Ghavami[1], FarahnazBodaghkhan[2], Saeedreza Vazirian[3], Zahra Amirghofran.[4]

*1. Department of Immunology and Autoimmune Diseases Research Center, Shiraz University of Medical Sciences*
*2. Pathology Lab Section, Shiraz University of Medical Science Namazi Hospital*
*3. Physician of Fars Oil Industry,MD N.I.O.P.D.C*
*4. Professor of Immunology,Autoimmune Disease Research Center, Shiraz University of Medical Sciences*

**Background**: Interleukin-27 is a cytokine with important anti-cancer activity. This study has evaluated the effects of IL-27 rs153109 and rs17855750 single nucleotide polymorphisms (SNPs) on risk of acute lymphoblastic leukemia (ALL) development, as well as their impact on prognosis and patient survival.
**Methods**: A total of 200 patients and 210 healthy subjects were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). There were 40 new cases that had received no chemotherapy at enrollment. We used the samples from these patients for IL-27 serum measurements by enzyme-linked immunosorbent assay (ELISA). The laboratory and clinical characteristics recorded at presentation were sex, age, white blood cell counts, platelet counts, immunophenotype, hemoglobin (Hb) level, percentage of blast in the bone marrow and peripheral blood and extra-medullary involvement (EMI). During the period of follow-up, response to therapy was evaluated by measuring complete remission (CR) rate, CR duration (CRD) and overall survival. Statistical analyses were accomplished using SPSS version 23 for Windows. P-values less than 0.05 were considered statistically significant.
**Results**: We observed a higher frequency of rs153109 AG and rs17855750 TG genotypes and allele G in patients compared to controls ($p<0.001$). Combined G variant genotypes (AG+GG and TG+GG) also conferred significantly greater risk of ALL. There was a significant correlation between the genotypes of both SNPs with event-free survival (EFS). Patients with GG genotypes of both SNPs and those of rs153109 AG and rs17855750 TG had a shorter EFS than patients with rs153109 AA and rs17855750 TT genotypes ($p\leq0.035$). Combined G variant genotypes for both SNPs showed poorer response to therapy in all patients ($p<0.027$) as well as B-ALL (rs153109, $p<0.001$) and T-ALL (rs153109, $p=0.048$) patients. In multivariate analysis, rs153109 combined G variant genotype was associated with shorter EFS (relative risk=9.7, $p=0.026$). Among those who relapsed, 87.1% had the rs153109 AG genotype and 77.4% had the rs17855750 TG genotype ($p<0.01$). Patients had higher IL-27 serum levels compared to controls, but this did not differ between genotypes.
**Conclusions:** The association of IL-27 rs153109 and rs17855750 polymorphisms with risk of ALL development and their impact on EFS suggested an important role for this cytokine in biology and response to ALL therapy.

**Keywords:** Interleukin-27, Acute lymphoblastic leukemia, polymorphism

ICIA2018\Cancer Immunology\Poster Discussion\7571
**7571**

## Blimp-1 Expression as an Exhaustion Transcription Factor in Chronic Lymphocytic Leukemia

Saeid Taghiloo[1], Esmaeil Allahmoradi[1], Mohsen Tehrani[1, 2], Ghasem Janbabaei[3], Ramin Shekarriz[3], Hossein Asgarian Omran[1, 4]*

1. *Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
2. *Molecular and Cell-Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran*
3. *Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran*
4. *Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

**Background:** Based on the tumor immune-surveillance theory, various components of the immune system constantly survey the body for any malignant cell proliferation and eliminate them or slow their growth. In the course of chronic cancer and infections, T cells become “exhausted” with poor effector function characterized by the expression of multiple immune checkpoint inhibitory receptors and transcription factors. Here, we investigated the expression of Blimp-1, a transcription factor involving in T-cell exhaustion, in patients with chronic lymphocytic leukemia (CLL).
**Methods:** Peripheral blood mononuclear cells were collected from 25 untreated CLL patients and 15 sex- and age-matched normal subjects. CLL patients were clinically classified according to the Rai staging system. The relative expression of Blimp-1 mRNA was determined by quantitative Real time Polymerase Chain Reaction (qRT-PCR) after normalization with β-actin.
**Results:** Expression of Blimp-1 mRNA was much higher in CLL patients than in normal controls ($p=0.001$). Moreover, Blimp-1 was higher expressed in patients with advanced clinical stages of CLL compared to those with early stages of the disease ($p=0.01$). Interestingly, the Blimp-1 expression was correlated with the frequencies of exhausted Tim-$3^+$/PD-$1^+$/$CD4^+$ and Tim-$3^+$/PD-$1^+$/$CD8^+$ T cells in CLL patients.
**Conclusions:** Our study introduces Blimp-1 as a potential transcription regulator of T cell exhaustion in CLL patients. Better understanding of underlying mechanisms of T cell exhaustion in CLL could be helpful in finding new therapeutic strategies for immunotherapy.

**Keywords:** Exhausted T cell, Blimp-1, Chronic Lymphocytic Leukemia

ICIA2018\Cancer Immunology\Poster Discussion\7794
**7794**

# SDF-1α Reduces Human NK cell Cytotoxicity against K562 Cells *in vitro*

Alireza Mardomi[1], Saeid Abediankenari[1*]

*1. Immunogenetics Research Center, Mazandaran University of Medical Sciences, Sari, Iran*

**Background:** Stromal cell derived factor-1 alpha (SDF-1α) belonging to CXC family of chemokines is up-regulated in a variety of malignancies. The expression of SDF-1α has been associated with poor prognosis and invasiveness of these neoplasms. It was not clear whether the described poor prognosis attributed to SDF-1α is due to immunosuppression or not. Evidence on the effect of SDF-1αin shaping the immune response is controversial. Therefore, the aim of present study was to investigate the effect of SDF-1α on the cytotoxicity of NK cells, as important effector cells in tumor immune surveillance.
**Methods:** Human NK cells were freshly isolated from 5 healthy donors using MACSxpress system (Miltenyi) and cultured 24 hours in the presence or absence of recombinant human SDF-1α (100 nM). A CD107a degranulation assay was conducted by the exposure of NK cells to K562 cells at 2:1 effector to target ratio for 4 hours. The percentage of CD107a positive cells was assessed by FACS. Effect of SDF-1α was also examined on the relative mRNA expression of NKG2A and NKG2D in NK cells.
**Results:** Our results demonstrated that SDF-1αdecreased the degranulation of NK cells significantly ($p=0.04$). However, blockage of the SDF-1α receptor, CXCR4 using AMD3100 did not recover the SDF-1α induced CD107a down-regulation. The mRNA content of inhibitory and activating receptors, NKG2A and NKG2D as indicators of NK cell activity were both down-regulated in SDF-1α treated group ($p=0.04$ and $p<0.01$ respectively). Moreover, AMD3100 recovered the NKG2A mRNA level to that of the un-treated cells.
**Conclusion:** The present study revealed that SDF-1α, as one of the soluble factors up-regulated in numerous malignancies, has a negative impact on NK cell activity and probably, is involved in tumor-induced immune-suppression. Therefore microenvironment manipulations targeting SDF-1α may reinforce cancer therapies by disturbing one of the immune-suppressive components exsiting in the tumor microenvironment.

**Keyword:** NK cell, SDF-1, Tumor microenvironment

ICIA2018\Cancer Immunology\Poster Discussion\7974
**7974**

# Upregulation of Immune Checkpoint Inhibitors, PD1-PDLs, in Patients with Bladder Cancer

Ali Ariafar[1, 2, 3], SomayehRezaeifard[1], MaisamGhaedi[3], ShahriarZeighami[2], Zahra Faghih[1]

1. *Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Urology-Oncology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
3. *Department of Urology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Malignant cells escape from immune responses through different mechanisms including inhibition of tumor infiltrating lymphocytes by expression of inhibitory molecules. A major hallmark of T-cell exhaustion is enhanced expression of multiple immune checkpoints i.e. PD-1. In the present study, we investigated expression of one inhibitory checkpoint, PD1, and its ligands, PD-L1 and PD-L2, on tumor cells and tumor-infiltrating lymphocytes in bladder cancer (BLC) tissue as well as tumor draining lymph nodes (TDLNs).
**Methods:** Cells were obtained from 37 tissues and 48 TDLNs were obtained from 61 untreated patients with BLC. The tumor cells were counted and stained for CD45, PD-1, PDL-1 and PD-L2. 7-AAD vital dye was used to discriminate live from dead cells. The cells were then acquired on four-color flow cytometer and analyzed with FlowJo software.
**Results:**Our results indicated that in TDLNs of BLC patients, the prevalence of total CD45+ expressing PD1 was significantly higher in LN+ patients and patients with higher stage tumor in comparison to the ones with free lymph nodes and lower stage tumor ($P<0.05$). PD1 expressing CD45low and CD45hi cells were also significantly increased in patients with higher stage tumor ($P<0.05$). Moreover, PD1 and PD-L2 expressing CD45hi cells elevated in LN+ patients as well ($P<0.05$). Whilst in tumor tissues, just PD-L1 expressing CD45hi cells were increased in patients with higher stage and higher grade tumors ($P<0.05$).
**Conclusion:** Elevated frequency of PD1 expressing CD45+ cells in TDLNs of patients with advanced diseases implied that immune cells in draining lymph nodes underwent exhaustion or over-activation. This exhaustion leads to decreased production of their effector cytokine and cytolytic activities and hence the failure of cancer elimination, tumor progression and metastasis. These results emphasize the importance of these molecules in cancer biology and designing new anti-cancer treatments.

**Keywords:** Bladder Cancer, Lymph node, PD1, PD-L1, PD-L2

ICIA2018\Cancer Immunology\Poster Discussion\8016
**8016**

# Evaluation of Potential Therapeutic Effect of Anti-Siglec-F Antibody in a Mouse Dual Breast Cancer and Allergic Asthma Model

Sima Shahmohammadi Farid[1, 2], Nazanin Mojtabavi[3], Ali Anissian[4], Alireza Razavi[1], Amir Hassan Zarnani[5, 6]

1. *Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
2. *Hematology &Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
3. *Department of Immunology, School of Medicine, Iran University of Medical Sciences, IUMS, Tehran, Iran.*
4. *Veterinary department, agricultural faculty, Islamic Azad University, Abhar branch, Abhar, Iran*
5. *Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*
6. *Immunology Research Center (IRC), Iran University of Medical Sciences, IUMS, Tehran, Iran*

**Background:** Eosinophils have long been known to infiltrate tumors. Eosinophils from allergic and non-allergic patients have differential effects on induction of apoptosis in tumor cells. Here, we explore the potential immunotherapeutic effect of anti-Siglec-F (sialic acid-binding immunoglobulin-like lectin F), a mouse eosinophil-specific antibody, on the course of breast cancer in a mouse model of allergic asthma.
**Methods:** We generated a dual mouse model of allergic asthma and breast cancer and injected mice with anti-Siglec-F antibody to explore the role of eosinophils in breast cancer pathogenesis and metastasis in an asthmatic condition. Chronic and acute allergic asthma models were generated by timeline injection and nebulization of ovalbumin (OVA). Breast cancer was induced by subcutaneous injection of 4T1 cell line. Eight different groups of mice were used in this study.
**Results:** In cancer group, anti-Siglec-F Ab caused a significant increase of IL-4 and IL-5 level and a significant reduction of IL-10 level compared with cancer group receiving vehicle. In asthma group, anti-Siglec-F Ab significantly increased the level of IL-4, IL-5 and IL-12 and diminished the level of IL-6 and IL-10. In asthma and cancer group, Sieglec F Ab leads to increased level of IL-4, -5, -6, -10 and -12 cytokines. Interestingly, such intervention significantly decreased OVA specific IgE level in sera of both asthmatic and asthmatic/cancer mice. Pathological evaluation of lungs from animals received antibody showed extensive metastasis in cancer group which was associated with 25% mortality in mice; however antibody administration in asthma/cancer mice significantly decreased metastatic foci.
**Conclusion:** Our data showed that, compared to cancerous condition, eosinophils behave differently in the pathogenesis of breast cancer when asthmatic condition has already been established raising the possibility of dual influence of this cell type on cancer prognosis depending upon microenvironment.

**Keywords:** Allergy, Breast cancer, Eosinophil, Immunotherapy, Metastasis

ICIA2018\Cancer Immunology\Poster Discussion\8047
**8047**

# Proteomic Pattern of Mesenchymal Stem Cells in the Patients with Malignant and Benign Salivary Gland Tumors

Mohammad Reza Haghshenas[1], Zahra Mojtahedi[1], Mahboobeh Razmkhah[1], Nasrollah Erfani[1], Bijan Khademi[2], Mohammad Javad Ashraf[3] , Abbas Ghaderi[1,4]

*1. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
*2. Otolaryngology Research Center, Department of Otolaryngology, Shiraz University of Medical Sciences, Shiraz, Iran*
*3. Department of Pathology, Khalili Hospital, Shiraz University of Medical Sciences, Shiraz, Iran*
*4. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:**To improve the knowledge about molecular mechanisms involved in pathogenesis of tumors, this study aimed to investigate MSCs proteome pattern in the patients with malignant and benign salivary gland tumors (SGTs).
**Methods:** Mesenchymal and non-mesenchymal fluorochrome antibodies and flow cytometric analysis were used to verify MSCs obtained from malignant (mucoepidermoid carcinoma) and benign (pleomorphic adenoma) tumor tissues, and two-dimensional polyacrylamid gel electrophoresis (2DE) coupled with matrix assisted laser desorption ionization tandem time-of flight mass spectrometry (MALDI-TOF-TOF) was done to identify differentially expressed proteins.
**Results**: Our result showed that both MSCs were strongly positive for mesenchymal markers such as CD90, CD73, CD105, CD166, and CD44, but they were negative or weakly positive for non- mesenchymal markers such as CD14, CD34, and CD45. Our result also showed that the expression pattern of cytosolic platelet-activating factor acetyl hydrolase type IB subunit beta (PAFAH1B), S-formyl glutathione hydrolase (FGH), type II trans glutaminase (TG2), FK506 binding protein 9 (FKBP9), and Annexin A4 (Anxa4) was significantly higher among MSCs isolated from malignant tissues, while the expression pattern of *keratin, type II cytoskeletal 7*(CK-7), *heat shock protein 70 (Hsp70)* was higher in MSCs isolated from the benign tissues.
**Conclusion:**Our result introduces differential expression of Anxa4 as well as four enzymatic proteins related to malignant transformation,cancer metabolism, and cancer development in the tumor microenvironment of patients with malignant SGTs. The data suggest that MSCs may serve as a desirable therapeutic target in SGTs treatment.

**Keywords:** Salivary gland tumors, Mesenchymal stem cells, Proteomic pattern

ICIA2018\Cancer Immunology\Poster Discussion\8198
**8198**

# Carcinoma-Associated Fibroblasts Are a Common Finding in the Microenvironment of HPV-Positive Oropharyn geal Squamous Cell Carcinoma

Samira Derakhshan[1], Sedigheh Rahrotaban[1], Alireza Abdollahi[2], Farzad Yazdani[3]

*1. Department of Oral and Maxillofacial Pathology, School of Dentistry, Tehran University of Medical Science, Tehran, Iran*
*2. Department of Pathology, Imam Hospital Complex, Cancer institute, Tehran University of Medical Sciences, Tehran, Iran*
*3. Department of Pathology, Amiralam Hospital, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** The important role of the human papillomavirus (HPV) is widely established in oropharyngeal squamous cell carcinoma (OPSCC). The behavior of a OPSCCs especially induced by HPV might be influenced by the tissue microenvironment and its changes according to the tumor nature. Recognition of the role of the tumor microenvironment on the behavior of neoplastic cells has led to employment the microenvironment to use as therapeutic target. Carcinoma-associated fibroblasts (CAFs), the most abundant cells in the tumor microenvironment, show wide-spread expression of alpha-smooth muscle actin (a-SMA). We focused on CAFs, its presence in oropharyngeal squamous cell carcinoma and the relationship with HPV for the first time.
**Methods:** Expression of a-SMA protein in CAFs of the tumor microenvironment of the 44 formalin fixed paraffin-embedded tissue blocks from the primary tumor of OPSCC evaluated by immunohistochemistry between HPV-positive and HPV-negative tumors separated by nested polymerase chain reaction.
**Results:** In 44 samples 23 HPV positive cases were detected. Statistically there were significant differences between histopathologic grade, percent and final score of a-SMA and HPV expression. Significant difference between HPV expression and inflammation, intensity and clinical parameters was not identified in the present study.
**Conclusion:** Our results indicate that CAFs are a common finding in the microenvironment of HPV-positive oropharyngeal squamous cell carcinoma and associated with higher histopathologic grade. Therapeutic strategies to employ CAF-mediated drugs need to be considered and evaluated more for treatment of HPV-positive oropharyngeal squamous cell carcinoma.

**Keywords:** Carcinoma-associated fibroblasts, HPV, Oropharynx, Squamous cell carcinoma

ICIA2018\Cancer Immunology\Poster Discussion\8263
**8263**

# Docosahexaenoic acid and Eicosapentaenoic Acid Induce Cell Death in Peripheral Blood Mononuclear Cells of Multiple Myeloma Patients but Not Healthy Individuals

Milad Moloudizargari[1], Esmaeil Mortaz[1, 2, 3], Frank A. Redegeld[3]

*1. Department of Immunology, Faculty of Medicine, ShahidBeheshti University of Medical Sciences, Tehran, Iran*
*2. Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), ShahidBeheshti University of Medical Sciences, Tehran, Iran*
*3. Division of Pharmacology, Faculty of Science, Utrecht Institute for Pharmaceutical Sciences, Utrecht University , Utrecht, Netherlands*

**Background:** Poly-unsaturated Fatty Acids (PUFAs) have been shown to have anti-cancer effects in both solid and non-solid tumors. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are among the most studied PUFAs. We have shown in a previous study that EPA and DHA induce apoptotic effects in multiple myeloma cell lines via altering the expression of a wide array of genes involved in oxidative stress as well as NF-κB inhibition, increasing mitochondrial perturbation, and caspase 3 activation.
**Methods:** The aim of the present study was to further evaluate the apoptotic effect of these two fatty acids in primary peripheral blood mononuclear cells (PBMCs) obtained from untreated patients (new cases) with confirmed symptomatic multiple myeloma.
**Results:** The results showed that EPA at the concentration of 100 µM and DHA at both concentrations of 50 and 100 µM can induce potent apoptotic effects (up to 44, 76 and 98%, respectively) in the PBMCs of patients with multiple myeloma ($P \leq 0.05$), while they have no effect on the PBMCs isolated from healthy individuals ($P > 0.05$). The observed effect was concentration and time-dependent and 72h treatment with DHA at a concentration of 100 µM had the strongest effect ($P \leq 0.01$).
**Conclusion:** These findings confirm the results of our previous study and may suggest a promising future for the application of PUFAs as an adjunctive therapy in the treatment of multiple myeloma with fewer side effects. Further studies are warranted to define the underlying mechanisms shedding light on the possibility of exploiting apoptotic effects of PUFAs in cancer therapy.

**Keywords:** Docosahexaenoic acid, Eicosapentaenoic Acid, Multiple Myeloma

ICIA2018\Cancer Immunology\Poster Discussion\8441
**8441**

## Genetic variants of PD-1 co-inhibitory molecule (PD1.3 and PD1.5) in patients with basal cell carcinoma

Maryam Fakhmi[1], Maryam Abedi[1], Zahra Faghih[1], Mohamad reza Haghshenas[1], Faride Jowkar[3], Nasrollah Erfani[1,2]

1. *Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Departments of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
3. *Department of Dermatology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Basal Cell Carcinoma (BCC) is the most common type of skin cancer. About 2% of people above 60 years suffer from this type of cancer. *PD-1* is an inhibitory molecule belonging to immunoglobulin gene super family which strongly inhibits immune system responses after interaction with its ligands. The aim of present study was to investigate the association of two variants of *PD-1* (*PD1.3*G/A and*PD1.5*C/T) with susceptibility and/or progression of BCC in a population from Southern Iran.
**Methods:** 168 pathologically confirmed BCC patients (mean age: 61.24±13.09) as well as 167 age-sex-matched healthy individuals with no family history of cancer or autoimmune diseases were recruited. Genomic DNA was extracted from the white blood cells, and genotyping was performed using Polymerase Chain Reaction following by Restriction Fragment Length Polymorphism (PCR-RFLP).
**Results:** No deviation was observed from Hardy Weinberg Equilibration as confirmed by Arlequin 3.1 software package. Statistical analysis revealed the frequency of AA genotype and A allele of *PD1.3* polymorphism to be significantly lower in the patients comparing to controls (0% vs. 4.19% and 9.38% vs. 17.97% respectively, P=0.01). Conversely, the frequency of GG genotype at this position was observed to be significantly increased in the patients comparing to controls (81.25% vs. 68.26% respectively, P<0.01). However, no statically significant difference were found in the genotypes' and alleles' frequencies of *PD1.5*C/T between two investigated groups (P>0.05). No association was also observed between these genotypes and clinic-pathological parameters in the patients (P>0.05). Additionally, the haplotype frequencies were not significantly different between patients and control group (P>0.05).
**Conclusion:** Our results collectively suggest that AA genotypes and A allele at *PD1.3* position may play a protective role, whereas GG genotype at this position may increase the risk of BBC of the skin in Iranian population.

**Keywords:** Basal Cell Carcinoma, Genotypes, Haplotypes, *PD-1*.

ICIA2018\Cancer Immunology\Poster Discussion\8584
**8584**

## CD39/CD73 expressing B cells in breast cancer draining lymph nodes

Sahar Shariati[1, 2], Fereshteh Mehdipour[2], Morteza Samadi[1], Abdol-Rasoul Talei[3], Abbas Ghaderi[2, 4]

1. *Department of Immunology, School of Medicine, ShahidSadoughi University of Medical Sciences, Yazd, Iran*
2. *Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
3. *Breast Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
4. *Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:**CD39 and CD73 are two ectoenzymes which contribute to immune suppression by converting ATP to AMP and AMP to adenosine, respectively. These two molecules are expressed by different cell types including B cells. Some subsets of regulatory B cells were reported to express CD39 and/or CD73. As B cells are a major component of the breast tumor draining lymph nodes (TDLNs) which can influence the anti-tumor immunity, the expression of these molecules by B cells in TDLNs of breast cancer were investigated.
**Methods**: 20 axillary lymph nodes were studied. Mononuclear cells were isolated and stained with anti-CD19, anti-CD39 and anti-CD73 or their isotype matched antibodies and subjected to flowcytometry.
**Results:** We found that 94.4±3.1 % and 83.5±7.1 % of B cells were $CD39^+$ and $CD73^+$, respectively. Regarding the CD39 and CD73 expression we could detect four distinct B cell subsets in the breast TDLNs: Majority of B cells (79.4±7.5 %) were $CD39^+CD73^+$ while 14.5±5.8 %, 3.5±2 % and 2.1±1.8 % of $CD19^+$ cells had $CD39^+CD73^-$, $CD39^-CD73^+$ and $CD39^-CD73^-$ phenotypes, respectively. The frequencies of these B cell subpopulations did not show significant differences in the metastatic and non-metastatic lymph nodes, however the percentage of $CD39^-CD73^-$ B cells had a non-significant increasing trend in metastatic lymph nodes (P=0.081). Moreover, none of these B cell subsets had significant association with disease parameters such as stage, grade, or the tumor size; However, the frequency of $CD39^-CD73^+$ B cells was significantly lower in stage III compared with stage II (P=0.020) and showed significant reverse correlation with the number of involved lymph nodes (R=0.5, P=0.014).
**Conclusion:** According to CD39 and CD73 expression, we could detect different B cell subpopulations in the TDLNs of breast cancer. $CD39^-CD73^+$ B cells showed negative associations with poor prognosticators such as stage and the number of involved lymph nodes.

**Keywords:** Breast cancer, Tumor draining lymph nodes, B cells, CD39, CD73

ICIA2018\Cancer Immunology\Poster Discussion\9783
**9783**

## SiRNA-Mediated Silencing of Snail-1 Induces Apoptosisin Human Breast Cancer Cell Line

Seyed mohammad hossein Musavi shenas[1], Amin kamrani[1],Yunes Panahi[2*]

1. *Tabriz University of MedicalSciences*
2. *Pharmacotherapy Department, School of Pharmacy, Baqiyatallah University of Medical Sciences, Tehran. Iran*

***Background:****Snail-1 known as one of important Transcription factor is a mediator of survival and cell migration, and expression is raised in numerous cancer types. Snail-1 gene may show a role in recurrence of several cancers including breast cancer by down-regulating E-cadherin, inducing an epithelial to mesenchymal transition and its related miRNA.* The aim of this study was to investigate the effect *of a specific Snail-1 siRNA on apoptosis and alter EMT related miRNA of MDA-MB-468 (breast cancer) cells.*
**Methods:** The cells were transfected with siRNAs using transfection reagent. The cytotoxic effects of Snail-1 siRNA, on breast cancer cells were determined using MTT assay**.** Relative Snail-1 mRNA levels were measured by QRT- PCR, respectively. Apoptosis was measured by TUNEL test based on labeling of DNA strand breaks. We also evaluated miR-29b, E-cadherin, vimentin and mmp9 expression by QRT-PCR. To determine alteration in miRNA expression and gene expression involved in EMT.
**Results:** Snail-1 siRNA significantly reduced mRNA expression levels in a 48 hour after transfection at the concentration of 80 PM in breast cancer cells. We also showed that the silencing of Snail-1 led to the induction of apoptosis and mmp9, vimentin and miR-29b depression as well as E-cadherin overexpression has been shown in snail-1 suppressed group in MDA-MB-468 cells in vitro.
**Conclusions:** These results propose that Snail-1 might play an important role in the progression of breast cancer, and be a potential therapeutic target for trigger apoptosis and suppression EMT related miRNA and gene in breast cancer.

**Keywords**: microRNA, Brest cancer

ICIA2018\Cancer Immunology\Poster Discussion\10061
**10061**

# Mesenchymal stem cells derived from stage II of human breast tumor tissue promote the proliferation of thePBMCs: An in vitro assay

Koushansineh sepehr[1], Alireza Razavi[1*], Zuhair Mohammad Hassan[2], AbdolReza Fazel[3], Majid Mossahebi Mohammadi[4], Seyed Mahmoud Hashemi[5*]

1. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
2. *Department of Immunology, Faculty of Medical Sciences, TarbiatModares University, Tehran, Iran*
3. *Cancer research center, Golestan University of Medical Sciences, Gorgan, Iran*
4. *Sarem Cell Research Center (SCRC),Sarem Women's Hospital,Tehran,Iran*
5. *Department of Immunology, School of Medicine, ShahidBeheshti University of Medical Sciences, Tehran, Iran*

**Background:** Mesenchymal stem cells (MSCs), as a subpopulation of stromal cells found in the tumor microenvironment, are critical in promoting tumor progression and possess immunomodulatory properties. Therefore, the purpose of this study was to isolate MSCs from primary human breast tumor tissue, and to study the effect of soluble factor of MSC on the peripheral blood mononuclear cell (PBMC) proliferation.
**Methods:** The tumor tissues (n=8) obtain from patients with pathological stage II of breast tumor. MSCs were isolated by explant culture method and identified. The Mitogen-induced PBMC was treated with 25 and 50% MSC conditioned medium (CM) for 72h, and changes in proliferation evaluated by BrdU ELISA kit.
**Results:** We successfully isolated and identified MSCs from primary breast tumor tissues. Flow cytometry analysis demonstrated that isolated cells were positive for, CD73, CD44, CD29, CD105 and CD90 but negative for, CD11b, CD45, CD34, and HLADR. In addition, cells possessed the capability of multipotential differentiation into osteoblasts and adipocytes.
The results of theBrdU ELISA assay showed that 50% CM concentrations significantly increased the proliferation of PBMCs.
**Conclusion:** Collectively, our study showed that MSCs were confirmed to exist in stage II of human breast tumor and significantly promote the proliferation of PBMCsin vitro.

**Keywords:** Breast Cancer, Mesenchymal Stem Cells, Isolation, Immunomodulatory

# Cancer Immunotherapy

# Poster Discussion

ICIA2018\Cancer Immunotherapy\Poster Discussion\5233 cancer immuno
**5233**

# Evaluation of The Effect of TIM-3 Suppression by miR-498 and Its Effect on Apoptosis and Proliferation Rate of HL-60 Cell Line

Yaser Moghaddam[1], Mazdak Ganjalikhani Hakemi[1], AlirezaAndalib[1], MohammadrezaSharifi[2], Vida Homayouni[1], Maryam Mhammad Ganji[1]

*1. Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
*2. Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Science, Isfahan, Iran*

**Background:** Acute Myeloid Leukemia (AML) is a Cancer of hematopoietic stem cells with a rapid progression. TIM-3 is expressed on leukemic stem cells (LSCs) in most types of AML and might have a positive effect on maintenance of malignant phenotype. MicroRNAs play important roles in either cancer progression or suppression. In this study were evaluated, the inhibitory effect of miR-498 on TIM-3 expression and its impact on proliferation and survival of HL-60 cell line.
**Methods:** Firstly, the probable inhibitory effect of miR-498 on TIM-3 expression was predicted. HL-60 cells were cultured and expression of TIM-3 was induced on the cells using phorbolmiristate acetate. The cells were transfected with miR-498 and expression level of TIM-3 was measured using with q-RT-PCR and flow cytometry methods. In addition, the effect of suppression of TIM-3 expression in HL-60 cell line was analyzed with apoptosis and cell proliferation assays.
**Results:**Bioinformatics analyses predicted that miR-498 has high ability to silence TIM-3 gene expression. Our experiments confirmed that miR-498 was able to strongly silence TIM-3 expression (68% silencing) in HL-60 cell line ($P<0.002$). Also, the cells with suppressed expression of TIM-3 had a lower proliferation and higher apoptosis rates.
**Conclusion:** Based on our results, the miR-498 can effectively suppress TIM-3 expression in the AML cell line. TIM-3 suppression, in turn, inhibits malignant cell proliferation and induces its apoptosis. Collectively, suppression of TIM-3 by miR-498 can be considered as a potential powerful way for treatment of AML.

**Keywords:** Tim-3, mir-498, miRNA

ICIA2018\Cancer Immunotherapy\Poster Discussion\5247 cancer immuno
**5247**

## Investigating Molecular Mechanism Regulating Egr2 Expression and Its Role in the Immune Response

Sina Taefehshokr[1*], Su Ling Li[2], Amin Oveisi[3], YasharAzari Key[1], Sahand Fattahi[3], MohaddesehGhezelcheh Khamsiyan[3], Nima Taefehshokr[2]

1. *Young Researchers and Elite Club, Tabriz Branch, Islamic Azad University, Tabriz, Iran*
2. *Division of Bioscience, Department of Life Sciences, College of Health and Life Sciences, Brunel University, UK*
3. *Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran*

**Background:** The immune system is evolved to defend the body against pathogens and is composed of thousands of complicated and intertwined approaches, which are highly controlled by regulatory processes such as transcription and repression of cellular genes. Early growth response gene (Egr2) is important for maintaining immune stability and it has a vital role in controlling inflammation and preventing the development of autoimmune diseases. The aim of this study was to investigate the molecular mechanism regulating Egr2 expression in the adaptive immunity and to address the vital function of Egr2 in regulating the immune checkpoint markers under melanoma tumor model to obtain effective anti-tumor therapy.
**Methods:** To investigate the effect of Interferon gamma (IFNγ) and Interleukin 6 (IL-6) on Signal transducer and activator of transcription 1 (STAT1) and STAT3 mediated Egr2 induction, green fluorescent protein (GFP)-Egr2 knock-in cluster of differentiation 4 ($CD4^+$)T cells stimulated with IFNγ and IL-6 in the presence of STAT1 inhibitor (Fludarabine) and STAT3 inhibitor (Stattic), assessed for Egr2 expression. Moreover, to pinpoint the function of Egr2 under tumor microenvironment, Egr2/3 KO and GFP Egr2 Knock-in mice ranging from 8-10 weeks of age were used in this study. For experiments mice received $1 \times 10^6$ B16 melanoma tumor cells via subcutaneous injection on both left and right flank.
**Results:** In this study, we have found that Egr2 expression is regulated by antigens and cytokines, including INFγ and IL-6. Furthermore, it is shown that Egr2 can be significantly expressed in tumor infiltrating lymphocytes (TILs) as we observed in a mouse melanoma tumor model.
**Conclusion:** Collectively our results demonstrate that Egr2 is an intrinsic regulator in the immune system and tumor microenvironment. It can work as an immune checkpoint in adaptive immunity and tumor condition, suggesting its essential role in effective immune response and anti-tumor therapy.

**Keywords:** Egr2, INFγ, IL-6, Immune response

ICIA2018\Cancer Immunotherapy\Poster Discussion\6354 cancer immuno
**6354**

## Melatonin as an efficient adjuvant for cancer therapeutic DNA vaccines

Sanaz Baghban Rahimi[1], Amir Ghaemi[2,3]

1. *Department of Microbiology, GolestanUniversityof Medical Sciences, Gorgan, Iran*
2. *Department of Virology, Pasteur Institute of Iran, Tehran, Iran*
3. *Department of Microbiology, Infectious Diseases Research Center, Golestan University of Medical Sciences, Gorgan,Iran*

**Background:**The aim of this study is to investigate theeffects of melatonin as an adjuvant in E7 DNA vaccines in order to control HPV associated tumors and evaluate effective therapeutic cancer vaccines.
**Methods:** The anti-tumor efficacy of an HPV-16 E7 DNA vaccine adjuvanted with melatonin (MLT) was evaluated in a C57BL/6 mouse bearing TC-1 tumor model. Post vaccination tumor growth and survival rate of tumor-bearing mice was assessed. The specific lymphocyte proliferation response was measured in control and vaccinated mice by MTT assay. IFN-γ secretion in splenocyte cultures was assessed by ELISA and the antitumor effects were estimated by tumor growth curves.
**Results:** Our results showed that subcutaneous administration of C57BL/6 mice with a DNA vaccine and MLT dose dependency adjuvant significantly induced strong IFN-γ responses. The high level of E7-specific T-cell proliferation was found in the adjuvant-vaccine group. Furthermore, we found a considerable drop in the tumor volume after immunization mice with MLT plus E7 DNA vaccine and the survival time of TC-1tumor bearing mice was also significantly prolonged.
**Conclusion:** These data indicate that melatonin will be used as an effective adjuvant in E7 DNA vaccines and also in other gene vaccines and raised hopes that clinically safe cancer therapeutic vaccines could be designed.

**Keywords:** Melatonin, HPV, DNA vaccines, Tumor

ICIA2018\Cancer Immunotherapy\Poster Discussion\8541 cancer immuno.doc
**8541**

# NK cells Reactivation by Combined Blockade of Tim-3 and PD-1 Inhibitory Receptors for Immunotherapy of Patients with Chronic Lymphocytic Leukemia

Mojgan Astaneh[1], Hadi Hossein Nattaj[1], Ramin Shekarriz[2], Hossein Asgarian Omran[1,3*]

*1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
*2. Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran*
*3. Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

**Background**: Recent studies showed that programmed death-1 (PD-1) and T cell immunoglobulin and mucin domain containing molecule-3 (Tim-3) immune checkpoints receptors are more expressed on cytotoxic T lymphocytes and NK cells in some chronic conditions such as viral infections and malignancies. The exhausted phenotype induced by these receptors makes immune cells inactive as they have impaired cytotoxic activities, degranulation and cytokines secretion. Since the exhaustion process is reversible, in the current study, combined blockade of PD-1 and Tim-3 were tested to restore the functional properties of NK cells in chronic lymphocytic leukemia (CLL).
**Method**: Fresh peripheral blood mononuclear cells were isolated from 15 patients with CLL and NK cells were positively selected by anti-CD56 microbeads via magnetic cell sorting method. Following treatment of purified NK cells with blocking monoclonal antibodies against PD-1 and Tim-3, their functional activities were assessed in comparison to treatment with isotype matched control antibodies. Induction of apoptosis in K562 cell line was determined by Annexin V-PI flow cytometry method. Degranulation activity of isolated NK cells was evaluated by CD107a degranulation assay and the concentrations of IFN-γ and TNF-α were measured in culture supernatant by ELISA.
**Results**: The results showed improvement in functional properties of isolated NK cells from CLL patients following blocking of PD-1 and Tim-3 with specific monoclonal antibodies. NK cells treated with blocking antibodies represented more apoptosis induction of K562 cells, increasing in degranulation activity and more production of IFN-γ and TNF-α compared to treatment with control antibodies.
**Conclusion**: Our results suggested that exhausted NK cells in CLL patients can be reactivated by co-blockade of PD-1 and Tim-3 inhibitory receptors. This strategy could be a potential therapeutic approach for immunotherapy of CLL.

**Keywords**: Chronic lymphocytic leukemia, Tim-3, PD-1, Checkpoint Inhibitors, Exhaustion, NK

ICIA2018\Cancer Immunotherapy\Poster Discussion\8607 cancer immuno
**8607**

# Overexpression of MicroRNA-146a-5p Inhibits Migration of Human MKN-45 Gastric Cancer Cell Line via Down-regulating of Metastasis-related Target Genes

NavidShomali[1, 2], NaghmehShirafkan[1, 2], BehzadBaradaran[1]

*1. Immunology Research Center (IRC), Tabriz University of Medical Sciences, Tabriz, Iran*
*2. Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:**The second cause of cancer-related death worldwide is gastric cancer. MicroRNAs are important post-transcriptional regulators that acts as negative regulators of gene expression. They do this role through base-pairing with the 3-UTR (3-untranslated region) region of their target genes. MiR-146a is one of the important microRNAs that plays a role in pathogenesis of wide ranges of human disorders. Though evidences have shown miR-146a as a strong metastasis and invasion inhibitor of gastric cancer, but the molecular mechanisms and the related genes are mainly unknown.
**Methods:** After miR-146a transfection, quantitative RT-PCR was performed to detect expression level of miR-146a and related target genes including L1CAM, ROCK1, MMP9, HMGA2, CXCR4 and vimentin in GC cell line. Wound-healing assay was performed to measure MKN-45 cells migration after miR-146a transfection. MTT and Annexin V staining were used to determine the role of miR-146a in the regulation of MKN-45 cell viability and apoptosis induction, correspondingly.
**Results:** We showed that miR-146a has low expression level in MKN-45 gastric cancer cell line.in the same way, we showed increased expression of miR-146a can suppress MKN-45 metastasis capability. Effect of miR-146a replacement on target genes showed that except for the CXCR4, which was not meaningful in the result, other related target genes showed reduced expression. The results of miR-146a replacement on cell viability and apoptosis showed it had not a significant effect on cell viability and apoptosis induction.
**Conclusion:** Our results indicated that miR-146a, may act as tumor suppressor microRNA in MKN 45 cell line. The freshly identified miR-146a target genes in gastric cancer offers a novel insight into the biological basis of migration in gastric cancer and offers miR-146a as a potential therapeutic approach to suppress gastric cancer migration.

**Keywords:** Gastric cancer, microRNA, Metastasis, Cell viability, Apoptosis

ICIA2018\Cancer Immunotherapy\Poster Discussion\8640 cancer immuno
**8640**

# Polarized Mesenchymal Stem Cells-Microvesicles with Whole Tumor Lysates Pulsed DCs Promote T Cells Cytotoxicity against Glial Cancer Cells

RahmaniKukia Nasim[1], Abbasi Ardeshir[2], Delirezh Nowruz[3]

1. *Division of Immunology, Department of Microbiology, Veterinary Faculty, Urmia University, Urmia, Iran*
2. *Division of Immunology, TarbiatModares University of Medical Sciences, Tehran, Iran*
3. *Associate professor, Division of Immunology, Department of Microbiology, Veterinary Faculty, Urmia University, Urmia, Iran*

**Background:** Cancer immunotherapy using dendritic cells (DCs) and peptides is considered as a new promising treatment modality and capable of inducing an anti-cancer response. Mesenchymal Stem Cells (MSCs) migrate to tumor microenvironments and contribute many bioactive factors such as microvesicles (MVs). The MSC-MVs as native or engineered stem cells have similar protective and reparative properties as their cellular counterparts in tissue repair and possibly anti-cancer therapy. Recent studies indicate that polarization of MSCs with Lipopolysaccharide (LPS) can reduce tumor growth and metastasis.
**Methods:** Peripheral blood monocyte derived DCs were isolated by heart puncture of rats and incubated with GM-CSF, IL-4 and addition of LPS at day 6 to induce maturation of the DCs. MSC-MVs were purified by differential ultracentrifugation after isolation, proliferation and treatment of MSCs with LPS. Autologous T cells were incubated with whole tumor lysate pulsed DCs in the presence of polarized MSC-MVs. Delicate T cells were co-cultured with $B_{92}$ glial cancer cells in 96-well plates at final volume of 200 µl of medium at an effector:target ratio of 100:1 to evaluate specific cytotoxic activity.
**Results:** The flow cytometric analysis of cytotoxic T cells showed that using polarized MSC-MVs with tumor lysate induce glial cancer cells death. These results represented that early and late apoptosis was stimulated in tumor cells in compare to controls and were responsible for cells death. Also, the specific lysis of tumor cells was promoted by autologous tumor specific T cell responses *in vitro* in the presence of polarized MSC- MVs.
**Conclusion:** It appears that an alternative strategy for effective anticancer responses may be the use of polarized MSC- MVs and tumor lysate to elicit T cell responses in terms of proliferation and cytotoxicity.

**Keywords:** Polarized MSC- MVs, Cancer immunotherapy, Tumor lysate, Cytotoxic T cells, Glial cells, Apoptosis

ICIA2018\Cancer Immunotherapy\Poster Discussion\8650 cancer immuno
**8650**

# Antitumor Effects of Liposomes-encapsulated CTLA-4 Immunotherapy Combined with Doxil Chemotherapy in the B16 Large Established Tumors of Melanoma

Seyedamir Jalali[1], Razie Alibeigi[2]

1. *Immunology Department, Medical School, Shahid Beheshti university of medical sciences*
2. *Immunology Department, Medical School, Mashhad university of medical sciences*

**Background:** Cancer therapy is designed to specifically integrate distinct treatment modalities in the most effective way to achieve the highest cure rate. Chemotherapy has pleiotropic effects on the immune system. The vast majority of chemotherapeutic agents are myelosuppressive and therefore could deplete the very cells responsive for antitumor immunity. In the presented studies, we describe synergy between CTLA-4 blockade, Liposomes-encapsulated CTLA-4 blockade antibody and chemotherapeutic agents, Doxil, in large established tumors in the mice models of melanoma and we were shown the effect of timing immunotherapeutic agent (CTLA-4 blockade antibody) and chemotherapeutic agent (Doxil) on antitumor responses.
**Methods:** Liposomes were prepared by thin lipid film hydration plus extrusion. The stock chloroform solution of ingredients For PEGylated liposomes, mPEG2000-DSPE, and cholesterol. The lipid film was hydrated with 10 mM phosphate-buffered saline containing 1 mg/ml Anti CTLA-4 monoclonal antibody. Ten days after tumor challenges, mice with large stablished tumors were randomized into eight different treatment groups of five mice each as follows: 1) free CTLA-4 blockade antibody, 2) CTLA-4 PEG-liposomes, 3) Doxil, 4) Concomitant CTLA-PEG-liposomes and Doxil, 5) concomitant free CTLA-4 blockade antibody and Doxil, 6) First Doxil so free CTLA-4 blockade antibody, 7) free CTLA-4 blockade antibody, 8) PBS and mice were administered intravenously with 100 µg of free or liposomal forms of CTLA-4 blockade antibody and/or single dose of 5 mg/kg Doxil. Flow cytometer analysis of drainage lymph node, tumor infiltrated lymphocytes and spleen were liposomal CTLA-4 blockade antibody with Doxil group (6.17%, 26.1% and 5.5%, 27% respectively).
**Result:** Our results demonstrating that the optimal timing of CTLA-4 blockade antibody is before Doxil administration in compare with first Doxil CTLA-4 blockade antibody and concomitant administration of Doxil and CTLA-4 blockade antibody
**Conclusion:** In summary, our findings give strong preclinical rationale for clinical evaluation of Doxil.

**Keywords:** Doxil, Anti CTLA-4, Chemoimmunotherapy

ICIA2018\Cancer Immunotherapy\Poster Discussion\9793 cancer immuno
**9793**

## Accumulative strategy of FoxP3-specific recombinant vaccine and tumor-specific DC vaccine against melanoma in mice

NedaMousaviNiri [1], Maryam Naseroleslami [2], NosratollahZarghami [3], Nasimmohammad kheshtchin[4], Jamshid Hadjati[4]

*1. Department of Medical Biotechnology, School of Advanced Medical Sciences, Tehran Medical Sciences branch, Azad Islamic university, Tehran, Iran*
*2. Department of Cellular and Molecular Biology, School of Advanced Medical Sciences, Tehran Medical Sciences branch, Azad Islamic university, Tehran, Iran*
*3. Department of Medical Biotechnology, School of Advanced Medical Sciences and Department of Clinical Biochmemistry, Radiopharmacy Lab, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
*4. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Treg apparently have a substantial role in suppression of anti-tumor immune responses. Application of recombinant FoxP3-Fc vaccine inducing anti-Treg immune responses accompanied with tumor antigen expressing dendritic cell therapy is a hopeful approach in anti-tumor treatments.
**Methods:** Instead of anti Treg vaccine, FoxP3-Fc fusion construct was applied to produce DNA vaccine and its respective recombinant protein. Afterward they were subcutaneously injected into C57/B6 mice followed by melanoma tumor induction (two weeks after last vaccination) and dendritic cell-based anti-tumor immunotherapy. The improvement of immunotherapy results in mice that received anti-Treg vaccination was evaluated by different immune assays compared to other groups.
**Results:** combinational therapy by dendritic cells and anti-Treg vaccine showed increment in immunological results compared to sole vaccination of anti Treg. Proliferation and IFN-γ section of Tumor specific lymphocytes were augmented significantly in dendritic cell and anti-Treg vaccinated group of mice. Tumor growth and survival as well indicated more efficient results inthe same group of treatment.
**Conclusion:**Due to the prospering immune protection of FoxP3-Fc (IgG) vaccine against Treg andits impression in improvement of dendritic cell-based immunotherapy, there would be a good prospect for applied strategy and other means of immunotherapy.

**Keywords:** Treg, FoxP3, Dendritic cells, Melanoma, Immunotherapy

ICIA2018\Cancer Immunotherapy\Poster Discussion\10012 cancer immunotherapy
**10012**

# Recombinant HER2 Protein Vaccination Induces Cellular Immune Response and Tumor Growth Inhibition in Syngeneic Breast Cancer Mice Model

Fateme Sadri Ardalani[1,2], Moslem Ahmadi [1,2], Mohammad Mehdi Amiri[1], Mahmood Jeddi Tehrani[2], Fazel Shokri[1,2] , Mahdi Shabani[3,2]

1. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
2. *Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*
3. *Department of Immunology, School of Medicine, ShahidBeheshti University of Medical Sciences, Tehran, Iran*

**Background:** Cellular and protective immune responses against the full HER2 extracellular domain (fHER2-ECD) recombinant protein were investigated in breast cancer mice model.
**Methods**: Balb/C mice were immunized with produced recombinant eukaryotic fHER2-ECD protein in combination with or without CpG to investigate their potential for induction of HER2 specific cell-mediated immune responses and tumor growth inhibition in vivo.
**Results**: Immunization resulted in induction of antibody response and induced secretion of IL4 and particularly IFNγ and IL17 cytokines. Challenging of immunized mice with stable 4T1-HER2 transfected cells resulted in partial but significant tumor growth inhibition in the immunized mice (about 8% tumor free in with and without CpG groups) particularly those immunized with fHER2-ECD together with CPG.
**Conclusion**: The fHER2-ECD along with CpG was a successful formula to induce IFNγ production. The fHER2-ECD induces tumor growth inhibition in immunized micecompared to control groups.

**Keywords:**Breast Cancer, Cytokine, HER2, Protein immunization, Tumor challenge

# Computational Immunology and Systems Biology

# Poster Discussion

ICIA2018\Computational Immunology and Systems Biology\Poster Discussion\7986
**7986**

# Detection of Blood cells' Genes Involved in Responding Sepsis by Weighted Gene Co-expression Network Analysis

Rasoul Godini[1], Fatemeh Hadi[1], Hossein Fallahi[1]

*1. Department of Biology, Faculty of Science, Razi University*

**Background:** Sepsis, as a systemic inflammation disorder is known as a clinical syndrome caused by infection. Sepsis is a life threating condition and early recognition and proper treatment is vital to reduce mortality. In here, we use Weighted Gene Co-expression Network Analysis (WGCNA) to detect which genes are most important in Dendritic Cells (DCs) and Peripheral Blood Mononuclear Cells (PBMCs) responding to sepsis.
**Methods:** In order to do this, we used free available dataset GSE49758. This data contains more than 80 microarray analysis from samples of two cell types, DCs and PBMCs. After preprocessing the matrices for genes with variation, outliers were removed. Then WGCNA analysis was performed to detect associated genes to disease symptoms. Soft threshold of 0.8 was used to detect modules. Hub genes in each module detected and their expression values determined.
**Results:** Our results showed that there were 4 related modules with the sepsis in each cell type. Searching for the correlations between genes' module membership and significance for the sepsis, we found that two modules with a correlation = 0.44 (p-value = 3.7e-10) and correlation = 0.47 (p-value = 1.6e-31) that are related to immune and inflammatory response in DCs and PBMCs, respectively. Looking for hub genes among members of these modules, 185 in DCs and 549 PBMCs modules, ADORA2A and CD163 in DCs and VIPR1 and LTB in PBMCs were detected as hub genes, which were also differentially expressed. We found 27 and 99 differentially expressed genes in modules regarding to DCs and PBMCs, respectively, however, only a few of them was hub differentially expressed genes, as well.
**Conclusion:** Results of this study can help not only to find markers to detect sepsis and possibly proper treatments, but also shed lights upon molecular information of sepsis.

**Keywords**: Dendritic cells, PBM cells, Sepsis, WGCNA

ICIA2018\Computational Immunology and Systems Biology\Poster Discussion\8300
**8300**

## *In silico* Design of Dodecameric Human Soluble CD40L Using Surfactant Protein-D

Bahare Shokoohian[1], Mahshoid Mohammadipour[1], Fatemeh Yari[1]

*1. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran*

**Background:** CD40L is a membrane protein and a member of TNF family that plays an important role in cell signaling in innate and adaptive immunity. Since this protein plays its role through clustering the receptors, CD40, on the target cell surface and also natural soluble form of CD40L is not stable, it is expected that the multimeric form of CD40L can have a stronger effect than the natural trimeric form.
**Methods:** To design the dodecameric form (four-trimer) of CD40L, aminoacid sequences of CD40L and surfactant protein D (SPD), were obtained from the NCBI protein data bank. To identify consensus sequence, alignment was performed by ClustalW method. Extracellular domain of CD40L was replaced with lectin domain of SP-D and primary construct with the length of 472 aminoacid was built. Physicochemical characteristics and stability of chimeric protein, SPD-CD40L, were determined by ProtParam software. To determine protein localization in eukaryotic expression host, HEK293, PSORTII software was used. Secondary and tertiary structures of the SPD-CD40L were predicted using PSIPRED server and I-TASSER software respectively. Validity of the predicted 3D structure was evaluated by the Ramachandran plot drawn by RAMPAGE. To obtain nucleotidic sequence and optimization of sequence for expression in eukaryotic cells, OPTIMIZER server was used.
**Results:** Molecular weight was measured 49145.4 Da and the half-life were greater than 30 hours in eukaryotic cells. SPD-CD40L was classified as stable. PSORTII results indicated that protein is secretory. Tertiary structure prediction revealed distinct folding of two parts of SPD-CD40L. RAMPAGE results showed that 95.3% residues clustered in favored region, therefore formation of desired structure between the predicted structures is probable.
**Conclusion:** We designed the stable soluble multi-trimeric structure of CD40L using SPD that seems to increase clustering of CD40 on the target cells surface and mimics the membranous CD40L orientation.

**Keywords:** CD40L, Multimeric protein, Surfactant protein D, TNF family

ICIA2018\Computational Immunology and Systems Biology\Poster Discussion\8451
**8451**

# Assessment the Structure and Function of The Model of Cintredekinbesudotox Based on *In-silico* Investigation

Fatemeh Samiezadeh Mashadi[1], Bahareh Hasani Sabzevar[*2], Nazanin Gholampour Faroji[2], Mohammad Rastegar Moghadam[1,2], Aliakbar Haddad Mashadrizeh[3], Mehran Akbari[1]

*1. Pharmaceutical Research Group, Khayyam Bioeconomy Institute, Mashhad, Iran*
*2. Structural biology and Bioinformatics Research Group, Khayyam Bioeconomy Institute, Mashhad, Iran*
*3. Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran*

**Background:** Immunotoxins are effective and promising drugs for target therapy of cancer, which in turn in nowadays has a special prospective in the healthcare system. In this regard, IL-13-PE38QQR which named as Cintredekinbesudotox is one of them which has developed for Glioma healing up to level 2 therapies. Therefore, recognizing the structure and function of this drug could be provide approach for its production based on knowledge, as well as its optimization for achieve higher levels of therapies which are considered in this study based on in-silico biology.
**Methods:** In this study, NCBI and RCSB databanks were used to achievement to the protein sequences and 3D structures of this drug. Modeller software were employed to assembling the fragment sequences of drug via homology modeling method. Moreover, ERRAT, and RAMPAGE programs were used to determine the quality of the structures of the models. Evaluation the thermostability of the model of this drug in quasi-physiological condition was carried out by GROMACS using GROMOS force fields. Finally, the functionality features of this drug were assayed by their affinity to IL13-R as corresponding antigen as well as immunogenicity properties by HADDOCK and IEDB programs, respectively. Visualizing the structures and interactions was performed via The PYMOL software.
**Results:** Surveying the sequence of IL-13-PE38QQR has led to reveal domains of IL-2 and *Pseudomonas* Exotoxin A in its context. Modeling and assembling of these fragments with flexible linker led to the production 10 models with various quality and structure. The best model showed stability structure under quasi-physiological circumstance with reasonable immunogenicity. Moreover, its affinity to corresponding antigen compared to negative control was confirmed.
**Conclusion:** Generally, the results of this study has led to introduced a model of IL-13-PE38QQR immunotoxin, which is suitable in the structure and function in quasi-physiological condition, so can using for its production.

**Keywords:** Immunotoxin, Cintredekinbesudotox, Glioma, Databases, GROMACS

ICIA2018\Computational Immunology and Systems Biology\Poster Discussion\9866
**9866**

## Common Gene Networks Contribute in Hashimoto and Urticaria Disorders

Nazanin Atieh Kazemi sefat[1] , Saeed Talebi[2]

*1. Department of Medical Immunology, Faculty of Medical Sciences, Tarbiat Modares University (TMU), Tehran, Iran*
*2. Department of Medical Genetics and Molecular biology, Iran University of Medical Sciences (IUMS), Tehran, Iran*

**Background:** Urticaria is common in some patients with Hashimato thyroiditis. In both disorders the immune system function is affected. In this study we have determined involved common gene networks in these disorders according to the gene ontologies (GO) and molecular pathways.
**Methods:** A comprehensive literature search was done to find important suggested genes in hashimato thyroiditis or urticaria. In order to determine the gene networks that were involved, network based enrichment analysis for KEGG and NCI pathway, Biological process, Molecular function, Cellular component was done (on common genes in these disorders) using "EnrichNet" Online tool. Pathways or processes by 5 or more affected genes and significant fisher exact test ($p<0.05$) were analyzed.
**Results:** We have found 144 important genes contribute in urticaria and 132 genes involved in hashimato thyroiditis. 28 genes were common in both disorders. In KEGG pathways most genes were contributed in "Cytokine-cytokine receptor interaction. In both NCI pathways and GO biological processes, IL-12 mediated signaling pathway covered most genes. Gene products typically localized to both extracellular space and plasma membrane in GO cellular component. Gene products with protein binding and cytokine activity were more common in analysis with GO molecular function. IFNG, IL18, IL2 and IL4 were most important cytokine genes and IL2RA and IL2RB were the most common cytokine receptor genes in these pathways.
**Conclusion:** Cytokine-cytokine receptor gene network specifically the interaction between Interleukin 2 and Interleukin 2 receptor in IL12-mediated signaling pathway may contribute in both urticaria and hashimato thyroiditis.

**Keywords:** Enrichment Analysis, Urticaria, Hashimato thyroiditis

ICIA2018\Computational Immunology and Systems Biology\Poster Discussion\9986
**9986**

# Accurate Prediction Approach Result in Increased Probabilities of Victory: has-miR-27 is a New Potential Autoimmune-Deregulated miRNA

Vahedeh Hosseini[1], Abdolhakim Hosseini[2], Kobra Mehdinejadiani[3], Shayesteh Mehdinejadiani[4], Vahid Kia[5], Samira Mohammadi Yeganeh[1]

*1. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Department of Biotechnology, School of Advanced Technologies in Medicine,, Hamedan University of Medical Science, Hamedan, Iran*
*3. Department of Immunology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*4. Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.*
*5. Zanjan University of Medical Sciences, Zanjan, Iran*

**Background:** Th17 cells, a subset of CD4 $^{+}$ T helper cells, are attracting considerable interest due to their critical role in the pathogenesis of different autoimmune disorders. MicroRNAs are a category of small non-coding RNA that function in a variety of cellular processes by silencing the target mRNA. Several positive and negative regulators govern the Th17 differentiation. Dysregulation of miRNA expression is associated with different autoimmune diseases. The aim of our research was to expand current knowledge of miRNA role as a Th17 differentiation regulator.
**Methods:** Our proposed search direction for predicting an appropriate miRNA that regulate Th17 differentiation is as follows: systematic literature mining; search in miRvestigator; predict the target genes of the selected miRNA in TargetScan and miRWalk, and final approval in Gene Expression Omnibus databases.
**Results:** We carried out a systematic literature mining for autoimmune-deregulated mRNAs of Th17 differentiation regulators. Then, using miRvestigator toll, we predicted a miRNA that can target over-represented sequence motif of submitted genes (negative regulators of Th17 differentiation). Among all suggested miRNAs, hsa-miR-27 can target over-represented sequence motif of submitted genes (*P-value*= 1.5e-05). Further bioinformatics studies were performed by TargetScan toll and integrative miRWalk database. To identify the expression pattern of has-miR-27 in Th17 differentiation, microarray dataset GSE75909 was downloaded from the Gene Expression Omnibus database and this data was analyzed by GEO2R to determine differentially expressed miRNAs (DEMs). The result of microarray data shows that has-miR-27 can promote the differentiation of Th17.
**Conclusion:** Target predictions of has-miR-27 and microarray data indicate its interaction with negative regulators of Th17 differentiation. Therefore, it can be reasonably assumed that inhibitors of hsa-miR-27 can be used as a treatment for autoimmune diseases.

**Keywords:** miRNA, Th17 cells, autoimmune disease

# Diagnostic Methods in Immunology

# Poster Discussion

ICIA2018\Diagnostic Methods in Immunology\Poster Discussion\7573
**7573**

## A Novel Sampling and Diagnostic Method for Cutaneous Leishmaniasis

Yasaman Taslimi[1], Pardis Sadeghipour[1], Sima Habibzadeh[1], Vahid Mashayekhi[2], Hossien Mortazavi[3], Housein Malekafzali Ardakani[1], Ingrid Muller[4], Pascale Kropf[4*], Sima Rafati[1*]

1. *Department of Immunotherapy and Leishmania Vaccine Research, Pasteur Institute of Iran, Tehran, Iran*
2. *Skin center, Mashhad Medical School, Mashhad, Iran*
3. *Razi Skin Center, Tehran Medical School, Tehran, Iran*
4. *Section of Immunology, Department of Medicine, Imperial College London, London, United Kingdom*

**Background:** *Leishmania major* and *tropica* are causative agents for CL in Iran causing either permanent lesions or scars in the skin. Accurate diagnosis of CL is crucial in order to the use of chemotherapy with highly toxic drugs such as glucantime. The gold standard for CL diagnosis involves invasive sampling and recognition of parasite in smears and cultures. Albeit these techniques are highly specific but they are not sensitive enough and require technical expertise. Additionally these approaches run the risks of secondary infection and considerable discomfort, especially for children and sensitive body areas. In this report, we present a novel rapid (>1minute), user-friendly and painless sampling method (tape-stripping) of skin to collect DNA from the lesion to perform accurate non-invasive diagnosis method.
**Methods:** More than 100 patients suspected of CL, 20 fungal-infected lesions and 50 areas of normal skin were examined. DNA isolated from the skin tape-stripping samples, were amplified using kDNA1 minicircle primer. In parallel, DNA was subjected to ITS1 PCR-RFLP for species identification. Standard reference strains of *L. major* and *L. tropica* were used as positive controls.
**Results:** Among 80 acute cases, half of them were diagnosed as *L. tropica*the rest diagnosed as *L. major*, additionally among 30 chronic CL samples, more than half of them were diagnosed as *L. tropica*, 11 samples as *L. major*. Among 8 healed cases, 7 cases were diagnosed as *L. tropica* and one sample as *L. major*.
**Conclusion:** In this study, we obtained DNA from around 170 CL patients and healthy control for PCR performance using of non-invasive diagnostic sampling. We confirmed the *Leishmania* infection as well as the species in both acute and chronic form. Our next step is to design a point of care diagnosis method for CL based on mentioned non-invasive sampling method.

**Keywords:** Cutaneous Leishmaniasis, Non-invasive Diagnosis, Skin-tape Stripping

ICIA2018\Diagnostic Methods in Immunology\Poster Discussion\7697
**7697**

# Detecting Epithelial Cell Adhesion Molecule in Human Carcinoma Cell Lines Using a Developed Polyclonal Antibody

Mehrzad Ghari[1], Saeideh Milani[2], Gholamreza Javadi[1], Ali Ahmad Bayat[2], Reza Hadavi[2], Hodjattallah Rabbani[2], Fatemeh Ghaemimanesh[2]

*1. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
*2. Antigen and Antibody Engineering Department, Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*

**Background:** Epithelial cell adhesion molecule (EpCAM) is a transmembrane glycoprotein mediating epithelial-specific intercellular celladhesion. EpCAM is also involved in cell signaling, migration, proliferation and differentiation. The overexpression of EpCAM in various types of human carcinoma makes this molecule to be considered as an interesting diagnostic marker. Therefore, the aim of this study was to produce a specific polyclonal antibody (pAb) against EpCAM to be used as a diagnostic tool in human carcinomas.
**Methods:** A white New Zealand rabbit was immunized using keyhole lympethemocyanin (KLH)-conjugated peptide derived from EpCAM molecule. The antibody was purified from immunized rabbit sera using a peptide- affinity chromatography column. The capability of pAb in detection of EpCAM was investigated in different protein read-out systems.
**Results:** Our results showed that anti-EpCAMpAb specifically detects EpCAM in various human carcinoma cell lines of MCF7, A549, SW480 and A431 by ICC. The IHC experiments showed the expression of EpCAM in human prostate and bladder carcinoma tissues.
**Conclusion:** The developed pAb might be used as a diagnostic tool in human carcinomas in ICC and IHC applications.

**Keywords:** Polyclonal Antibody, EpCAM, Cancer, Detection

ICIA2018\Diagnostic Methods in Immunology\Poster Discussion\8099
**8099**

# Determination of *Helicobacter pylori* Antigen in Stool Samples and Comparison with Rapid Urease Test in Patients Suspected of Helicobacter Infection

Reza Marvinam[1], HosseinHadinedoushan[1], Mahdi Dehghanmanshadi[1], FatemeZare[1]

*1. Department of Immunology, Reproductive Immunology Research Center, ShahidSadoughi University of Medical Sciences, Yazd, Iran*

**Background:** *Helicobacter pylori (H.Pylori)* is the microorganism that infects nearly half of word's population. There are several invasive and non-invasive methods for diagnosis of infection. The main objective of this study was to evaluate antigen of *H. Pylori* in feces with *H. pylori* stool antigen (HpSA) test and comparison with rapid urease test (RUT) in the patients suspected to be infected.
**Methods:** one hundred thirty-seven subjects (56 males, 81 females) were recruited from those patients undergoing a gastrointestinal endoscopic examination in the endoscopy units of Shahid Sadoughi University of medical sciences. One biopsy specimen was obtained from the stomach and each biopsy specimen per subject was tested for the presence of urease using the commercially available CLO test. Stool specimens were taken concurrently with the endoscopic examination and tested by Enzyme-linked immunosorbent assay (ELISA) method for presence of HpSA. In this study, RUT was considered as a gold standard test.
**Results:**The mean age was 40.4±1.12 years. 13.3% of samples were shown HpSA-positive and negative RUT and 12.9% were shown HpSA-negative and positive RUT. Sensitivity and specificity of HpSA test was 86.6% and 87.1%, respectively. Positive and negative predictive values and accuracy were 89%, 84.3%, 86.2%, respectively.
**Conclusion:** Our findings showed that the stool enzyme immunoassay for *H.pylori*is a useful method for the primary diagnosis of *H. pylori* in the patients suspected to be infected.

**Keywords:** ELISA, *H.Pylori,* HpSA, Rapid Urease Test

ICIA2018\Diagnostic Methods in Immunology\Poster Discussion\8570
**8570**

## Optimization of HER2-based ELISA Immunoassay Using Trastuzumab

Maryam Ahmadzadeh[1,2], Elham Mohit[1], Leila Nematollahi[3], Farzaneh Farshdari[4]

*1. Department of Pharmaceutical Biotechnology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Student's Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Biotechnology Research center, Pasteur Institute of Iran, Tehran, Iran*
*4. Department of Biotechnology, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic azad University, Tehran- Iran (IAUPS)*

**Background:** Enzyme-linked immunosorbent assay (ELISA) is a sensitive, specific, reproducible and fast method for detection of antigen-antibody reactions. Currently, ELISA is the most common immunoassay approach used to evaluate the biological activity of recombinant whole or fragment of antibody. However, due to the complex nature of immunoassays that involve multiple assay steps using multiple reagents, many factors affect the ELISA performance and should be optimized.
Trastuzumab, a humanized monoclonal antibody directed against the epidermal growth factor receptor 2 (HER2), is used for the treatment of breast cancer patients who overexpress the HER2 receptor. Herein, commercial Trastuzumab was used as a positive control in developing HER2- based ELISA immunoassay for anti-HER2 antibodies/antibody fragments targeting the same epitope as Trastuzumab. The aim of this study was to develop an acceptable HER2-based ELISA procedureusing trastuzumab as positive control. Therefore, different factors such as antigen concentration, the type of blocking agent and sample concentration were optimized.
**Methods:** Accordingly, serial dilution of HER2 antigen (7.8 to 500 ng/µL), skim milk 5% and BSA (1 & 3 %) two type of blocking agent and different concentration of trastuzumab (0, 1, 10, 100 ng/µL) were analyzed. Anti-human IgG (whole molecule) peroxidase-conjugate (1:5000) and 3, 3′, 5, 5′-Tetramethylbenzidine (TMB) were used as detection antibody and substrate, respectively. The reaction was stopped using sulphuric acid and absorbance was recorded at a wavelength of 450 nm.
**Results:** The lowest concentration of HER2 antigen which has the ability to detect trastuzumab was 7.8 ng/µL. Skim milk 5% demonstrated minimal non-specific background and was selected as the optimum blocking agent. Also, the optimal concentration of trastuzumab was between 10 and 100 ng/µL which fallen within the linear portion of the curve (absorbance against concentration of trastuzumab).
**Conclusion:** It was demonstrated that the type of blocking agent is an important factor affecting the performance of the HER2-based assay. The result of this study led to the improvement of the HER2-base ELISA assay and can be used for developing the ELISA method for other anti-HER2 antibodies.

**Keyword:** ELISA, Trastuzumab, HER2 antigen, Optimization.

ICIA2018\Diagnostic Methods in Immunology\Poster Discussion\8622
**8622**

# Assessment of Serum Level of Stromal Derived Factor–1α (SDF–1α) in Serum of Patients with Sepsis at Admission and Discharge in Ali–ebne–Abitaleb at 2013

Marzie Motaghy[1], Mohammad Kamali[2], Gholamhossein Hasanshahi[3]

1. *Postgraduate student of Hematology, Kerman University of Medical Sciences, Kerman, Iran*
2. *Medical Student, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
3. *Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

**Background:** Sepsis is a major cause of death and the most common cause of death in the intensive care unit at a rate of 30 to 70 percent. SDF-1α as an ELR negative CXC chemokine is effective in Call of inflammatory cells in the blood and the role of chemokine activity by a process known as angiogenesis. This study compared the levels of these chemokines in patients with sepsis at admission and discharge based on age and gender studies.
**Methods:**Fifty four sepsis patients who were enrolled to the study Peripheral blood samples were collected from patients in parallel with demographic data. Methods: SDF-1α (CXCL12) levels in the samples were measured by ELISA. SPSS, T-test and Chi-square and ANOVA were utilized for data analysis.
**Results:** The 54 patients participating in the project, 41 were male (75/9%) and 13 females (24/07%) respectively. The minimum age of participants was 51 years and a maximum of 90 years in the project and the mean age was 69/13 years. The mean values of serum chemokines in patients with severe sepsis in the peripheral blood at the time of hospital discharge was significantly reduced and the differences were statistically significant differences (P <0/0001). Average concentrations at admission and discharge were 222/780 and 58/980 respectively. Compared to the overall mean concentration of serum chemokines in patients with severe sepsis at admission by sex and age in peripheral blood concentrations were approximately equal in terms of differences not statistically significant (P =0/564, P =0/818). Compared to the overall mean concentration of serum chemokines in peripheral blood of patients with sepsis at the time of discharge based on sex, age and concentration was almost the same in terms of the differences was not statistically significant (P = 0/193 and P =0/816).
**Conclusion:** According to the results of our study showed that the concentration of this chemokine was not directly associated with age and sex. Finally, our study suggest that SDF-1α (CXCL12) could possibly use as an early diagnosis marker for sepsis and perhaps other systemic inflammatory syndrome as a new treatment.

**Keywords:** Sepsis, SDF-1α, Inflammatory cells.

ICIA2018\Diagnostic Methods in Immunology\Poster Discussion\9750
**9750**

## The Exact Method for Determining the Dose and Type of Chemotherapy Drug

Amirhossein Alimohammadian[1], Negin Maleki[2]

1. *Member of the brilliant talent of young and elite peoples youth club of Ardabil Islamic Azad University, Iran*
2. *Department of medical laboratory, Ardabil branch, Islamic Azad University, Ardabil, Iran*

**Background:** One of the ways to cure various types of cancer and even after chemotherapy. All types of chemotherapy drugs are somehow inhibitors of cancer cells, but the main problem is that no chemotherapy drug has any significant effect on any type of cancer cell and There is no general method for testing the type and dosage of chemotherapy and the oncologist, based on the type of cancer and its origin, is always using an experiment with an error test, despite the multiplicity of chemotherapy drugs. Especially in malignant tumors, the passage of time with the initial test and error is very unfortunate and irreparable.
**Methods:** An ELISA plate containing tissue and cell in a suitable medium from RPM or any other medium (for breast cancer cells, prostate cancer, esophageal stomach and leukemia and ...) and a supplement plate containing a chemotherapy drug that was placed on an ELISA plate after 24 hours. Ultimately, an NBT resuscitation spectrometer was detected in aqueous ampullazer with specific wavelengths controlled by the drug and determined by regimen of the drug.
**Results:** This method makes it possible to customize the chemotherapy of each patient and monitor the stages of treatment, predict and control possible complications, and prescribe appropriate drugs for the continuation of treatment.
**Conclusion:** With this method, it is possible to diagnose the effect of the drug that is requested by the oncologist and the physician, and calculated by the laboratory.

**Keywords:** Cancer, Chemotherapy, NBT, Diagnose

ICIA2018\Diagnostic Methods in Immunology\Poster Discussion\9818
**9818**

# Urinary levels of Endothelin-1, Monocyte Chemotactic Peptide-1 and N-acetyl- Glucosaminidase as Prognostic Markers for Severity of Obstruction in Hydronephrotic Neonates

Fatemeh Ezzatifar[1,2], Hamid Mohamad Jafari[1], Alireza Rafiei[1]

*1. Molecular and Cell biology Research Center , Faculty of Medicine , Mazandaran University of Medical Sciences , Sari , Iran*
*2. Department of Immunology Mazandaran University of Medical Sciences, Sari, Iran*
*3. Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran*

**Background:** Antenatal hydronephrosis (AH) is found in 0.5% of neonates, the majority of them had obstructive entity such as UOJO or UVJO. In order to diagnose severity of obstruction, we required invasive testing and exposure of the neonate to radiation. The urinary levels of Endothelin-1(ET-1), monocyte chemotactic peptide-1(MCP-1) and N-acetyl- glucosaminidase (NAG) and their ratios to creatinine were assessed in severely hydronephrotic newborns and were compared to those with milder forms and those without obstruction.
**Methods:** The neonates with a history of prenatal hydronephrosis were enrolled in three groups based on imaging study results, group 1 the neonates with severe forms of obstruction who need surgical intervention because of functional impairment and group 2 those with milder forms of obstruction without functional impairment. We measured the random urinary levels of ET-1, MCP-1and NAG, their ratios to creatinine and cut-off values were calculated and finally ROC curve was drawn.
**Results:** Fifty-nine neonates were enrolled in three groups; 24 patients with severe obstruction (Group 1), 18 neonates with milder forms of obstruction (group 2), and 17 neonates without any obstruction (group 3). The urinary ET-1, NAG, MCP-1 and normalization to creatinine, ET-1/Cr and NAG/Cr values were not significantly different between group 1 and 2 patients but urinary MCP-1/Cr ratio was significantly higher in severely obstructive patients than those with milder obstruction. To compare groups 1 and 2 a cut-off value of ET-1/Cr was measured as 0.5709 ng/mg (sensitivity [sens] 75 %, specificity [spec] 67 %) , of MCP-1/Cr was 0.927 pg/ml (sens 77 %, spec 72%) and the cut-off value of NAG/Cr ratio was 1.1913 (sens 62 %, spec 67 %) .
**Conclusions:** Evaluation of urinary levels of MCP-1/Cr ratios may help us to differentiate the newborns with severe obstructive hydronephrosis that are candidates of surgical intervention.

**Keywords:** Antenatal hydronephrosis, Obstruction, Neonates, Endothelin-1

ICIA2018\Diagnostic Methods in Immunology\Poster Discussion\9877
**9877**

# Determination of CD4, CD8 and IL-8 Level in Serum and BAL Fluid of Patients with Pulmonary Anthracosis

Atefeh Abedini[1]; Arda Kiani[2]; Mehdi Ramazanpour[3]; Hamidreza Jamaati[1]; Esmaeil Mortaz[4]; Fatemeh Razavi[1]

*1. Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Tracheal Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Department of Pulmonology, Qom University of Medical Sciences, Qom, Iran*
*4. Clinical Tuberculosis and Epidemiology Research Center, National Research Institute for Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Anthracosis is the black pigmentation of bronchial mucosa. This disease is caused by the deposition of carbon particles, silica, quartz, etc. In the mucosa, sub mucosa, and inside the macrophages and in some cases have been reported with pulmonary tuberculosis (TB). The aim of the present study was to compare serum levels and fluid BAL of CD4/CD8 ratio and IL-8 as diagnosis and prognosis biomarkers for anthracosis.
**Methods:** 60 patients who referred to Masih Daneshvari Hospital, 30 anthracosis patients that confirmed with bronchoscopy were included as the cases. 5 ml of BAL sample and 5 ml of blood sample containing anticoagulants were taken from these individuals and sent to the Immunology Laboratory for analysis. Patients without anthracosis on bronchoscopy who were suspected to have tuberculosis were included in the control group.
**Results:** Thirty cases and 30 controls were included in the study. Among them, 60% were women and 40% were men. There were no significant differences in CD4/CD8 ratios in anthracosis compared to control group and no significant differences was observed in IL-8 as well. Pulmonary tuberculosis was confirmed in 88.9% of patients with anthracosis, which was significantly higher than control group.
**Conclusion:** These results suggest that changes in serum levels and BAL fluid of CD4/CD8 and IL-8 do not play an important role in the diagnosis or pathogenesis for anthracosis. Also due to the strong association of anthrocosis and pulmonary tuberculosis, TB should be considered in patients with anthracosis, which in turn can lead to the early diagnosis and treatment of their patients.

**Keywords:** Anthracosis, CD4/CD8, IL-8, Pulmonary tuberculosis

ICIA2018\Diagnostic Methods in Immunology\Poster Discussion\9971
**9971**

# Study of STING Gene Expression in Patients with Multiple Sclerosis in ChaharmahalvaBakhtiyari in 2015

KambizYousofzadeh Eshkevari[1], NahidJivad, HoseinTeymoori

*1. Cellular and molecular research center, Shahrekord University of medical sciences, Shahrekord, Iran*

**Background:** Multiple Sclerosis (MS) is an autoimmune disease which begins by exposure to a pathogen in genetically susceptible individuals. There is still no specific approved gene or collection of genes for MS. STING (Stimulator of interferon genes) also known as transmembrane protein 173 (TMEM173) for induction of type I interferon. In this study, STING gene expression levels in MS patients were measured.
**Methods:** This study is a comparative cross-sectional study. Patients were selected from the entire ChaharmahalvaBakhtiyari Province. 25 new diagnosed, 25 treated by interferone beta-1α, 25 treated by interferone beta-1β MS patients as well as 25 healthy individuals who referred to Kashani Hospital located in Shahrekord city in Iran, were participated in the study. Gene expression levels were determined by Real-Time PCR Device with using the ΔΔCt method. The data were analyzed with statistical software GraphPad Prism version 5 and SPSS version 22.
**Results:** STING gene expression in newly diagnosed patients was more than the control group ($P<0.0001$) and in patients treated with interferon was lower than the control group ($P<0.0001$).
**Conclusion:** This study showed that gene expression of STING can be used to diagnose and treat MS patients by using Interferon beta-1α and Interferon beta-1β, especially among high-risk patients (such as first degree relatives). It is also possible to use this method to better determination of prognosis of disease.

**Keywords:** Multiple Sclerosis, STING Gen Expression, Interferon

ICIA2018\Diagnostic Methods in Immunology\Poster Discussion\10004
**10004**

# Urinary Levels of Lipoarabinomannan Do not Correlate with Active Tuberculosis in HIV-infected Patients Even in Those with Advanced Immunosuppression

*EsmaeilMortaz[1, 2, 3], MiladMoloudizargari[4], MajidMarjani[1], Afshin Moniri[1], Parissa Farnia[5], Mohammad Varhram[5], Johan Garssen[3,6], Mehdi KazemporDizaji[5], Ian M. Adcock [7], Payam Tabarsi[1,]*

*1. Clinical Tuberculosis and Epidemiology Research Center, National Research Institute forTuberculosis and Lung Disease (NRITLD), Shahid Beheshti University of Medical Sciences,Tehran, Iran,*
*2. Department of Immunology, school of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran,*
*3. Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, Utrecht, Netherlands ,*
*4. Department of Immunology, school of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** The diagnosis of active tuberculosis (TB) in HIV-positive patients remains a global challenge due to paucibacillary presentations, low sensitivity with sputum based diagnostics, and high rates of extra-pulmonary TB. These issues have led to the pursuit of non-sputum based diagnostics, including the development of a commercially available ELISA-based urinary assay as well as a Lateral Flow (LF)-LAM assay for the detection of mycobacterial cell wall glycolipid lipoarabinomannan (LAM). We examined the specificity and sensitivity of urinary LAM ELISA in the diagnosis of TB in Iranian HIV-positive patients undergoing evaluation for TB. We also assessed whether there was an association between urinary LAM levels and total CD4+T cell count in HIV-TB co-infected patients.
**Methods**: Urine and blood samples were collected from HIV-positive patients with suspected TB infection. Urinary LAM levels were measured by ELISA and CD4+T cell counts were measured using a flow cytometer. Urinary LAM levels were compared between groups to determine the sensitivity and specificity of the test.
**Results:** 98 HIV-infected patients were eligible to be included in the study and were able to provide urine samples for LAM analyses. No significant association was found between the levels of urinary LAM in HIV-infected patients and the presence of TB infection. The urinary LAM test to discriminate between HIV-TB co-infected patients and HIV patients without TB had a sensitivity and specificity of 52% and 34%, respectively.
**Conclusion:** Our results do not support the use of urinary LAM ELISA test for the diagnosis of TB in HIV-positive patients due to low specificity even in patients with severe immunosuppression.

**Keywords:** LAM, TB, HIV, *Mycobacterium Tuberculosis*

ICIA2018\Diagnostic Methods in Immunology\Poster Discussion\10005
**10005**

## CD4 to CD8 Ratio as a Valuable Diagnostic Test in Iranian Pulmonary Sarcoidosis Patients

ArdaKiani*[1], Atefeh Abedini[2], Kimia Taghavi[2], Mihan Pourabdollah Toutkaboni[2], Esmaeil Mortaz[3], Mehdi Kazempour Dizaji[4]

*1. Tracheal Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Disease (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Department of Immunology, Faculty of Medicine, Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*4. Mycobacteriology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Sarcoidosis is a multi-organ disease with noncaseating granulomas that are comprised of T-helper/inducer ($CD4^+$) lymphocytes and scant cytotoxic ($CD8^+$) T-lymphocytes. This study is the first report from Iran that evaluates the ability of CD4:CD8 to detect sarcoidosis from other interstitial lung diseases (ILDs) in a large population.
**Methods:** Fifty patients with pulmonary sarcoidosis and 50 patients with non-sarcoidosis interstitial lung diseases (nsILDs) were included in the present study. Bronchoalveolar lavage (BAL) was performed using a flexible fiber optic bronchoscopy. After that, the samples were examined by flow cytometry method.
**Results:** The non-sarcoid group consisted of 50 patients in eight subgroups of the non-sarcoidosis interstitial lung diseases. As expected, CD4/CD8 ratio was significantly higher in sarcoidosis patients than non-sarcoidosis patients ($p<0.001$). The best cut-off point in detecting sarcoidosis from other interstitial lung diseases was 1.1, which had a sensitivity of 92% and aspecificity of 80%.
**Conclusion:** Bronchoalveolar Lavage flow cytometry is a safe and fast test and its use as the first stage of diagnosis of sarcoidosis confirms the disease in 92% of patients (sensitivity to the current study). Hence, only a few patients need to undergo an aggressive procedure.

**Keywords:** Sarcoidosis, Bronchoalveolar lavage, lymphocyte, $CD4^+$:$CD8^+$ ratio

# Immune Cell Therapy

# Poster Discussion

ICIA2018\Immune Cell Therapy\Poster Discussion\8079
**8079**

# Leukemia Inhibitory Factor (LIF) Modulates the Maturation of Murine Bone Marrow (BM)-derived Dendritic Cells

Atefeh Yaftiyan[1], Maryan Skandaryan[1], Amir Hossein Jahangiri[1], Seyyed Mohammad Moazzeni[1]

*1. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

**Background:** Dendritic cells (DCs) are professional antigen presenting cells which their activation and function have a major role in establishment of specific immunity responses. Different biological factors such as cytokines are involved in the development of immunogenic or tolerogenic functions of DCs. Leukemia Inhibitory factor (LIF) is a pleotropic cytokine from interleukin-6 family that play various roles in cell proliferation, differentiation and survival. Previous studies demonstrate that LIF is linked to several tolerogenic events and also to tolerogenic phenotype and function of immune cells; yet the exact effect of this cytokine on dendritic cells is not particularly identified. In current study, we investigate the probable effect of LIF on DC maturation.
**Methods:** In current study, immature dendritic cells were differentiated from mouse bone-marrow (BM) in a GM-CSF and IL-4 containing medium. To determine the role of LIF in maturation, the immature differentiated DCs were treated in four groups: with TNF-α (maturation inducer), TNF-α and LIF, LIF only, or without cytokine. The harvested cells were assessed for the expression of phenotypic surface markers, antigen uptake function and allogenic T-cell proliferation capacity.
**Results:** LIF treatment of dendritic cells while stimulating by TNF-α caused a significant decrement in the expression of MHCII, CD40 or CD86 molecules in comparison with TNF-α only stimulated cells; meanwhile it was accompanied with an increase in the antigen uptake function (an indication for a reduced functional maturation). The ability for induction of allogenic T-cell proliferation proved no statistically significant difference.
**Conclusion:** Based on these findings, LIF is efficiently effective in modulating the levels of phenotypic and functional maturation. Such overall state can lead to the development of semi-mature and tolerogenic DCs. This inquiry can lead to a more precise understanding of the mechanisms of dendritic cell-dependent regulation of immune responses.

**Keywords:** Leukemia Inhibitory Factor (LIF), Dendritic cell, Tolerogenic DC, Maturation

ICIA2018\Immune Cell Therapy\Poster Discussion\8331
**8331**

# Engineering Recombinant Lentivirus Expressing MBP Targeted Chimeric Auto Antibody Receptor (CAR) for Treatment of Multiple Sclerosis

Maryam sahlolbei[1], Jafar kiani[2], Seyed Mahmoud Hashemi [3]

*1. Department of Molecular Medicine Faculty of Advance Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran.*
*2. Department of Molecular Medicine Faculty of Advance Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran.*
*3. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Cancer treatment has been the subject of many researches for many years. Using and reinforcement your own immune system is a new way of treating the disease, which has made significant advances in the field today. In CAR T cell therapy, T cells are engineered and injected against specific antigens. For treating different types of cancer, the results are fairly good and many clinical trials are underway.
Recently, the treatment of autoimmune diseases has been considered by researchers in the field of biology. To treat autoimmune diseases, pathogenic autoimmune cells should be eliminated.
Chimeric auto-antibody receptor (CAR) is a strategy for the removal of autoreactive B-cell in autoimmune diseases. CAR-T cells may be an effective and global strategy for targeting auto-reactive B cells in antibody-mediated autoimmune diseases.
One of the reasons that causes multiple sclerosis, MS, as an autoimmune disease, is the auto-reactive B cells that make antibodies against the MBP protein. Our goal is to engineer T cells, which express CAR at their surface to target the autoreactive B cell expressing the anti-MBP antibody.
**Methods:** The CAR structure was designed and synthesized via bioinformatics, transmitted to the HEK293T cell via a third generation lentivirus.
**Results:** The CAR-MBP design was expressed at the cell surface of HEK293T and its expression was confirmed by flowcytometry.
**Conclusion**: Finding new ways to treat MS is a new hope for patients, and maybe MS can be converted into a treatable condition by increasing studies in this field and simultaneous use of different immunotherapy techniques.

**Keywords**: MS, chimeric antigen receptor, CAR T cell, autoimmune,

ICIA2018\Immune Cell Therapy\Poster Discussion\8338
**8338**

# Study of the Effect of Mesenchymal Stem Cell Derived Exosome- treated Dendritic Cells on T Cell Proliferation

Mehri Shahir[1], Esmaeil Mortaz[1], Seyed Mahmoud Hashemi [1]

*1. Department of Medical Immunology ,School of Medicine ,Shahid Beheshti University of Medical Sciences, Tehran ,Iran*

**Background:** DCs are professional antigen-presenting cells with functional plasticity. Regulatory DCs play an important role in the maintenance of immunological tolerance via the induction of T cell unresponsiveness or apoptosis, and generation of regulatory T cells. Mesenchymal stromal cells that are adult multi-potent cells have also been isolated from virtually all postnatal organs. Exosomes play an important role as a messenger in communications of cells. They are releasing from cells and activates/suppress important signaling. In this study we aim to investigate the effects of isolated exosomes from MSCs on the proliferation of T lymphocytes.
**Methods:** Culture of C57BL/6 mouse AD-MSCs**:** Murine adipose-derived MSCs were isolated from C57BL/6 Mice. Extracellular matrix was digested with type I collagenase, centrifuged and the pellet was then cultured in DMEM.
Isolation and purification of exosomes: Exosome was isolated by Exo-spin kit and evaluated by SEM. The protein concentration was detected by BCA kit.
Preparation bone marrow derived DCs: BM mononuclear cells were prepared from C57BL/6 mouse femur and tibia and then cultured in 6-well plates containing RPMI-1640 medium supplemented with 20 ng/mL GM-CSF and 20 ng/mL IL-4.
Lymphocyte proliferation assay: The BALB/C mouse derived splenocytes were labelled by carboxy fluorescein diacetate succinimidyl diester (CFSE) and then suspended in RPMI 1640 with DC groups (immature DC (iDC), iDC+LPS(1µg/ml), iDC+exosome(100µg/ml), iDC+exosome(100µg/ml ) +LPS(1µg/mL)) and cocultured at ratio of 1:3, 1:10 and 1:30 for 3 days in 96-well U-bottom plates. Finally 10000 cells were analyzed by Flowcytometry.
**Results:** The results showed that the T cell proliferation was decreased in the presence of MSC derived exosomes treated- DCs in comparison with control cells.
**Conclusion:** Our data suggests that the exosomes of MSC could effect on the proliferation of T cells in presence of DCs and modulates immunologic consequences.

**Key words:** Mesenchymal Stem Cell, Exosomes, Tolerogenic Dendritic Cell, In Vitro

ICIA2018\Immune Cell Therapy\Poster Discussion\8422
**8422**

**The Effects of Caffeine and Nicotine on Mesenchymal Stem Cell Functions**

Zahra Zeinaly[1], Seyyed Meysam[1], Abtahi Froushani[1]

*Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Mesenchymal stem cells (MSCs) have especially used in the cell therapy plans for autoimmune and inflammatory diseases. MSCs functions are controlled by various factors in the microenvironment that surround them, like nicotinic receptors and adenosine receptors. Here, we investigated the effect of caffeine (as adenosine antagonist) or nicotine on some functions of MSCs.
**Methods:** After the isolation, MSCs were incubated with different concentrations of caffeine (0, 0.1, 0.5 and 1 mM) and or with different concentrations of nicotine (0, 0.1, 0.5 and 1µM) for 48 hours. The survivability of cells was determined by MTT and neutral red uptake method over a 7-day period. After 90% confluence, the cultured plates were scratched. MSCs were visualized using a time-lapse microscope and the cells were maintained at 37ºC throughout the imaging period. Images were taken every 15 min over a 2-h period to estimate the regenerative potential of MSCs.
**Results:** Attained results indicated that caffeine at low to moderate doses cannot change the rate of vitality, proliferation and regenerative potential of MSCs. Caffeine at 1 mM and nicotine at any doses significantly reduced the vitality, proliferation and regenerative potential of MSCs.
**Conclusion:** Nicotine at any doses and caffeine at the logarithmic dose have shown to adversely affect the vitality, proliferation and regenerative potential of MSCs.

**Keywords:** Mesenchymal Stem Cell, Nicotine, Caffeine.
.

ICIA2018\Immune Cell Therapy\Poster Discussion\9839
**9839**

# The effect of Phagocytosis of Apoptotic Mesenchymal Stem Cells by Mouse Peritoneal Macrophage on iNOS and Arginase Activation

Maryam Ghahremani Piraghaj[1], Seyed Mahmoud Hashemi[*1], Hosein Ghanbarian[2], Sara Soudi[3]

*1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Faculty of Medical Sciences, Department of Immunology, Tarbiat Modares University, Tehran, Iran*

**Background**: Efferocytosis or phagocytosis of apoptotic cells by macrophages is very important to protect tissues inflammation because of releasing pro inflammatory intracellular ingredients. Mesenchymal stem cells have substantial immunosuppressive properties and many of studies have focused on the immunomodulatory properties of MSCs and its therapeutic implication for autoimmune diseases.
**Methods**: MSCs were isolated from abdomen adipose tissue of C57/BL6 mice. After optimal confluency of MSCs, macrophages were obtained from peritoneal cavity of C57/BL6 mice four days after thioglycollate injection. MSCs were exposed to UV irradiation at 254 nm for 20 min and followed by incubation in 37 with 5% CO2. After that apoptotic MSCs were added to macrophages and after phagocytosis of apoptotic cells by them (nearly 1.5 h), macrophages were washed with PBS and were incubate for additional 48 h for analysis of activation of iNOS and Arginase enzymes.
**Results**: Our study showed that phagocytosis of apoptotic MSCs limit activation of iNOS and production of Nitric Oxide and promotes activation of Arginase enzymes and production of Urea.
**Conclusion**: The phagocytosis of apoptotic mesenchymal stem cells, induce non-inflammatory phenotype in macrophages. So, injected Mesenchymal Stem Cells maintain their immunomodulatory properties even if they apoptosis in the body.

**Key words**: Macrophage, Mesenchymal stem cell, Efferocytosis, Phagocytosis

# Immunodeficiency

# Poster Discussion

ICIA2018\Immunodeficiency\Poster Discussion\5178
**5178**

# Role of Treg Cells (CD4 + CD25 + FOXP3 +) in Allergic CVID Patients

Nahid Eskandari[1], Roya sherkat[2] , Zahra Adnani Sadati[1], Vajiheh Ostadi[1]

*1. Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. neskandari@med.mui.ac.ir*
*2. Acquired Immunodeficiency Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*

***Background*:** Common Variable Immunodeficiency (CVID) is a primary immunodeficiency which is characterized by the failure of antibody responses such as reduction in antibody levels and an increased sensitivity to bacterial infections. The most important immunologic deficiency is the lack of immunoglobulin production by B cells. In addition, the defect and disturbance of the T cells, including the regulatory T cells (Treg), have also been proven to play a role in some clinical manifestations such as autoimmunity and splenomegaly. Protein P-STAT5b plays a key role in the function, survival and differentiation of Treg cells. The purpose of this study is to evaluate the role of Treg cells in CVID patients with allergic clinical symptoms.
**Methods:** Peripheral blood mononuclear cells were isolated from blood of 10 healthy volunteers and 13 patients with CVID before treatment with intravenous immunoglobulin and 10 allergic patients using *Ficoll density* gradient. The percentage of Treg cells in these individuals was measured using flowcytometry. Then Treg cells were isolated by immunomagnetic method and after culture and stimulation, percentage of expression of P-STAT5b protein in Treg cells was measured using flowcytometry.
**Results:** The percentage of Treg cells in CVID patients was significantly lower than control groups. The percentage of P-STAT5b in the CVID patients with allergic symptoms was significantly lower than the control group.
**Conclusion:** Some of the clinical manifestations of CVID diseases are not only due to the defect in the production of antibody, but also due to the defect in Treg cells, which can play a role in the pathogenesis of the disease.

**Keywords:** Common Variable Immunodeficiency, Regulatory T cells, P-STAT5b protein, Allergy

ICIA2018\Immunodeficiency\Poster Discussion\5300
**5300**

# Mannose-Binding Lectin Protein Deficiency among Patients with Primary Immunodeficiency Disease Receiving IVIG Therapy

Sina Abdolrahim Poor Heravi[1], Gholamreza Azizi[1, 2], Asghar Aghamohammadi[1,2]

*1. Research Center for Immunodeficiency, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran*
*2. Primary Immunodeficiency Diseases Network (PIDNet), Universal Scientific Education and Research Network (USERN), Tehran, Iran*

**Background:** Primary immunodeficiencies (PIDs) are inherited disorders in which one or several components of the immune system are defective. Immunoglobulin replacement therapy is the mainstay of treatment for patients with impaired antibody production. However, recurrent infections would continue to occur in some patients due to the other high frequent concomitant defects, such as mannose-binding lectin (MBL) deficiency.
**Methods***:* A total of 51 PID patients participated in this cross-sectional study. A detailed questionnaire was completed by interviewing patients in order to record demographic, clinical and laboratory data. The levels of MBL were determined in the serums of patients by a sandwich enzyme-linked immunosorbent assay (ELISA) technique.
**Results***:* MBL deficiency was found in 29.4% of cases; 11.8% patients had mild, 3.9% patients had moderate and 13.7% patients had severe MBL deficiency. In patients with MBL deficiency, the rate of meningitis, sepsis, pneumonia, and otitis were higher than patients with normal MBL levels. Immunoglobulin replacement therapy reduced the rate of infectious complications in PID patients; however, these reductions were more apparent in patients with normal MBL levels than patients with MBL deficiency.
**Conclusion***:* Antibody deficient patients with a concomitant immune defects in MBL production have higher rates of recurrent infections despite receiving Immunoglobulin replacement therapy.

**Keywords:** Primary immunodeficiency, mannose-binding lectin, infection.

ICIA2018\Immunodeficiency\Poster Discussion\7836
**7836**

# Increased Expression of B lymphocyte Induced Maturation Protein 1 (BLIMP1) in Patients with Common Variable Immunodeficiency (CVID)

Faezeh Abbasi Rad[1], Nafiseh Esmaeil[1], Mazdak Ganjalikhani Hakemi[1], Roya Sherkat[2]

*1. Department of Immunology, school of Medicine, Isfahan Isfahan University of Medical Sciences, Isfahan, Iran*
*2. Aquired Immunodeficiency Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background:** Common variable immunodeficiency (CVID) is a primary immune deficiency disorder characterized by hypogammaglobulinemia and defect in response to vaccines. B cells maturation and differentiation are defective in this disorder, and CVID patients demonstrate commonly reduced memory B cells and antibody-secreting plasma cells numbers. Since the BCL6 and BLIMP1 molecules are two important transcription factors in the maturation of B cells to plasma cells, we evaluated the expression levels of BCL6 and BLIMP1 in B lymphocytes from peripheral blood in patients with CVID.
**Method:** Blood samples were collected from 14 patients with CVID and 14 healthy donors. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll density gradient separation. Subsequently, CD19 + B cells were purified using MACS. The expression of protein and transcriptional levels of BCL6 and BLIMP1 were assessed using flowcytometry and Real-time PCR, respectively.
**Results:** Our results showed that the percentage of plasma cells in patients was significantly lower than control subjects. Also the expression levels of BLIMP1 were significantly higher in patients compared to control subjects. In addition, we found that although the expression of BCL6 was slightly higher in patients compared with, this difference was not significant.
**Conclusion:** Our findings suggest that increased expression levels of BLIMP1 and BCL6 could be involved in defective maturation of B cells in CVID patients and provide mechanistic insights into the pathogenesis of this disorders.

**Keywords:** CVID, Plasma cell, BCL6, BLIMP1

ICIA2018\Immunodeficiency\Poster Discussion\8319
**8319**

# Toll like Receptors Pathway in Common Variable Immune Deficiency (CVID) and X-linked Agammaglobulinemia (XLA)

Parsova Tavasolian[1], Asghar Aghamohammadi[2, 3], Farshid Noorbakhsh[1], Rouzbeh Sanaei[4], Mahsima Shabani[2,5], Nima Rezaei[1, 2, 6*]

*1. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
*2. Research Center for Immunodeficiency, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran*
*3. Primary Immunodeficiency Diseases Network (PIDNet), Universal Scientific Education and Research Network (USERN), Tehran, Iran*
*4. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*
*5. International Hematology/Oncology of Pediatrics Experts (IHOPE), Universal Scientific Education and Research Network (USERN), Tehran, Iran*
*6. Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Tehran, Iran*

**Background:** Common variable immunodeficiency (CVID) and X-linked agammaglobulinemia (XLA) are two major humoral immunodeficiencies, causing a high burden of morbidity and early age mortality in children. Although many studies have investigated the involved etiologic factors, the exact impaired pathway is yet to be elucidated. The activation of Toll-like receptors (TLRs) is considered to be essential in B cell activation, co-stimulatory molecule up-regulation, class-switch recombination, and antibody and cytokine production. Current studies focus on the role of TLRs and demonstrate the defects in different TLR pathways in immune cells of CVID and XLA patients.
**Methods:** Herein, we measured TLR-4 and TLR-9 RNA levels in peripheral blood mononuclear cells (PBMCs) of patients with CVID and XLA. Moreover, for understanding the functional defects in associated TLR pathways we evaluated the level of TNF-α and IFN-α production in PBMCs of patients with CVID and XLA. **Results:** We have found that in CVID and XLA patients, TLR-9 expression is not increased significantly after stimulation with CpG, the TLR-9 ligand, whereas lipopolysaccharide (LPS) induced TLR-4 expression is increased similar to PBMCs derived from healthy control group. IFN-α production was increased significantly in patients and healthy individuals after CpG stimulation but LPS stimulated TNF-α production was only increased in CVID patients and healthy controls.
**Conclusion:** Our data suggests that defects in TLR-9 activated pathways may be a result of decreased TLR-9 expression but defects in TLR-4 activated pathways are due to defects in TLR-4 downstream molecules activity. Although LPS induced TNF-α production is defected in XLA patients, the preserved IFN-α production in CVID and XLA patients despite decreased TLR-9 expression suggests that TLR-9 is not the key modulator in INF-α production in these patients.

**Keywords:** TLR-4 (Toll-Like Receptor-4), TLR-9 (Toll-Like Receptor-9), CVID (Common Variable Immunodeficiency), XLA (X-linked Agammaglobulinemia),

ICIA2018\Immunodeficiency\Poster Discussion\9954
**9954**

## Differentiation Classification of CVID Patients Based on B cell Subpopulation

Reza Yazdani[1, 2*], Mazdak Ganjalikhani Hakemi[2], Zahra Hamidi Esfahani[3], Nima Rezaei[1], Asghar Aghamohammadi[1]

*1. Research Center for Immunodeficiency, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Science, Tehran, Iran*
*2. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
*3. Department of Immunology, School of Medicine, Tarbiat Modares University, Tehran, Iran*

**Background:** Common variable immunodeficiency (CVID) is a heterogeneous disease, characterized by hypoaglobulinaemia leading to recurrent infections and various complications. The aim of this study was to classify CVID patients based on four known classifications (Paris, Freiburg, EUROclass, and B-cell patterns) by measurement of B-cell subsets and to assess the relation of each classification with clinical manifestations.
**Methods:** We measured all B-cell subsets as both absolute count and percentage in 30 CVID patients and 30 healthy individuals using four-color flow cytometry. Moreover, we evaluated antibody responses to pneumococcal vaccine in patients.
**Results:** A significant reduction in percentage of terminal B-cell subsets (total, marginal zonelike, switched memory, IgM-only memory, total memory B-cells and plasmablast) and absolute count of all B-cell subsets along with a strong increase in CD21 $^{low}$ B-cells has been observed in patients. Patients with splenomegaly and hepatomegaly were clustered in group Ia, smB + 21 low and group 1 based on known classifications, and significantly tended to have a decreased transitional and marginal zone-like B-cells count, as well as an increase in CD21$^{low}$ B-cell counts. Patients with lymphadenopathy, bronchiectasis and allergy had a significant decrease in absolute count of total memory, switched memory and total B-cells, respectively.
**Conclusions:** Classification of patients could provide useful information to guide clinicians in long-term follow-up of CVID patients. Our data demonstrate that it may be more accurate to use absolute counts of B-cell subpopulations in CVID patients because absolute counts of B-cell subsets are more associated with clinical manifestations compared with their percentages and also four known classifications

**Key words:** B-cell subsets, Common variable immunodeficiency, Classifications

ICIA2018\Immunodeficiency\Poster Discussion\9977
**9977**

## Heterogeneous features of Lipopolysaccharide-responsive Beige-like Anchor (LRBA) Deficiency in Siblings

Fereshte Salami [1], Gholamreza Azizi [2], Hassan Abolhassani [3, 4], Reza Yazdani [4], Asghar Aghamohammadi [4]

*1. University of medical sciences, Shahid Sadoughi yazd*
*2. Non-Communicable Diseases Research Center, Alborz University of Medical Sciences, Karaj, Iran; and Department of Laboratory Medicine, Imam Hassan Mojtaba Hospital, Alborz University of Medical Sciences, Karaj, Iran*
*3. Department of Laboratory Medicine, Karolinska Institute at Karolinska University Hospital Huddinge, Stockholm, Sweden; Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran*
*4. Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** LPS-responsive beige-like anchor protein (LRBA) deficiency is a novel primary immunodeficiency disorder (PID) caused by biallelic mutations in the LRBA gene that abolish its protein expression. Immune dysregulation as a consequence of LRBA deficiency leads to recurrent infections, autoimmunity, and enteropathy. Affected individuals mainly show reduced levels of at least two Immunoglobulin (Ig) isotypes. We reported on siblings from two different families with LRBA deficiency who were carrying the same mutation but had discordant serum Ig levels.
**Methods:** In this case report Whole Exome Sequencing (WES) has facilitated identification of genetic defects in these PID patients with the same mutation and variable clinical phenotypes.
**Results:** Mutations of LRBA result in diminished expression and a spectrum of clinical phenotypes that includes hypogammaglobulinemia, enteropathy, autoimmune disorders, respiratory infections, and combinations of these phenotypes. Patients with the same mutation in LRBA may present different clinical phenotypes or even be asymptomatic .We reported two immunologic phenotypes of LRBA deficiency in two female siblings, including hypogammaglobulinemia and normal Ig levels with autoimmunity. In summary, we reported two male siblings with LRBA deficiency with the same mutation (c.C4814G [p.S1605X, exon 30]) and discordant clinical and immunological presentations. The index patient had severe clinical complications, including chronic diarrhea, organomegaly, respiratory tract infection, and hypogammaglobulinemia, whereas his brother had no clinical complications and normal serum Ig and specific antibody levels.
**Conclusion:** Our findings are consistent with those of a report confirming that the same genetic mutation in the LRBA gene can manifest with a broad phenotypic spectrum and no genotype–phenotype correlation. Further studies are required to define the exact role of genetic and nongenetic parameters in the penetrance of LRBA deficiency.

**Keywords:** LRBA, Immunodeficiency, Hypogammaglobulinemia, Immunoglobulin

ICIA2018\Immunodeficiency\Poster Discussion\9979
**9979**

# Association of Mycobacterium Infections in Patients with Mendelian Susceptibility to Mycobacterial Disease with Venous Thromboembolism

Maryam Alinejad Dizaj [1], Esmaeil Mortaz [2]

*1. Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** An association between a hypercoagulable state and Mendelian susceptibility to mycobacterial disease (MSMD) has been established in a few studies; resultant thrombosis is considered rare.
**Methods:** In a case-control study, the prevalence of factor V Leiden, prothrombin G20210A and methyleneetertetrahy-drofolate reductase (MTHFR) C677T, A1298C mutations were investigated in mycobacterium-infected patients. The study comprised 30 patients with mycobacterial infections (invasive, disseminated and/or recurrent infections with Bacille Calmette–Guerin or non-tuberculosismycobacteria and Mycobacterium Tuberculosis with positive results for acid-fast bacilli and tuberculin skin tests) and 30 normal healthy controls. Forty female (66.7%) and 20 male subjects (33.3%) aged from 3 to 70 years were recruited into this study. Genotyping of targeted genes was performed by RT-PCR and cytokine TNF-a concentrations were quantified using a commercially available ELISA kit.
**Results:** Significant associations between mycobacterial infection and TNF- α production after stimulating peripheral blood mononuclear cells with LPS alone and with IFN- ϒ plus LPS were identified. Moreover, genotyping analysis in the studied population revealed a significant association between MTHFR c.677C>T (OR, 3.28; 95% CI, 1.35–7.92; $P < 0.05$), MTHFR c.1298A>C (OR, 2.33; 95% CI, 1.10–4.93; $P < 0.05$) and mycobacterial infection in affected patients, indicating susceptibility to venous thromboembolism according to previous studies.
**Conclusion:** Mycobacterium-infected patients had a significantly greater prevalence of MTHFR C677T and A1298C mutations than controls.

**Key words:** MSMD, MTHFR A1298C, MTHFR C677T, TNF- α.

ICIA2018\Immunodeficiency\Poster Discussion\9982
**9982**

**Susceptibility to Mycobacterial Disease Due to Mutations in IL-12Rβ1 in Three Iranian patients**

Maryam Alinejad Dizaj [1,2], Esmaeil Mortaz [3]

*1. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** In the last decade, autosomal recessive interleukin-12 receptor β1 (IL 12Rβ1) deficiency, the most common cause of Mendelian susceptibility to mycobacterial disease (MSMD), has been diagnosed in a few children and adults with severe tuberculosis in Iran.
**Methods:** Here, we report three cases referred to the Immunology, Asthma and Allergy ward at the National Research Institute of Tuberculosis and Lung Diseases (NRITLD) at Masih Daneshvari Hospital from 2012 to 2017 with Mycobacterium tuberculosis and nontuberculous mycobacteria infections due to defects in IL12Rβ1 but with different clinical manifestations.
**Results:** All three were homozygous for either an IL-12Rβ1 missense or nonsense mutation that caused the IL-12Rβ1 protein not to be expressed on the cell membrane and completely abolished the cellular response to recombinant IL-12.
**Conclusion**: Our findings suggest that the presence of IL-12Rβ1 deficiency should be determined in children with mycobacterial infections at least in countries with a high prevalence of parental consanguinity and in areas endemic for TB like Iran.

**Keywords**: IFN-γ , IL12RB1 , IL-12Rβ1 , IMD30 , MSMD , PID

# Immunogenetics

# Poster Discussion

ICIA2018\Immunogenetics\Poster Discussion\7687
**7687**

# Susceptibility Pattern of HLA-DRB1 and DQB1 Alleles and Haplotypes for Pemphigus Vulgaris in an Iranian Population

Ghasem Solgi[1], Fatemeh Ahmadi Motamayel[2], Mahsa Mahdian[2], Hamzeh Rezaei[1], Zohreh Zarei Ghane[1], Mehrdad Hajilooi[1], Hamidreza Abdolsamadi[2], Ali Mahdavinezhad[3]

*1. Department of Immunology, Medical School, Hamadan University of Medical Sciences, Hamadan, Iran.*
*2. Dental Research Center, Department of Oral Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.*
*3. Department of Genetic and Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*

**Background:** Genetic factors, particularly *HLA* class II genes have been implicated in pathogenesis of pemphigus vulgaris (PV). This study aimed to evaluate any correlation between HLA-DRB١ and DQB١ alleles and haplotypes frequencies and PV disease presentation in an Iranian population.
**Methods:** In this case-control study, 39 PV patients that diagnosed based on clinical and pathological criteria as well as 98 ethnic-matched healthy controls were included. HLA-DRB1 and HLA DQB1 specific alleles were determined by PCR-SSP methods. The alleles and haplotype frequencies were compared between both groups of the study as well as subgroups of the PV patients.
**Results:** Among *HLA*-DRB1 alleles, DRB1*04:02 ($P=2.3\times10^{-7}$), DRB1*04:03 (P=0.02) and DRB1*14 ($P=6.2\times10^{-5}$) were positively associated with PV and DRB1*15:01 (P=0.01) and DRB1*11:01 (P=0.007) showed negative association with PV disease. Also, HLA-DQB1*03:02 ($P=3.07\times 10^{-11}$) and DQB1*05:03 ($P=7.1\times10^{-6}$) showed positive and DQB1*02:01, DQB1*03:01 and DQB1*05:01 alleles showed negative association with disease incidence. Two frequent haplotypes, HLA-DRB1*04:02-DQB1*03:02 ($P=2.3\times10^{-7}$) and HLA-DRB1*14-DQB1*05:03 ($P=9.3\times10^{-5}$) conferred susceptibility to PV whereas, DRB1*11:01-DQB1*03:01 and DRB1*13:01-DQB1*06:03 showed protective role for PV.
**Conclusion:** Our finding corroborates somewhat a different pattern for susceptible/protective HLA class II alleles and haplotypes among PV patients in Iranians.

**Keywords:** HLA-DRB1, HLA-DQB1, Alleles, Pemphigus Vulgaris

ICIA2018\Immunogenetics\Poster Discussion\8286
**8286**

## rs352140 & rs187084 Polymorphisms in TLR9 Gene and Risk of Multiple Scrlosis

Saiedeh Erfanian[1], Maliheh Moradzadeh[2]

*1. Department of Advanced Medical Sciences and Technologies, School of Medicine, Jahrom University of Medical Sciences (JUMS), Jahrom, Iran*
*2. Golestan Rheumatology Research Center, Golestan University of Medical Sciences, Gorgan, Iran*

**Background:** Multiple sclerosis (MS) is a chronic inflammatory disease that degenerate myelin cells of the central nervous system (CNS), particularly in advanced societies
**Methods:** This study was conducted on 100 MS patients and 100 healthy people that were selected randomly by molecular study. After explaining the project and obtaining informed consent from patients and healthy subjects, 5 to 10 mL blood sample was taken from the cases. DNA samples were extracted using DNA extraction kit. To identify the gene region containing the polymorphism rs1044243, PCR method was used and then to enzymatic digestion, Afl II restriction enzyme for rs187084 polymorphisms and Bst UI restriction enzyme for rs352140 polymorphisms, was added to make a cutgel electrophoresis technique was used. The data collected were processed using the software spss 15.
**Result:** In genotype distribution, frequency of rs187084 polymorphism in healthy and patient groups, respectively was CC 10(10%) and 11(11.0%) (P=0.609), CT 65(65.0%) and 68(68.0%) (P=0.522) and TT 25(25.0%) and 21(21.0%) (P=reference) and no significant difference was observed between the two groups in any of the states. But in terms of the c allele frequency in rs187084 polymorphism among healthy subjects and patients with MS is 85(%42.5) and 110(%55) that two groups showed significant difference (P=0.016). In genotype distribution frequency of rs352140 polymorphism in healthy and patient groups, respectively was CC 25(%25) and 19(%19) (P=Reference), CT 53(%53) and 56(%56) (P=0.360) and TT 22(%22) and 25(%25) (P=0.340) and no significant difference was observed between the two groups in any of the states. The T allele frequency in rs352140 polymorphism among healthy subjects and patients with MS is 97(48.5%) and 94(47%) that the two groups showed no significant difference (P=0.841).
**Conclusion:** Despite the proof of association of rs352140 and rs187084 polymorphisms on TLR9 gene with MS in some study of out of Iran, our findings did not show any association in population of Fars province. However, we must consider this issue that besides the difference in terms of selection of the patients and controlling different study, some factors like: nationality, strips, nutrition, environmental factors and etc, must be analyzed and more studies need to be done to prove the role of this polymorphisms as an independent risk factor of MS.

**Key words:** Polymorphism rs352140, Polymorphism rs187084, TLR9 gene, MS disease

ICIA2018\Immunogenetics\Poster Discussion\8373
**8373**

**Bioinformatics Analysis of Vitiligo Relationship with Allergies**

Elmira shiuokhi[1, 2*], Seyed saadi hosseini[2, 3]

1. *Department Science and Research .University of Islamic Azad. Central Tehran*
2. *Lab. of Regenerative Medicine and Biomedical Innovations, Pasteur institute of Iran*
3. *Department of Biotechnology Faculty of Bioscience University of Shahid Beheshti*

**Background: Vitiligo is an autoimmune skin disease with depigmentation.** Many of vitiligo genes identified. These genes encode proteins that involved in immune regulation, several others play roles in cellular apoptosis and others involved in regulating functions of melanocytes. vitiligo involving a complex relationship between programming and function of the immune system. The purpose of this study is to investigate the bioinformatics of vitiligo disease and its relation with allergic diseases.
**Methods:** nucleotide and proteins sequences of 35 genes that were candidates for vitiligo formation and 31 genes involved in allergic diseases that were likely to be associated with vitiligo were retrieved from the GenBank and UCSC. Then bioinformatics analyzes were performed with different software such as; Bioedit2007, Mega7, string-db, PROTPARAM, PROTSCALE, UNIPROT, InterPro.
**Results:** Phylogenetic analyzes showed that most of these genes are susceptible to single nucleotide mutations. Protein analyzes and genetic network showed that FOXP3, PTGS2, IL10, TNF and TSLP genes have the highest interactions and a key role in the network. Also, the analysis shows that TNF, FOXP3and IL-10 genes has a direct effect on the interactions between genes. Analysis of the vitiligo gene network with the genes involved in the allergy disease showed that these genes, including the FASLG, STAT6, FAS, IRF4, CD44, CD80, CTLA4, STAT4, PTPRC, HLA-A , KiTLG and IL17 which may indicate that Vitiligo is associated with allergies, proves the need comprehensive bioinformatics and in vitro studies. Furthermore, the results of the genetic network showed that the FOXD3, C12orf10, TXNDC2, UVRAG, FBXO11 genes did not interact with other genes, which could indicate that these genes to Vitiligo disease had not meaning.
**Conclusion:** The results of this study showed that a number of genes had a significant effect on the vitiligo mechanism, and these genes have significant interactions with a number of allergenic agent genes which indicates the association of these diseases.

**Keywords:** Vitiligo, autoimmune, allergy. Bioinformatics

ICIA2018\Immunogenetics\Poster Discussion\8376
**8376**

# Appropriate Housekeeping Gene in C57/B6 Bone Marrow Cells in Serum Starvation Condition for Real-time PCR

Gilda Parsamanesh[1], Amrolah Mostafazade[1], Davar Amani[2], Kaveh Baghaei[3], Samaneh Tokhanbigli[3], Hamid Asadzadeh Aghdayi[3]

*1. Department of immunology, School of medicine, babol University of medical scienc, Babol, Iran*
*2. Department of immunology, School of medicine, shahid beheshti University of medical sciences, Tehran, Iran*
*3. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research institute for Gastroenterology and Liver Diseases, Shahid BeheshtiUniversity of Medical Sciences, Tehran, Iran*

**Background:** Real-time PCR (qPCR) assay is a conventional method for determining RNA transcription and gene expression. In this regard, housekeeping gene in Real-Time PCR is considered as a gene which its expression does not undergo too many changes in experimental conditions. Moreover, the reliability of a Real-Time PCR depends on a decent reference gene for normalization of the results. In some study, MHC class I, HPRT (Hypoxanthine Phosphoribosyl transferase) and GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) are used as a reference gene. In current study, we aim to show that which of mentioned genes is a suitable candidate for serum starvation condition.
**Methods:** Bone marrow cells from C57/B6 were isolated and cultivated in complete media as a control group and without FBS as a starved group for 24, 48 and 72 hours. Consequently, after each time point RNA extraction and DNA synthesis were performed and given data were analyzed.
**Results:** HPRT and MHC class I expression after 48 and 72 hours starvation were decreased, whereas, GAPDH in 24, 48 and 72 hours had no significant difference in expression.
**Conclusion:** According to Real-Time PCR analysis, HPRT and MHC class I had shown substantial changes in their expression in serum starvation in comparison with the non-starved condition which suggests that these two genes could not be reliable housekeeping genes in Real-Time PCR in serum starvation circumstances.

**Keywords:** housekeeping gene/ MHC class I/ HPRT/ GAPDH/ Serum starvation/ Real-Time PCR

ICIA2018\Immunogenetics\Poster Discussion\8581
**8581**

# V617F-independent Upregulation of JAK2 Gene Expression in Patients with Inflammatory Bowel Diseases

Khadijeh Koushki[1], Pedram Azimzadeh[1], Mohammad Rostami Nejad[2], Kazem Mashayekhi[3], Davar Amani[4], Hamid Asadzadeh Aghdaee[1], Mohammad Reza Zali[2].

*1. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Immunology Department, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*4. Immunology Department, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Inflammatory bowel disease (IBD) is one of the most important immune mediated disorders that affecting the GI tract. However, IBD can also be associated with numerous extraintestinal complications such as VTE or have an increased risk of vascular complications that leads to significant morbidity and mortality. The JAK2 (Janus kinase 2) V617F mutation is a well-known point mutation that involved in VTE and one of the most important gene involved in IBD pathogenesis. Therefore, the aim of this study was to evaluate the JAK2 V617F mutation detection and JAK2 gene expression in Iranian IBD patients.
**Methods:** A total of 452 individuals including 246 IBD patients (209 UC and 37 CD) and 206 healthy controls were enrolled in the study. The genomic DNA and total RNA were extracted from PBMCs. JAK2 V617F mutation detection was performed using Restriction Fragment Length Polymorphism (RFLP) method and JAK2 mRNA expression was analyzed by a Real-time PCR based on relative quantification method. The data were analyzed using SPSS software.
**Results:** Of 246 IBD (mean age36±SD=2.11, 128 Male and 118 Female) and 206 controls (mean age 32±SD=3.42, 97 male and 109 female), our results showed no distinguishable relationship of JAK2 V617F mutation in IBD patient with thrombosis compared with IBD patients without thrombosis and healthy control. However, the mRNA expression was significantly different between IBD patients and controls enrolled in the study (P=0.0001).
**Conclusions:** Our results suggest a JAK2 V61F -independent upregulation of JAK2 mRNA expression in IBD patients. The absence of JAK2 V617F mutation in the thrombotic IBD patients suggests that other mechanisms such as transcriptional level play an important role in the pathogenesis of thrombosis in IBD. Our data confirms that thromboembolism in IBD is associated with elevated JAK2 mRNA level.

**Keywords:** Inflammatory Bowel Diseases (IBD), Janus Kinase 2 (JAK2), V617F, Thromboembolism

ICIA2018\Immunogenetics\Poster Discussion\9838
**9838**

# Evaluation of Inflammasome Receptors Genes Expression of AIM2 and NLRP1 in Peripheral Blood of SM Injured Patiants by Real-Time PCR

Mitra Sabetghadam Moghadam [1], Jafar Salimian [2] , Jamileh Norouzi [3]

1. *Department of biology, Islamic Azad University, North Tehran Branch*
2. *Department of Chemical Injury Research , Baqiyatallah University of Medical Sciences, Tehran, Iran*
3. *Department of Microbiology, Islamic Azad University, North Tehran Branch*

**Background**: Sulfur mustard (SM) exposed patients suffer from chronic lung injuries after 30 years exposure to mustard gas. It seems that the imbalance in the immune system is one of the most important molecular mechanisms involved in pathophysiology of late complications in mustard lung patients. Inflammasome is a protein complex that promotes inflammation and secretion inflammatory cytokines IL-18 and IL-1β. NLRP1 and AIM2 molecules are innate immune cells receptors that can contribute to the formation of the inflammasome complex. In this study, the genes expression levels of NLRP1 and AIM2 receptors were evaluated in peripheral blood sample of SM patients and compared with chronic obstructive pulmonary disease patients (COPD) and healthy controls.
**Methods**: In this study, 15 SM patients as a case group, 15 COPD patients as control that referred to the lung department of Baqiyatallah hospital were selected, as well as 15 subjects as healthy controls. After extracting of RNA from their blood samples, cDNA was synthesized. The genes expression levels of NLRP1 and AIM2 were assayed using Real-Time PCR technique.
**Results**: The NLRP1 gene expression level was higher in SM patients (7±), and COPD patients (14±), compare to HC group ($P < 0.05$). There was no significant difference between the AIM2 gene expression level in SM and COPD patients group compared to the control group.
**Conclusion**: Increased gene expression level of NLRP1 in SM and COPD patients, proposed the inflammasome complex to be active in these patients. This indicates the role of NLRP1 in the inflammation process in SM and COPD patients. Due to the lack of significant difference in expression of AIM2 gene, it seems that the AIM2 is not active in these patients.

**Keywords:** SM, Inflammasome, NLRP1, AIM2

ICIA2018\Immunogenetics\Poster Discussion\9859
**9859**

# Analysis of the Frequencies of HLA-A, B, and DRB1 Alleles in the Three Ethnic Groups, in East Azerbaijan, Iran

Ava Nasrolahi[1, 2], Roya Dolatkhah[2], Hossein Nikkhah[2], Sima Shahmohammadi Farid[2]

*1. Department of Molecular Medicine, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran*
*2. Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Human Leukocyte Antigen (HLA) refers to the major histocompatibility complex (MHC), are one of the highly polymorphic loci of human genome. The HLA allele frequency and linkage disequilibrium patterns are different among human populations. The donors-recipients HLA matching for HLA-A, -B and -DRB1 loci have an important impact in the outcome prediction of allogeneic bone marrow transplantation. Aim of this study was calculated allele frequencies of the HLA-A, -B and -DRB1 genes and their relationship with ethnicity in 293 healthy individuals in East Azerbaijan province of Iran.
**Methods:** In this cross-sectional analytic study, HLA typing was performed using PCR-SSP method. Allele frequencies were estimated for the HLA-A, -B and -DRB1 classes for of 293 cases. For assessment of any relationship between different HLA class and mentioned variables Chi-square-test was performed.
**Results:** from 293 cases 132 (45.1%) were female and 161 (54.9%) were male, with the mean age of 30.30 (±8.70%) years. A total of 16 HLA-A alleles, 26 HLA-B alleles and 13 HLA-DRB1 alleles were detected. Among them, the most common were: HLA class I alleles A*02 with 97(16.6%), B*35 with 98(16.7%), and HLA class II alleles DRB1*11 with 96(16.4%) frequencies. there was not any significant association between ethnicity of the cases and HLA-A (P = 0.88), HLA-B (P = 0.31) and HLA-DRB1 (P = 0.85).
**Conclusion**: This was the first comprehensive study of frequency of different HLA types in East Azerbaijan, but any relationship between different HLA classes and ethnic groups was observed.

**Keywords:** Human Leukocyte Antigen, HLA typing, Transplantation

ICIA2018\Immunogenetics\Poster Discussion\9937
**9937**

## Lack of association between Vitamin D binding protein (VDBP) gene polymorphisms and endometriosis

Mohammad Jafari[1, 2], Soheila Arefi[1], Sahel Heydari[2], Hadi Mohagheghian Yaghoubi[1], Ali Akbar Delbandi[1, 2], Nesa Rashidi[1, 2]

*1. Immunology Research Center, Immunology and Infectious Disease Institute, IRAN University of Medical Sciences*
*2. Immunology Department, School of Medicine, IRAN University of Medical Sciences*

**Background:** Vitamin D binding protein (VDBP) is a multifunctional, polymorphic protein that is a carrier of vitamin D and its metabolites in serum. In addition, this protein enhances the neutrophil chemotactic activity of complement component 5a. In the previous studies observed variants of the VDBP gene seem to be connected to levels of the main circulating vitamin D metabolite [25(OH) D]. On the other hand, low levels of 25(OH)D in women associated with endometriosis. Then, polymorphisms in VDBP gene may be affected anti-inflammatory effects of vitamin D. The percent study aimed to investigate the distribution of variants of VDBP gene in Iranian endometriotic women.
**Methods:** We conducted an association study and included 118 Iranian endometriotic patients and 116 matched healthy controls. We examined the associations between the common variants of the VDBP (rs4588 and rs7041) gene using Restriction Fragment Length Polymorphism (RFLP) method after DNA isolation.
**Results:** There was no significant difference in genotypes and allele frequency of the investigated VDBP polymorphisms between patients and healthy women controls.
**Conclusion:** Our finding revealed that rs4588 and rs7041 polymorphisms of VDBP are not risk factor for susceptibility to endometriosis.

**Keywords:** Endometriosis, Vitamin D, Vitamin D binding protein, Genetic polymorphism

ICIA2018\Immunogenetics\Poster Discussion\10017
**10017**

# Functional Promoter Polymorphism of Matrix Metalloproteinase (MMP)-3 5A/6A and Its Interaction with MMP-7 A-181G Polymorphism in Multiple Sclerosis

Ardeshir abbasi[1], zohreh rahimi[2, 3], ziba rahimi[3]

*1. Department of Immunology, Tarbiat Modares University of Medical Sciences, Tehran, Iran*
*2. Department of Clinical Biochemistry, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*3. Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran*

**Background:** Multiple sclerosis (MS) is a progressive autoimmune disease of the central nervous system (CNS) in which axonal inflammation, demyelination and damage occurs. The aim of present study was to investigate the influence of matrix metalloproteinase (MMP)-3 5A/6A and its interaction with MMP-7 A-181G polymorphism on the risk and the clinical course of MS.
**Methods:** We studied 121patients and 106 healthy individuals without family history of MS or any other autoimmune diseases from Kermanshah province. The MMP-3 and MMP-7 genotypes were detected using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP).
**Results:** The MMP-3 6A allele was more prevalent in patients (70.2%) than that in the controls (67%, p=0.45). The frequency of MMP-3 6A/6A genotype was higher in secondary progressive-MS (SP-MS) patients (57.9%) than that of relapsing remitting-MS (RR-MS) patients (39.2%) but it did not reach to a statistical significance. The concomitant presence of both MMP-3 6A and MMP-7 -181 G alleles compared to the combined presence of MMP-3 6A and MMP-7 -181A alleles increased the risk of MS by 1.64-fold (p=0.05).
**Conclusion:** finally, our study for the first time among a homogenous ethnic group of Iranians (Kurds) indicated the absence of an influence of MMP-3 5A/6A polymorphism on the risk of MS or its clinical course. However, we detected a gene-gene interaction betweenMMP-3 and MMP-7 that increased the risk of MS in the presence of MMP-3 6A and MMP-7 -181 G alleles.

**Keywords:** multiple sclerosis, MMP-3 5A/6A, MMP-7 A-181G, polymorphism, clinical course.

# Immunohematology

# Poster Discussion

ICIA2018\Immunohematology\Poster Discussion\7478
**7478**

# The Effect of *Tribulus terretris* Aqueous Extract on Production and Secretion/Activity of Platelets Related Factors in Immune Thrombocytopenic Purpura Patient

Zahra Koolivand[1], Ali mostafaie[2], Mehrdad Payandeh[3]

1. *Abadan School of Medical Sciences, Abadan, Iran.*
2. *Medical Biology Research Center, Kermanshah University of medical sciences, Kermanshah, Iran*
3. *Department of Hematology and Medical Oncology, Kermanshah university of medical sciences, Kermanshah, Iran*

**Background:** Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disease with decreased platelet levels. First line therapy consists of corticosteroids such as prednisone and intra venous immunoglobulin (IVIG) and anti-Rho (D) and in the case of unresponsiveness, the second line includes splenectomy and rituximab and thrombopoietin (TPO) receptors agonist. Both therapy plans are associated with side effects which could be avoided by using herbals such as *Tribulus terrestris*.
**Methods:** Aqueous extract of *Tribulus terrestris* was prepared, dried and final powder was distributed between twenty newly diagnosed ITP patients (40% of which were Helicobacter pylori positive) to take as pills three times a week for fourteen consecutive days. Blood samples were taken from patients on days 1, 14 and 28, after stop taking the pills to analyze plasma PF4, serotonin and VWF.
**Results:** In all of patients, PF4 was decreased and VWF showed significant increased on all three days. The serotonin was exhibited the increase on days 1 and 14.
**Conclusion:** Altogether, *Tribulus Terrestris* seems to be remarkable safe choice for treatment of ITP patients.

**Keywords:** *Tribulus terretris*, ITP, serotonin, VWF, PF4

ICIA2018\Immunohematology\Poster Discussion\8150
**8150**

## Percentage of Anti-A and Anti-B Hemagglutinins in "Dangerous O Blood Group Donors"

Hajar Shali1 [2], Vahid Baghdadi[2], Mohammad Hossein Baradaran[2]

1. *Department of Immunology, School of medicine, Tabriz University of medicine Science, Tabriz, Iran*
2. *Qazvin Blood Transfusion Organization, Qazvin, Iran*

**Background:** Dangerous O blood group donors have high titers of anti-A, anti-B and/or anti-AB hemagglutinins in their plasma. These antibodies are mostly in class of IgM and IgG that causes agglutination of the erythrocytes of non-O recipients. Transfusion of blood products from these groups can cause severe complication such as an acute hemolytic transfusion reaction (HTR). To prevent transfusion reactions, titration of these antibodies is recommended.
**Methods:** For titration of anti-A and anti-B hemagglutinins (IgM class), serial dilutions were prepared with donor plasma collected in (EDTA) in saline solution (from 1:1 until 1:1024). To destroy IgM class and then titration of anti-A and anti-B hemagglutinins (IgG class), the donor plasma was treated with Mercaptoethanol (2ME) .When the titers of anti-A or anti-B hemagglutinins were ≥128 and ≥256 related to IgM and IgG, respectively, donors were considered to be in the dangerous O blood group.
**Results:** Considerably, the percentage of cases with titer ≥256 for anti-A (IgG) and anti-B (IgG) are 4% and 3%, respectively. Percentage of anti-A (IgM) only was 2%. No one detected with anti-B (IgM).
**Conclusion:** To prevent the occurrence of dangerous transfusion reactions, O blood group products such as fresh-frozen plasma and cryoprecipitate, especially platelet concentrates obtained by apheresis should only be given to group O recipients.

**Keywords:** Dangerous O blood group, hemolytic transfusion reaction, hemagglutinins.

# Immunology of Chemical Victims and Environmental Pollution

# Poster Discussion

ICIA2018\Immunology of Chemical Victims and Environmental Pollution\Poster Discussion\8573
**8573**

# Evaluation of Serum Level of IL-8 in Chemical Victims and Its Relationship with Severity of Pulmonary Complications

Maryam Khabareh[1], Tooba Ghazanfari[2]

1. *Shahed University Tehran*
2. *Department of Research Center of Regulation the Immunology Response*

**Background:** Sulfur mustard (SM) is a toxic and chemical agent. One of the most important long-term effects of sulfurmustard exposure is pulmonary complication that chemical victims are suffering for years. Interleukin 8(IL-8) is one of the important cytokines contributes in chronic pulmonary inflammatory diseases. In this study, the serum level of IL-8 and its correlation with severity of pulmonary complications was evaluated in SM exposed individuals about three cascades after exposure.
**Methods:** In this work, 73 SM exposed cases and 86 unexposed as control were studied. Pulmonary function test was performed using spirometry. Chemical victims were classified into three groups of normal, moderate and severe pulmonary damage, according to classification of the medical committee of the foundation of martyr and veterans Affair. The serum level of IL-8 was measured by ELISA method.
**Result:** There was significant decrease in the serum level of IL-8 in the exposed group compared to control group also there was significant decrease in the serum level of IL-8 in all three exposed groups compared to control group. But no significant difference observed between the moderate and severe pulmonary damage groups with normal ones.
**Conclusion:** The serum level of IL-8 in chemical victims is affected by exposure to SM. But the serum level of IL-8 cannot play an important role in the severity of pulmonary Complications as an inflammatory factor.

**Keywords:** IL-8**,** Pulmonary Complications**,** sulfurmustard**.**

ICIA2018\Immunology of Chemical Victims and Environmental Pollution\Poster Discussion\8715
**8715**

# Evaluation of Serum Level of IL-18 Binding Protein in Chemical Victims and Its Relationship with Severity of Pulmonary Complications

Somaye Mami[1], Tooba Ghazanfari[2]

*1. Shahed University Tehran*
*2. Department of Research Center of Regulation the Immunology Response*

**Background**: Exposure to mustard gas leads to acute and chronic toxic effects on the pulmonary. Results from several studies have been suggested the role of immune mediators including IL 18BP in the pathogenesis of pulmonary diseases. IL 18BP by binding to IL18 prevents its binding to the receptor and acts as a natural inhibitor for this cytokine.
**Methods**: In this study, the serum level of IL 18bp and its correlation with severity of pulmonary complications was evaluated in SM exposed individuals about three cascades after exposure. In this work, 93 SM exposed cases and 86 unexposed as control were studied. Pulmonary function test was performed using spirometry. Chemical victims were classified into three groups of normal, moderate and severe pulmonary damage, according to classification of the medical committee of the foundation of martyr and veterans Affair. The serum level of IL-18bp was measured by ELISA method.
**Result**: There was significant decrease in the serum level of IL 18bp in the exposed group compared to control group also. There was significant decrease in the serum level of IL-8bp in all three exposed groups compared to control group. But no significant different observed between the moderate and severe pulmonary damage groups with normal ones.
**Conclusion**: The serum level of IL-8bp in chemical victims is affected by exposure to SM, but the serum level of IL-8bp can ‘not play an important role in the severity of pulmonary Complications as an inflammatory factor.

**Keywords:** IL-18, Pulmonary Complications, mustard gas.

# Immunology of Exercise, Aging, and Nutrition

# Poster Discussion

ICIA2018\Immunology of Exercise, Aging, and Nutrition\Poster Discussion\8071
**8071**

# Effect of Inhibition of D2 Dopamine Receptors on Several Functions of Monocytes of Peripheral Blood in Rat under Food Restriction

Khatereh Noormohammadi[1], Farrin Babaei Balderlou[1], Seyyed Meysam Abtahi Foroushani[2]

1. *Department of Biology, Faculty of Sciences, Urmia University, Urmia, Iran*
2. *Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background**: In previous studies, the effects of food restriction on the changes in immune responses and brain dopamine content have been reported. On the other hand, it has been shown that immune cells, in addition to dopamine production, also have dopamine receptors. The purpose of this study was to evaluate the effect of inhibition of D2 dopamine receptors on several functions of monocytes of peripheral blood in rat under food restriction.
**Methods**: In this experimental study, 36 male Wistar rats (weighing 200-250 gr) were allocated into six groups (n=6), including control groups, food restriction (25%), food restriction (50%), food restriction (75%), food restriction 75% and Sulpiride and rats treated with Sulpiride. Sulpiride was injected Intracerebroventricular at a concentration of 50 µg / rat on day 21 after the study initiation. At the end, the mice were bled and peripheral blood mononuclear cells were isolated by ficoll gradient method.
**Results:** Food restriction caused a significant decrease in the activity of monocyte cells like neutral red uptake test and respiratory burst (NBT reduction test) simultaneously with decreasing lymphocytes proliferation after stimulation with phytohemagglutinin. Administration of Sulpiride with a 75% Food Restriction resulted in the improvement of these functions of monocyte cells as well as lymphocyte proliferation.
**Conclusion:** Intracerebroventricular administration of dopamine D2 receptor antagonists (sulpiride) effectively inhibited the effects of a severe dietary restriction on the suppression of immunity system.

**Key words:** Dopamine, Monocyte, Food Restriction Sulpiride, Rat.

ICIA2018\Immunology of Exercise, Aging, and Nutrition\Poster Discussion\8135
**8135**

## An in vitro Evaluation of Anti-aging Effect of Guluronic Acid (G2013) Based on Enzymatic Oxidative Stress Gene Expression Using Healthy Individuals PBMCs

Mahsa Taeba, Seyed Shahabeddin Mortazavi Jahromib, Abdollah Jafarzadeha, Mohammad Reza Mirzaeic, Abbas Mirshafieyd

*1. Department of Immunology, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
*2. Department of Cellular and Molecular Biology, Kish International Campus, University of Tehran, Kish, Iran*
*3. Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Box: 771664-8798, Rafsanjan, Iran*
*4. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Box: 14155-6446, Tehran, Iran*

**Background**: Aging is usually associated with increased levels of oxidants, and may result in damages caused by oxidative stress. There is a direct relationship between aging and increased incidence of inflammatory diseases. The present research intended to study the anti-aging and anti-inflammatory effects of the drug G2013 (guluronic acid) at low and high doses on the genes expression of a number of enzymes involved in oxidative stress (including SOD2, GPX1, CAT, GST, iNOS, and MPO) in peripheral blood mononuclear cells (PBMCs) of healthy individuals under in vitro conditions.
**Methods**: Venous blood samples were taken from 20 healthy individuals, the PBMCs were isolated and their RNAs extracted and their cDNAs were synthesized, and the genes expression levels were measured using the qRT-PCR technique.
**Results**: Our results indicated that this drug could, at both low and high doses, significantly reduce the expression of the genes for SOD2, GPX1, CAT, and GST compared to the LPS group ($p < 0.0001$). Moreover, it was noticed that the drug is able to significantly reduce gene expression levels at the high dose and at both doses (low and high), for iNOS and MPO compared to the LPS group ($p < 0.0001$), respectively.
**Conclusions**: The present research showed that G2013, as a novel NSAID drug with immunomodulatory properties, could modulate the expression levels of the genes for SOD2, GPX1, CAT, GST, iNOS, and MPO, to the level of healthy gene expression, and possibly it might reduce the pathological process of aging and age-related inflammatory diseases.

**Keywords**: G2013 Guluronic acid Anti-aging Oxidative stress NSAID Aging

ICIA2018\Immunology of Exercise, Aging, and Nutrition\Poster Discussion\8271
**8271**

## Effect of Saturated and Unsaturated Fatty Acids on T Helper Differentiation in Vitro

Ayssouda Hossein zade[1], Maryam Moogooei[1], Shayan Mostafaei[2], Hossein Rahavi[1], Reza Mansouri[1]*, Sepideh Soukhtezari[3], Mojgan Ghaedi[4]

1. *Department of Immunology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
2. *Department of Biostatistics, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
3. *Department of Medical Biotechnology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
4. *Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Autoimmunity shows a concerning growth recently. It seems that role of diet is important for development of autoimmunity. Nutritional elements can affect immune system functions. Oversupply or deficiency of specific metabolites may change performance of immune cells, especially that of T helper lymphocytes. For example, glucose, omega 3 and poly unsaturated fatty acids can induce specific Th subsets which play a critical role in autoimmune diseases. T lymphocytes, mostly T CD4+ (Th cells) not only play a critical role in orchestrating immune responses, but also they have major role in pathogenesis of some autoimmune disease.
**Methods:** We designed a research to find out effects of oleic and palmitic acids on differentiation of Th cells. We added oleic acid, palmitic acids and combination of them on peripheral blood mononuclear cells (PBMC) culture and cells were harvested for RT-PCR and flow cytometry analysis after seven days.
**Results:** Our results showed that palmitic and oleic acids induce Th1 and Th17 subsets (p-value<0.05).
**Conclusion:** All data showed that adding only1mM of oleic and palmitic acids change Th cells metabolism and induce Th1 and Th17 induction. In this study saturated fatty acid, unsaturated fatty acid, and combination of both of them result in inflammatory Th subsets induction.

**Keywords:** T Helper Cells, Differentiation, Metabolism, Fatty Acids, mTOR

# Immunology of Infectious Diseases

# Bacteria and Fungi

## Poster Discussion

ICIA2018\Immunology of Infectious Diseases Bacteria and Fungi\Poster Discussion\7943
**7943**

# OmpA Gene Targeted Real-time PCR Compared with the Conventional Culture Method for Detection of *Acinetobacter baumannii* in Pneumonic and *Zataria multiflora*-treated Balb/c Mice

Niloofar Hassannejad[1], Abbas Bahador[2], Kazem Parivar[3], Hadi Ghazanfari[4]

*1. Department of Cellular and Molecular Biology, Faculty of Sciences, Science and Research Branch of Islamic Azad University, Tehran, Iran.*
*2. Department of Microbiology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
*3. Department of Biology, Faculty of Sciences, Research and Science Branch, Islamic Azad University, Tehran, Iran.*
*4. Department of Immunology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** *Acinetobacter baumannii* is an important pathogen in health care-acquired infections and has free existence of multidrug-resistance responsible for severe nosocomial and community-acquired pneumonia. Currently, mouse model for *A. baumannii* pneumonia is essential for designing novel therapeutic agents.
**Methods:** In this report, for the first time we described a mouse model of *A. baumannii* using clinical and 19606R standard strains for developing a quantitative real time PCR. The qRT-PCR was used for rapid identification of *A. baumannii* infection from lung tissues of Balb/c mice with pneumonia and also for evaluation of antibacterial effects of *Zataria multiflora* extract on pneumonic Balb/c mice.
**Results:** Three doses of bacterial strains (0.5 $\times 10^8$, 1 $\times 10^8$, 1.5 $\times 10^8$ cfu/ml) were used intranasally for three days to infect the mice. To treat the pneumonic mice, one day after infection with 1×108 cfu/ml bacterial strains, mice were treated with 60 mg/kg of Z. multiflora ethanolic extract I.P. for 3 days. Lung tissues of pneumonic and treated mice were cultured conventionally and the results were compared with the evaluation of *A. baumannii ompA* gene expression using qRT-PCR**.** In all experiments, clinical isolate had better positive results at day three with highest dose than ۱۹۶۰۶ strain either in culture (4 versus 3) or in qRT-PCR assay (5 versus 4). However, qRT-PCR detection was 100%, the specificity was 70%, and the positive predictive value was 27%. Our data also shown that *Z. multiflora* extract significantly decreased the number of positive samples in both clinical (from 4 to one) and in standard isolates (from 3 to zero).
**Conclusion:** The qRT-PCR detection of *A. baumannii* in lung tissue of Balb/c mice model had a high sensitivity as compared to the culture-based method. Also, *Z. multiflora* extract could be a new therapeutic agent for *A. baumannii* pneumonic infection.

**Keywords:** *Acinetobacter baumanii,* OmpA, Quantitative Real time PCR, *Zataria multiflora Boiss*, Pneumonia, Balb/c mice

ICIA2018\Immunology of Infectious Diseases Bacteria and Fungi\Poster Discussion\8084
**8084**

# Stimulation of PBMCs of Patients with Abdominal Aortic Aneurysm with *Helicobacter pylori* Antigens Increases IL-21 Production

Hamid Aria[1,2], Mehdi Kalani[3], Hossein Hodjati[4], Mehrnoosh Doroudchi[1]

1. *Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Bioinformatics and Computational Biology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
3. *Prof. Alborzi Clinical microbiology research center, Nemazee hospital, Shiraz University of Medical Sciences, Shiraz, Iran*
4. *Department of Vascular Surgery, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Abdominal Aortic Aneurysm (AAA) is the local dilatation of abdominal aorta and an inflammatory condition in which cytokines may play a pathogenic role. AAA shares several pathogenesis criteria of atherosclerosis, therefore, we asked if stimulation with CagA+ and CagA- *Helicobacter pylori* has different effects on the level of cytokines produced by AAA patients' PBMCs compared to controls.
**Methods:** PBMCs were isolated from 5 men with diagnosis of AAA and 5 men with normal/insignificant angiography, CT-Scan and Ultrasonography results. Then, PBMCs were cultured in separate plates with bacterial extract of CagA+ and CagA- *Helicobacter pylori.* The supernatants were then removed to measure IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F, IL-21, IL-22, IFN-ɣ and TNF-α using a commercial fluorescent-labeled bead assay.
**Results:** After stimulation with CagA+ *Helicobacter pylori*, patients' PBMCs (18.43±12.91 Pg/ml) produced higher IL-21 compared to untreated PBMCs (10.84 ± 4.92 Pg/ml) (P=0.06). While after stimulation with CagA+ *Helicobacter pylori*, controls' PBMCs (9.90 ± 5.95 Pg/ml) produced higher levels of IL-13 compared to untreated PBMCs (4.59 ± 2.26 Pg/ml) (P=0.06). A remarkable observation was the huge production of IL-9 by the controls' PBMCs but not controls after stimulation by both CagA+ and CagA- *Helicobacter pylori* (P=0.05).
**Conclusion:** *Helicobacter pylori* is suggested to be one of the bacterial causes of endothelial lesion at the beginning of atherosclerotic process. Because Th2 cytokines are associated with the progression of AAA, the possible role of IL-13 in the onset of disease is noteworthy. Also it can be suggested that the lack of IL-9 may have an effect on AAA disease.

**Keywords:** Abdominal aortic aneurysm, cytokines, Helicobacter pylori

ICIA2018\Immunology of Infectious Diseases Bacteria and Fungi\Poster Discussion\8196
**8196**

## Correlation between Recurrent Vulvovaginal Candidiasis and Dectin-1 Y238X Gene Polymorphism

Hamid Badali[1], Fardin Ahmadkhani[2*]

*1. Department of Medical Mycology and Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
*2. Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran*

**Background:** Vulvovaginal candidiasis is a frequent disease affecting approximately more than 75 % of all childbearing women at least once in their lifetime by overgrowth of opportunistic *Candida* species. Recurrent vulvovaginal candidiasis (RVVC) is common in otherwise healthy individuals. Several risk factors were reported to contribute to RVVC susceptibility. A polymorphism in Dectin-1 (Y238X, rs16910526) was identified in patients with RVVC and hypothesized that genetic factors play an important role in susceptibility to RVVC. Herein, we aimed to survey the polymorphisms in the Dectin-1 gene, linked to susceptibility to RVVC.
**Methods:** In the current study, blood samples were obtained from 25 patients who had frequent vulvovaginal candidiasis relapses and were diagnosed as RVVC. In addition, blood cultures were obtained from control group comprising of healthy individuals (n=25) with no history of RVVC, vaginal discharge, or itching on the day of examination. Dectin-1 Y238X gene polymorphism was investigated using DNA sequencing and bidirectional polymerase chain reaction (PCR) amplification of specific alleles (Bi-PASA), as previously described by Carvalho et al.
**Results:** The analysis revealed that all of the patients were wild-type homozygous for Dectin-1 Y238X polymorphisms. None of the individuals showed heterozygous or mutant homozygous Dectin-1 polymorphism.
**Conclusion:** No significant correlations were observed between the susceptibility to RVVC and Dectin-1 Y238X polymorphism in the Iranian population, which was not previously studied.

**Keywords:** *Candida* species, Dectin-1 Y238X gene polymorphism, RVVC.

ICIA2018\Immunology of Infectious Diseases Bacteria and Fungi\Poster Discussion\8260
**8260**

# The Effects of Opium Addiction on the Immune System Function in Patients with Fungal Infection

Alireza Izadi [1], Seyyed Amin Ayatollahi Mousavi[2], Gholamreza Asadikaram[3], Nouzar Nakhaee[4]

*1. Department of Medical Parasitology and Mycology, School of Health, Tehran University of Medical Sciences, Tehran, Iran*
*2. Department of Medical Mycology and Parasitology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran*
*3. Endocrinology and Metabolism Research Center, Institute of Basic and Clinical Physiology Sciences, and Dept. of Biochemistry Kerman University of Medical Sciences, Kerman, Iran*
*4. Neuroscience Research Center, Kerman University of Medical Sciences, Kerman, Iran*

**Background:** The use of narcotics such as opium exposes addicts as susceptible targets of different diseases so that they might easily be exposed to different diseases such as fungal infections. The present study aimed to investigate the effects of addiction to opium and fungal infection on plasma levels of certain cytokines including IL-4, IL-6, IL-17, IFN-γ and TGF-β.
**Methods:** Present study includes 72 individuals who were divided into 4 groups: 1) opium addicted with fungal infection; 2) opium-addicted without fungal infection; 3) non-opium-addicted with fungal infection; and 4) normal individuals (non-opium-addicted and non-fungal infection). The fungal samples, after being detected and confirmed by a physician, were prepared based on clinical symptoms and then analyzed by direct smear and culture method. The measurement of the plasma level of cytokines was done by ELISA method.
**Results:** The comparison of the mean of the plasma level of cytokines shows that addiction to opium and fungal infection had respectively the significant effects on the plasma levels of IL-17, IFN-γ, TGF-β cytokines in all studied groups. The interaction of addiction to opium and fungal infection was only significant in the case of plasma level of IL-6.
**Conclusion:** Addiction to opium and fungal infection, either separately or simultaneously with each other, pose significant effects on the immune system and cause disorders in the cytokine network and the immune system and also provides a suitable environment for fungal infection.

**Keywords:** Opium; Addiction; Fungal infection; Cytokine

ICIA2018\Immunology of Infectious Diseases Bacteria and Fungi\Poster Discussion\8569
**8569**

## Investigating the Association between *Helicobacter pylori* Infection and IgE Increase in Children Under the Age of 15 Years

Nima Rostami Koshki[1], Mehdi Ebrahimiyan[2], Mohammad Salehi[3]

1. *Yong Reserch and Elit Club, Marand Branch, Islamic Azad University, Marand, Iran*
2. *Department of Biology ,Naein Branch, Islamic Azad University,Naein, Iran*
3. *Department of Biology ,Urmia Branch, Islamic Azad University,Urmia, Iran*

**Background:** Almost half of the world population is infected with *Helicobacter pylori*, a major risk factor for chronic gastritis, peptic ulcer and gastric cancer. The most effective way to improve the eradication of *H. pylori* peptic ulcers is recommended. Recent studies indicate that there is a relationship between infection with *Helicobacter pylori* and an increase in the level of IgE in the blood of patients. The purpose of this study was to determine the association between total IgE and *Helicobacter pylori* infection as a reliable diagnostic method.
**Methods:** From 45 patients referred to Children Hospital Tabriz, suspected Gastric biopsies were obtained. Biopsies were transported to the laboratory in Stuart transport medium. First, endoscopy and then biopsy specimens were prepared and further confirmed using urease test. Subsequently, the samples were confirmed by the PcR test. Anti-H. Pylori IgG anti-H. Pylori-CagA IgG, and total plasma IgE levels were measured using ELISA and then statistically analyzed.
**Results:** 19 out of 45 patients had H. pylori infection, out of which 16 patients were CagA positive.
**Conclusion:** The findings of the present study demonstrated that there is a significant relationship between *Helicobacter pylori* infection and elevated levels of IgE in the blood of patients.

**Keywords:** *Helicobacter pylori*, IgE, ELISA, Endoscopy

ICIA2018\Immunology of Infectious Diseases Bacteria and Fungi\Poster Discussion\9768

**9768**

# Immediate Hypersensitivity and Serum IgE Antibody Responses in Patients with Dermatophytosis

Pro.AR.Khosravi[1], Hadis khamevar[2]

1. *Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
2. *Student of Mycology, Faculty of Veterinary, University of Tehran, Tehran, Iran*

**Background:** The association of dermatophytes with atopic patients and improvement in allergic signs with antifungal treatment suggest a possible link between chronic infection and atopy. The purpose of this study was to determine skin reactivity and serum IgE antibody responses in patients with chronic and acute dermatophytosis.

**Methods:** One hundred and sixty-three patients with chronic dermatophytosis, 35 patients with acute dermatophytosis, 41 atopic patients and 49 healthy subjects were enrolled in this study. Sensitization to *Trichophyton mentagrophytes* (*T. mentagrophytes*), *Candida albicans* and *Aspergillus fumigatus* antigens has been evaluated in patients by skin prick test (SPT) and by the presence of specific IgE antibody in enzyme-linked immuno-sorbent assay (ELISA).

**Results:** Positive immediate hypersensitivity (IH) reactions were obtained in 95.1% of the atopic patients with chronic infection for *T. mentagrophytes,* representing a significant difference from other patient groups ($P < 0.05$). Specific anti-*T. mentagrophytes* IgE antibodies were detected in atopic patients with chronic (65.9%) and acute (50%) dermatophytosis, while none of the atopic subjects had positive IgE reactions to T. mentagrophytes.

**Conclusion:** The results showed significant higher positive IH and specific anti-*T. mentagrophytes* IgE responses in atopic patients with chronic dermatophytosis than the other groups.

**Keywords:** IgE, hypersensitivity, dermatophytosis, *T. mentagrophytes*

ICIA2018\Immunology of Infectious Diseases Bacteria and Fungi\Poster Discussion\9796
**9796**

# Probiotic *Lactobacillus* Strains Stimulate the Innate Immune Response and Modulate the TLR Expression against *Salmonella* Enteritidis Infection *in vitro*

Mona Moshiri[1] (Presenter author), Mohammad Mehdi Soltan Dallal [1,2], Farhad Rezaei[3], Masoumeh Douraghi [1], Laleh Sharifi [4], Zahra Noroozbabaei [3], Mehrdad Gholami [5], Abbas Mirshafiey[6]

1. *Division of Microbiology, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
2. *Food Microbiology Research Center, Tehran University of Medical Sciences, Tehran, Iran*
3. *Department of Virology, Tehran University of Medical Sciences, Tehran, Iran*
4. *Uro-Oncology Research Center, Tehran University of Medical Sciences, Tehran, Iran*
5. *Department of Microbiology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran*
6. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Lactic acid bacteria (LAB) have been observed to modify the immune responses and could have therapeutic effects in inflammatory disorders. The aim of the present work was to evaluate the effects of *Lactobacillus acidophilus* (*L. acidophilus*) and *Lactobacillus casei* (*L. casei*) strains on toll-like receptor (TLR2 and TLR4) expressions in HT29 cell line infected with *Salmonella enterica* serovar Enteritidis (*Ses*E).
**Methods:** HT29 cells were cultured in Roswell Park Memorial Institute medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. The cells were treated with the *Lactobacillus* strains after or before challenge with *Ses*E. At 2 and 4 hours post-infection, we measured changes in the expression levels of TLR2 and TLR4 via real-time polymerase chain reaction method.
**Results:** Probiotic strains increased the proportion of TLRs expressions and stimulate the innate immune response, when uninfected HT29 cells were treated with lactobacilli. While, the administration of the probiotic strains reduced the TLRs expression previous and also after the challenge with *Ses*E.
**Conclusion:** Our findings show that both of the L. *acidophilus* and *L. casei* boosted innate immune responses, as following the stimulation of TLR signalling and the downregulation of a broad array of pro-inflammatory cytokines after infectin with *Ses*E. Although, *L. acidophilus* display greater anti-inflammatory activity than *L. casei* in this work. Further in vivo and in vitro studies are required to further elucidate the mechanisms underlying this anti-inflammatory effect.

**Keywords:** Probiotics, *Lactobacillus*, Toll-like receptors, Innate immunity, *Salmonella* Enteritidis

ICIA2018\Immunology of Infectious Diseases Bacteria and Fungi\Poster Discussion\9801
**9801**

## Gastrointestinal Inflammation: Microbial and non-Microbial Factors

Shila Jalalpour[1], Vahid Mirzaee[2], Abdollah Jafarzadeh [3,4,5] Afshin Tahmasebi[2]

1. *Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran, shilla.*
2. *Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
3. *Immunology of Infectious Diseases Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
4. *Department of Immunology, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
5. *Department of Immunology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran*

**Background**: Gastrointestinal inflammation can be caused by microbial and non-microbial factors, such as life style: alcohol use, stress, or the use of certain medications such as aspirin or other anti-inflammatory drugs and Helicobacter pylori . *H. pylori* is common, infecting around 50% of the global human population. The aim of present study was investigate and compare prevalence of microbial and non-microbial gastrointestinal inflammation in visited patients in endoscopy of unit of Aliebn-Abitaleb Hospital in Rafsanjan city. This descriptive cross-sectional study was conducted in the second half of 1396 in patients refer to gastroenterologist. Gastrointestinal inflammation diagnosis by a specialist physician with endoscopy method.
**Results**: According to the result, of the 87 patients with inflammation, 51 (58.62%) patients had *H. pylori* infection and 36 (41.37%) patients were negative *H. pylori* infection.
**Conclusion**: The results of this study indicate a higher prevalence of inflammation in people who are infected with *H. pylori* colonization. Due to the importance and role of *H. pylori* in gastrointestinal inflammation and the fact that the persistence of gastrointestinal inflammation can lead to peptic ulcers and cancer, it is important *H. pylori* treatment and elimination in patients.

**Keywords**: Gastrointestinal inflammation, Gastric, Helicobacter pylori, Endoscopy, Colonization

ICIA2018\Immunology of Infectious Diseases Bacteria and Fungi\Poster Discussion\9891
**9891**

**Evaluation of Antibacterial and Cytotoxic Effects of K4 Synthetic Peptide**

Mohammad Mehdi ADIBZADEH SERESHGI[1]. Kazem AhmadI[1], Mahdi Fasihi-Ramandi*.[1]

*1. Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*

**Background**: With increasing antimicrobial resistance to common antibiotics, development of alternative therapeutic strategies is necessary. Also, antibacterial peptide with numerous behavior and different properties such as net charge, hydrophobicity, length, etc. could act against pathogenic microorganism. In recent years, novel peptides with activity against wide range of bacteria have been introduced.
**Methods**: In this study, seventeen pathogenic bacteria were chosen to study the antibacterial effect of K4 peptide using MIC and MBC assays. The therapeutic index (TI) of this peptide experimentally calculated by the ratio of HC50 to MIC as a parameter to represent the specificity of AMP. *In silico* analysis was performed to predict the physico-chemical properties, structures, and behavior of this peptide. *In vitro* cytotoxic effect of peptide was evaluated on the Hela cell line using MTT assay, and the amount of macrophage nitric oxide production was measured by Griess method on the J774 cell line.
**Results**: Peptide concentrations of 25-400 µg/ml was seen as the MIC value results for different bacteria. MBC assay showed such a result with concentration of more than 25-400 µg/ml. The result of hemolysis assay was 24 percent at 1 mg/ml concentration. The amount of nitric oxide production of macrophage cell line J774 was 25.9873 µM at 6.3 µg/ml peptide concentration.
**Conclusion**: K4 peptide had strong antibacterial effect on some bacteria such as *B. melitensis*. This peptide may have a role in immunity with nitric oxide production. Additionally, it enhances bacterial killing mechanism of macrophage, making this peptide a potential agent against pathogens.

**Keywords:** Antimicrobial peptide; Cationic peptide; MIC; MBC; Nitric oxide.

ICIA2018\Immunology of Infectious Diseases Bacteria and Fungi\Poster Discussion\9913
**9913**

# Egg Yolk Immunoglobulin Against *ETEC*, Prevention and Treatment of Infections

Razieh Dawodi[1], Seyed. Latif Mousavi Gargari[2], Shahram Nazarian[3]

1. *Student Master, Department of Cell Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran*
2. *Professor, Department of Cell Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran*
3. *Assistant Professor, Biology Research Center, Imam Hossein University, Tehran, Iran*

**Background**: Diarrheal illnesses are a severe public health problem and a major cause of morbidity and mortality in diarrhea in developing countries and travelers to these countries. 30-40% of cases of diarrhea in the world due to infection with *E. coli* strains such as *ETEC* occurred infection with ETEC is the leading cause of travelers' diarrhea and a major cause of diarrheal disease in lower-income countries, especially among children Irrational use of antibiotics for the treatment of diarrhea is a global problem.
Due to the increasing prevalence of antibiotic resistance around the world and spreading to humans, concern about the presence of antibiotic residues in the environment, the protection of intestinal flora against the side effects of antibiotic and considering that vaccines have not been effective in treating the infection so far, the use of antibodies as an agent for passive immunity to prevent and treat infections.
The properties of IgY such as easy and inexpensive production compared to mammalian serum antibodies, non-invasive and animal rights protection, non-activating the complement system and non-response to rheumatoid factors, have paved the way for its utilization as an anti-infectious agent within the mammalian gut.
**Methodes**: Chickens were immunized intramuscularly with *ETEC* formalin killed emulsified with an equal volume of Freund's adjuvant to obtain ETEC-specific IgY loaded eggs. After collecting eggs the water-soluble fraction was isolated from egg yolk to utilize for the analysis of IgY properties. the antibody titer was measured by ELISA technique.
**Results**: In examining ELISA results, increased antibody titers showed a significant increase compared to control samples. The produced bird's immunoglobulin has the ability to detect and attach to the antigen and, by means of the neutralization mechanism, prevents the binding, colonization and replication of the animal's intestines and causes the disease.

**Keywords**: Formalin killed, *ETEC*, Egg yolk immunoglobulin

# Immunology of Infectious Diseases

# Viruses

# Poster Discussion

ICIA2018\Immunology of Infectious Diseases Viruses\Poster Discussion\6316
**6316**

# Study of the Effects of Combined Therapy with Sodium Valproate, Peg-interferon and Corticosteroid on Proviral Load of HTLV-1 and Expression of Rel-A, Creb and IL-1 Genes in Patients with HTLV-1 Associated Spastic Paraparesis (HAM/TSP)

Davoud Ghaffarian[1], Reza Farid Hosseini[2], Hadi Atabati[1], Reza Boostani[3], Abdolrahim Rezaei[4*], Houshang Rafat Panah[5]

1. *Department of Immunology, Mashhad University of Medical Sciences, Mashhad, Iran*
2. *Department of Immunology, Mashhad University of Medical Sciences, Mashhad, Iran*
3. *Department of Neurology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
4. *Inflammation and Inflammatory Diseases Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
5. *Department of Immunology, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** Human T-Cell Lymphotropic Virus Type I (HTLV-I) is endemic in northeast of Iran. Although chronic inflammation of spinal cord seems to play a major role in the disease pathogenesis, immunomodulatory treatments have not shown significant improvement in disease symptoms. This study was conducted to evaluate the effect of sodium valproate in combination with Peg-Interferon (Peg-IFN) and prednisolone on proviral load and inflammatory factors of patients with HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP).
**Methods:** In this pilot clinical trial, 10 HAM/TSP patients, referred to HTLV- 1 clinic of Qaem Hospital of Mashhad, Iran in 2012, were treated with sodium valproate, Peg-IFN and prednisolone for 6 months. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll Hypaque and the RNA was extracted from PBMCs. Complementary DNA was synthesized using TaqMan Gold RT-PCR Kit. A real-time PCR TaqMan method was designed and optimized for evaluation of Rel-A, Creb and IL-1 genes expression.
**Results**: The analysis in this study indicated that proviral load and IL-1 mRNA expression in patients were significantly lower after treatment. Despite this decline, Creb and IL-1 mRNA expression was not significant.
**Conclusion:** The combination of sodium valproate, Peg-IFN and prednisolone seems an effective treatment for proviral load and relatively efficient for damaging inflammatory factors of HAM/TSP; however, further studies with sufficient sample sizes are necessary.

**Keywords:** Sodium valporate, Peg-Interferon, prednisolone, HAM/TSP

ICIA2018\Immunology of Infectious Diseases Viruses\Poster Discussion\7877
**7877**

# The Effects of Curcumin on Apoptosis and Cytotoxicity-Related Molecules in HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP) Patients

Houshang Rafatpanah[1], Asadollah Mohammadi[1], Reza Boostani[2], Zohre Poursina[1], Shadi Zamanian[1]

*1. Immunology Research center, Division of Inflammation and Inflammatory Diseases, Mashhad University of Medical sciences, Mashhad, Iran*
*2. Department of Neurology, Mashhad University of Medical sciences, Mashhad, Iran*

**Background:** Apoptosis is a universal cellular defense mechanism against viral infection. Curcumin, an anti-inflammatory phytochemical, induces apoptosis through mitochondrial and receptor-mediated pathways, as well as activation of caspase cascades. Here, we investigated the impact of supplementation with curcumin on the expression of a panel of apoptosis- and cytotoxicity-related genes in patients suffering from human T lymphotropic virus Type 1 (HTLV-1)- associated myelopathy/tropical spastic paraparesis (HAM/TSP), a progressive demyelinating neuroinflammatory disease caused by HTLV-1 infection.
**Methods:** We enrolled 21 HAM/TSP patients in this study. Curcumin nanomicelles (80 mg/day, orally) were administered once a day for 12 weeks. The mRNA levels of total Fas (tFas), membrane-bound Fas (mFas), Fas-Ligand (FasL), TNF-related apoptosis-inducing ligand (TRAIL), perforin, granzyme A, granzyme B and granulysin were analyzed before and after treatment in peripheral blood lymphocytes. Protein levels of Fas, FasL, TRAIL and granulysin were also measured in serum using ELISA.
**Results:** Curcumin supplementation inhibited FasL mRNA production and up-regulated the expression of pro-apoptotic molecules granzyme A (at the mRNA level) and granulysin (at the protein level), suggesting degranulation of granulysin-bearing cells following curcumin supplementation. Conversely, Curcumin did not affect Fas, TRAIL, perforin, granzyme B at the mRNA level, and anti-apoptotic molecules sFas, sFasL and sTRAIL at the protein level.
**Conclusions:** The present results suggest that curcumin supplementation increases cytotoxicity-related molecules granzyme A and granulysin in patients with HAM/TSP.

**Keywords:** HTLV-1; Curcumin; Apoptosis; HAM/TSP

ICIA2018\Immunology of Infectious Diseases Viruses\Poster Discussion\7892
**7892**

# Sequence and Phylogenetic Analysis of LTR region of Human T-Cell Lymphotropic Virus Type 1 from Mashhad, Iran

SeyedAliMirhosseini[1], MehranMoharreri[1], SeyedRohollahArab[1], Baratali Mashkani[2], SeyedAbdolrahimRezaee[3], RezaFaridHosseini[4], HoushangRafatpanah[1]

*1. Inflammation and Inflammatory Diseases Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*2. Biochemistry and Nutrition Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad,Iran*
*3. Immunology Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*4. Allergy Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** Human T-cell lymphotropic virus type 1 (HTLV-1) is widespread around the globe, with endemic areas in Southern part of Japan, Africa, the Caribbean, South America and Northeast of Iran. The virus is responsible for causing adult T-cell leukemia (ATL) and the neurological disorder HTLV-1 associated myelopathy or tropical spastic paraparesis (HAM/TSP) in less than 5% of the infected individuals. Variations in the provirus sequence of HTLV-1 have been used as a tool for molecular epidemiology investigation of HTLV-1 infection.
**Methods:** In current study, the amplified LTR region was purified, cloned and sequenced from 15 infected individuals living in Mashhad, Iran. The sequences were compared with the sequences from Neyshabur, Torbat-e Heydarieh and Sabzevar. It was also compared with a reference sequence obtained from GenBank (Accession No. J02029) from Japan.
**Results:** The LTR sequence in the samples belonged to the transcontinental subgroup A. There was no mismatch in the LTR sequences in 13 out of 15 samples collected from Mashhad with those from Neyshabur, Torbat-e Heydarieh, and Sabzevar. However, these LTR sequences were difference in 2.6% of the nucleotides from the J02029.
**Conclusion:** These results indicate that HTLV-1 infection in the Northeast of Iran had a common origin. However, further investigation is required to identify the source of small differences were observed in those two samples.

**Keywords:** HTLV-1, HAM/TSP, ATL, LTR, DNA sequencing

ICIA2018\Immunology of Infectious Diseases Viruses\Poster Discussion\8015
**8015**

# Evaluation of Gene and Protein Levels of IL-33 and Soluble Receptor (ST2-Receptor) Patients with HAM/TSP and HTLV-1 Carriers

Saeedeh Mehraban Moghadam[1], Reza Boostani[2], Seyed Abdolrahim Rezaee[1], Bahareh Fazeli[1], Narges Valizadeh[1], Houshang Rafatpanah[1]

*1. Immunology Research Center, Division of Inflammation and Inflammatory Diseases, Mashhad University of Medical Sciences, Mashhad, Iran*
*2. Department of Neurology, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** HAM/TSP is a chronic, progressive and neurologic disease in CNS which is caused by HTLV-1. Secretion of pro and inflammatory cytokines such as: IFN-γ and TNF-α have been shown to be associated with HAM/TSP. IL-33 is an inflammatory cytokine released from necrotic tissue as an alarmin because of its high expression in endothelial and epithelial cells exposed to tissue damage or pathogen. In the present study we examined the role of IL-33 and its soluble receptor named sST2 in hTLV-1 infection.
**Methods:** The population study was included HAM/TSP patients, asymptomatic HTLV-1 carriers (ACs) and healthy controls (HCs). Peripheral blood mononuclear cells were collected and RNA was extracted and cDNA was synthesized. The mRNA expression of IL-33 was quantified by real time PCR. IL-33 and soluble ST2 serum levels was measured by ELISA.
**Results:** The IL-33 expression was higher in AC group compared with HAM/TSP patients ($p=0.001$) and HCs ($p=0.012$). There was no significant difference in IL-33 expression between HAM/TSP patients and HCs ($p>0.05$). IL-33 serum level was higher in HAM/TSP patients compared with AC group ($p=0.009$). There was no significant difference in IL-33 serum level between HAM/TSP patient and HCs ($p=0.345$). No significant difference in IL-33 serum levels was observed between ACs and HCs groups ($p=0.158$). Finally, there was no significant difference in sST2 serum levels was detected among there groups ($p=0.385$).
**Conclusion:** The serum levels of IL-33 was markedly elevated in HAM/TSP patients compared to HTLV-1 carriers which might suggest it might be involved in the pathogeneses of HTLV-1 infection.

**Keywords:** HTLV-1, HAM/TSP, IL-33, sST2

ICIA2018\Immunology of Infectious Diseases Viruses\Poster Discussion\8117
**8117**

## Predictive Factors of Treatment Failure in Patients with Chronic Hepatitis C

Maryam Khosravi[1], Hamid Salimi[2], Amir Mohammad Alborzi[3], Pedram Nazari[3], Farzaneh Chahardahcherik[3]

1. *Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
***2.*** *Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO, USA*
*3. Cancer Research Center Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

**Background:** Drug resistance and relapse of disease are the main challenges encountered when treating patients with chronic Hepatitis C Virus (HCV) infection. Production of interferon gamma (IFN-γ), IL-29 and other inflammatory cytokines appears to play a critical role in limiting virus replication and thus impacting on patients' response to antivirals therapies. The aim of this study was to assess effects of the above mentioned factors on the lack of response to treatment among a group of chronically infected HCV patients.
**Methods:** This study included 57 HCV patients who were admitted to *Shariati* Hospital for treatment. Blood samples were collected from each subject before the onset of treatment with ribavirin and peg-interferon. Levels of IL-29, IFN-γ, liver enzymes as well as HCV titer were then measured in each serum sample. To determine HCV titer, RNA was extracted from peripheral blood mononuclear cells (PBMCs) of patients using High Pure Viral RNA Kit. RT-nested PCR was performed on the RNA extracts using primers designed for the Core region of HCV genome. Patients were followed for 24 months post-treatment. Significance level was considered as $P < 0.05$.
**Results**: While all non-responder (NR) patients carried HCV in their PBMCs, 41.1% and 58.7% of those in sustained virologic response (SVR) group and relapse group were positive for the virus genome, respectively. The level of liver enzymes was significantly higher in patients who contained HCV genome in their PBMCs compared to those with no detectable viral load. The highest level of IL-29 and IFN-γ was detected in SVR .group, followed by the relapse and NR groups, respectively
**Conclusion**: The results from this study revealed a direct correlation between levels of IL-29 and IFN-γ in patients' sera and their response to antiviral therapy. Induction of these immune responses in HCV patients may, therefore, be beneficial in controlling virus replication and thereby preventing disease relapse/treatment failure.

**Keyword**: Hepatitis C Virus, treatment response, predictive factors

ICIA2018\Immunology of Infectious Diseases Viruses\Poster Discussion\8392
**8392**

# Effect of Aromatherapy Massage on Salivary Cortisol and Psychological Assessments in a Depressed Patient with Hepatitis B: A Reversal-to-Baseline Design

Bijan Pirnia[1], Dr. Kambiz Pirnia[2], Hamid Reza Taheri Nakhost[3], Dr. Ali Reza Moradi[4], Soheila Rahmani[5], Dr. Rasool Roshan[6]

1. *Department of Psychology, Faculty of Humanities, University of Science and Culture, Tehran, Iran*
2. *Technical Assistant in Bijan Center for Substance Abuse Treatment, Tehran, Iran*
3. *Iranian National Center for Addiction Studies and Tehran University of Medical Sciences, Tehran, Iran*
4. *Department of Psychology, Faculty of Humanities, School of Psychology, Kharazmi University*
5. *Islamic Azad University, Karaj, Iran*
6. *Department of Psychology, Faculty of Humanities, Shahed University, Tehran, Iran*

**Background**: Studies show that 33-78% of patients with chronic Hepatitis B (CHB) suffer from depression in Iranian society. Mental disorders such as depression may affect the immune response in these patients, hence it is clinically important. This was conducted aimed to evaluate the efficacy of aromatherapy as a complementary therapy on the level of cortisol and mood index in an opioid dependent patient.
**Methods**: The patient was a 41 years old man with hepatitis B with a history of pseudoaneurysm and a long history of heroin using who was under methadone treatment. In an ABAB reversed design with a multiple baseline, methadone treatment was evaluated over a 24-week period and aromatherapy massage was presented at two periods last for forty minutes each. The level of salivary cortisol was considered as primary outcomes and the changes in anxiety, stress and depression indices were considered as secondary outcomes. The results were analyzed by linear mixed model (LMM) and Kolmogorov-Smirnov (K-S) tests.
**Results**: The primary outcomes showed that inhalation aromatherapy led to a decrease in salivary cortisol levels. Secondary outcomes showed that aromatherapy has a significant effect on the improvement of anxiety, stress and depression. Also, there was a significant correlation between the levels of cortisol and triple indices (all<0.01).
**Conclusion**: Aromatherapy as an alternative and complementary therapy can be used in the management of psychological indices in drug dependent individuals.

**Keywords**: aromatherapy, hepatitis B**,** cortisol**,** pseudoaneurysm**,** depression**,** anxiety

ICIA2018\Immunology of Infectious Diseases Viruses\Poster Discussion\8633
**8633**

## Detecting the Effects of Anti-HCV Drug "Daclatasvir" on Apoptosis' Factors

Somaieh Nasereslami [1]*, Taravat Bamdad [1], Asghar Abdoli [2]

1. *Department of Virology, School of Medical Sciences, University of Tarbiat Modares, Tehran, Iran*
2. *Department of Virology, Pasture Institute, Tehran, Iran*

**Background:** Direct-acting antiviral agents (DAAs) have revolutionized the treatment of hepatitis C virus (HCV) infection over the last 5 years. As a result of our better understanding of the HCV life cycle, specific DAAs have been developed for HCV that are able to target the viral proteins implicated in replication of the virus, i.e., the NS3/4A protease, NS5B polymerase, and multifunctional NS5A replication complex. The first-generation protease inhibitors significantly improved the sustained virologic response (SVR) in genotype 1-infected patients, but at the cost of increased side effects, a complex pattern of drug–drug interactions, and viral resistance. In addition, the first-generation drugs still required the use of PEGylated interferon (PEG-IFN) for 24–48 weeks. Oral IFN-free combinations containing at least two DAAs enabled less complex dosing, tolerable side effects, and fewer drug–drug interactions
**Methods:** In this study MTT dose of Daclatasvir that has minimum cytotoxicity for cell. Flow cytometry test in order to demonstrate. LC3 protein accumulation was carried out on Huh7 cells. After the culture of cells and incubation with 60 pM daclatasvir, transfected in pcDNA-Beclin1, NS5A, P53, Caspase, Bax and Bcl2. After cDNAs synthesis Real-time PCR was carried out on their expression (with HPRT), were T assay was applied to indicate evaluated. The result had been analyzed by Prism software.
**Results:** To examine the potential mechanism underlying the effects of daclatasvir on cell cycle and apoptosis, we analyzed the expression of genes involved in the cell cycle regulation and apoptosis. We evaluated P53, BCL-2, BAX, and CASP3 gene expression, 48 hours after the transfection with these factors. P53, a known target of daclatasvir, showed a 2.53 fold increase in the infected cells in comparison with the control cells. The expressions of CASP3 and BAX were upregulated in the infected cells in comparison with the control cells (3-fold and 2-fold increase, respectively) ($p < 0.05$) .The BCL-2, an anti-apoptotic gene, showed no significant change in response to the increased apoptotic factors expression level.
**Conclusion:** These findings suggest that DAA-based therapy (Daclatasvir) could be effective in advanced patients and could improve the current therapeutic strategies for treatment hepatocarcinoma.

**Keywords:** Daclatasvir, HCV, apoptosis, NS5A.

ICIA2018\Immunology of Infectious Diseases Viruses\Poster Discussion\8704
**8704**

## Accessing of TCD8+ Cells Response Against Adipophlin, Epstein - Barr virus, Cytomegalovirus, Influenza Derived Poly Peptide in Patients with Atherosclerosis Compared to Healthy Individuals

Solmaz khansalar[1], Zahra farzaneh[1], Hossein Golmoghadam[1] , Mohamad Ali asadi[1],
Saeed Malek Hosseini[1],Mehrnoosh Doroudchi[1], Kurosh kalantar[1]

*1. Department of Immunology, School of Medicine, Shiraz University of Medical sciences, Shiraz, Iran*

**Background:** Atherosclerosis, a chronic inflammatory process that affects the walls of large and medium-sized arteries, is the leading causes of morbidity and premature death worldwide. Many cell types, including endothelial cells, monocytes, dendritic cells (DCs), lymphocytes, eosinophils, mast cells and smooth muscle cells, contribute to the formation of atherosclerotic plaques. Inflammatory responses at the atherosclerotic plaques can damages self-tissue and subsequently release self-peptides that are engulfed by APCs and will be presented to T cells. CD8+ T lymphocytes may play an important role in atherogenesis. As adipose differentiation-related protein (ADRP, adipophilin) is a prominent component of the cytosolic lipid droplets (CLD) present in most of the cells of the atherosclerotic plaque, the possible use of HLA class I restricted peptide derived from this protein was analyzed as a T-cell epitope presented by APCs. In addition, several reports showed that the role of some pathogens in the immunopathology of atherosclerosis. So, Peptide from Epstein-Barr virus, Cytomegalovirus, influenza also was selected to explore this possibility.
**Methods:** 28 patients with atherosclerosis and 40 age and sex matched healthy donors were entered the study. PBMCs were isolated and HLA typing was done using flow cytometry. HLA-A2 positive PMCs were cultured and stimulated using specific peptides (Adipophilin 129-137, Flu 58-66, CMV 495–503, EBV 280-288). Cell culture supernatant was used to measure IFN-γ using human ELISA kit.
**Results:** 11 (39.3%) out of 28 patients with atherosclerosis and 16 (40%) out of 40 healthy individuals were positive for HLA-A02. PBMCs stimulation revealed no statistically significant differences in the levels of IFN-γ between HLA-A2 positive patients or healthy controls against selected peptides.
**Conclusion:** Adipophilin is not a self Ag presented by APCs at the site of atherosclerotic plaque, but further experiments are needed to completely rule out its importance in the etiopathogenesis of arthrosclerosis.

**Keywords:** Atherosclerosis, CD8+ T lymphocytes, adipose differentiation-related protein (ADRP), adipophilin, cytosolic lipid droplets (CLD).

ICIA2018\Immunology of Infectious Diseases Viruses\Poster Discussion\9810
**9810**

## HTLV-1 Infection Dampens NK cell Function

Asadollah Mohammadi[1], Reza Boostani[2], Houshang Rafatpanah[3]

*1. Cellular & Molecular Research Center, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran*
*2. Department of Neurology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*3. Inflammation and Inflammatory Diseases Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** HTLV-1 is the etiological agent of HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP). Natural killer (NK) cells are necessary for first defense against virally-infected and stressed cells. They kill target cells by receptor-mediated and granule exocytosis pathways.
**Methods:** A total of 21 asymptomatic carriers (ACs), 21 HAM/TSP patients and 8 healthy controls (HCs) were recruited. NK cell numbers, phenotype, function, and effector molecules expression were analyzed by flow cytometry. The cytotoxic activity of NK cells was assessed by CD107a degranulation. Also, the effects of NK cell anti-viral functions on HTLV-1 proviral load, Tax and HBZ mRNA expression were analyzed.
**Results:** Total NK cell numbers showed a decrease in HAM/TSP patients. NK cells from HAM/TSP patients and ACs showed substantial decrease in their function and had lower cytotoxic-related molecules such as Fas, FasL and TRAIL than HCs, but the levels of perforin, granzyme B, and granulysin were similar. No significant correlations between NK cell number, phenotype and function with proviral load, Tax and HBZ mRNA expression were found.
**Conclusion:** Lower numbers of NK cells and specific defects in their capacity to degranulate may play a role in HTLV-1 persistence. Also, lower numbers of NK cells and activity in HAM/TSP patients could be an effect of a high proviral load of HTLV-1, antigen abundance and/or persistence, rather than a cause. Restoration of NK cell capacity, as achieved by viral load reduction, could therefore contribute to viral control and subsequently HAM/TSP disease susceptibility.

**Keywords:** HTLV-1; NK cell; Neuroinflammation; HAM/TSP.

ICIA2018\Immunology of Infectious Diseases Viruses\Poster Discussion\9933
**9933**

# Comparison of IL-12, IL-18 and IFN-γ Gene Expression in HTLV-1-Associated Myelopathy / Tropical Spastic Paraparesis (HAM/TSP) Patients, HTLV-1 Carriers and HTLV-1 Negative Individuals

Hamid Reza Bidkhori[1], Houshang Rafatpanah[2*], Seyyed Abdolrahim Rezaee[2], Narges Valizadeh[2], Mohsen Tadayyon, Sanaz Ahmadi Gezeldasht[1], Rahele Miri[1], Arezo Gohari Shabgah[2], Mohammad Reza Hedayati Moghaddam[1*]

*1. Blood-borne Research Center, Academic Center for Education, Culture and Research (ACECR)-Khorasan Razavi, Mashhad, Iran*
*2. Immunology Research Center, Inflammation and Inflammatory Diseases Division, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** Chronic inflammation is among the parameters that may increase the risk of developing HAM/TSP in the HTLV-1 carriers. The imbalance between Th1 and Th2 cells is highly determinative due to variations in the level of cytokines. Interleukins 12 and 18, along with interferon gamma, induce Th1 response. The present study aimed to determine the gene expression and serum levels of IL-12, IL-18 and IFN-γ in patients with HAM/TSP and asymptomatic HTLV-1 carriers compared to healthy subjects.
**Methods:** In this study, 21 asymptomatic HTLV-1 carriers, 20 HAM/TSP patients and 21 HTLV-1-negative healthy volunteers were enrolled. Real time PCR technique was used to measure the expression level of IL-12, IL-18, IFN-γ genes and proviral load. The plasma levels of cytokines were obtained using ELISA assay.
**Results:** IL-12 gene was expressed in all healthy controls, nine asymptomatic HTLV-1 carriers and five HAM/TSP patients. The gene expression and the serum levels of IL-18 were lower in the HTLV-1-positive group than in the control group ($p = 0.001$ and $p = 0.012$, respectively). However, there was no significant difference between HTLV-1 carriers and HAM/TSP patients ($p = 0.335$ and $p = 0.695$, respectively). The mean viral load in HAM/TSP patients ($3693.2 \pm 2444$) was higher than non-symptomatic carriers ($1388.4 \pm 1365.2$) ($p = 0.002$). The correlation coefficient between serum level of IFN-γ and viral load in HAM/TSP patients was not statistically significant ($r = -0.142$, $p = 0.55$) but there was a positive correlation between serum level of IL-18 and viral load in this group ($r = 0.654$, $p = 0.002$).
**Conclusion:** In this study, increased HTLV-1 proviral load and high serum IFN-γ levels in the HAM/TSP group were determined in comparison to the asymptomatic carriers. Moreover, the expression level of IL-18 in the HTLV-1-positive group and the healthy control group was significantly different. Our findings highlight the fact that the proviral load and the inflammatory cytokine profile during HTLV-1 infection may be independent events leading to the occurrence of HAM/TSP.

**Keywords:** HTLV-1 infection, Non-symptomatic carriers, HAM/TSP, IL-12, IL-18 and IFN-γ.

# Immunology of Mycobacterium Tuberculosis

# Poster Discussion

ICIA2018\Immunology of Mycobacterium Tuberculosis\Poster Discussion\7961
**7961**

## Assessment of B cell Lymphoma 6 (BCL6) Expression Level in Peripheral Blood CD38+B Lymphocytes of Common Variable Immunodeficiency (CVID) Patients

Faezeh Abbasi Rad[1], Fahimeh Khadem[1], Nafiseh Esmaeil[1], Mazdak Ganjalikhani Hakemi[1], Shokrolah Farrokhi[2], Roya Sherkat

*1. Department of Immunology, school of Medicine, Isfahan Isfahan University of Medical Sciences, Isfahan, Iran*
*2. Department of Immunology and Allergy, The Persian Gulf Tropical Medicine Research Center, The Persian Gulf Biomedical Research Institute, Bushehr University of Medical Sciences, Bushehr, Iran*
*3. Aquired Immunodeficiency Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background:** Common variable immunodeficiency (CVID), the most common symptomatic primary immunodeficiency, is a heterogeneous set of immunological abnormalities including decreased serum levels of antibodies, impaired antibody response to infections or vaccination. The syndrome includes impaired B-cell maturation, impaired somatic hypermutation, reduced numbers of circulating memory B cells, and absent or reduced plasma cells. BCL6 (B cell Lymphoma 6) is a transcription factor which is important for the evolution and proliferation of B cells. This study aimed to investigate the expression of BCL6 in peripheral blood CD38+B lymphocytes of the patients with CVID.
**Methods:** Blood samples were collected from 14 CVID patients with substitutive immunoglobulin (Ig) therapy before immunoglobulin infusion and 14 normal controls. Then peripheral blood mononuclear cells were isolated by Ficoll-Hypaque density centrifugation. CD19+ B lymphocytes were purified from PBMCs by positive selection using B-cell isolation kit. Flow cytometry method was employed to determine the expression of the BCL6 in CD38+ B cells.
**Results**: In this study, the expression of BCL6 in CD38 +B lymphocytes in patients was 1.51% and in healthy subjects was 0.58%, respectively. According to the statistical analysis, the difference was not significant ($P>0.05$).
**Conclusion:** The results showed that there is no significant difference in the mean expression of BCL6 of the CD38 + B cells in the patients with CVID, compared with control group. However, the average BCL6 expression of CD38+ B lymphocytes in patients was more than in control group.

**Keywords:** B lymphocyte; Plasma cell; PBMC; BCL6; CD38

ICIA2018\Immunology of Mycobacterium Tuberculosis\Poster Discussion\7994
**7994**

# IL-10 -592 A/C Gene Polymorphism and Cytokine Plasma Levels Are Associated with Susceptibility to Drug Resistance Tuberculosis

Atefeh Sadeghi Shermeh[1], Mehdi Shekarabi[*1, 2], Ali Akbar Delbandi[1, 2], Payam Tabarsi[3], Esmaeil Mortaz[3], Mohammad Reza Bolouri[1], Majid Khoshmirsafa[1], Elham Alipour fayez[1]

*1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*
*2. Immunology Research Center, Institute of Immunology and Infectious Diseases Iran University of Medical Sciences, Tehran, Iran*
*3. Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background**: Tuberculosis (TB) as a global public health problem, still remains a major cause of mortality worldwide. In recent decades, Mycobacterium tuberculosis (Mtb) strains resistant to anti-tubercular agents have been emerging as critical issues in tuberculosis treatment. Immune responses to all pathogens are cytokine dependent, Mtb is no exception and variation in cytokine genes, especially single nucleotide polymorphisms (SNPs) may lead to abnormal or ineffective immune response.
**Methods**: So we investigated SNPs in *IFN-γ* (+874 T/A) and *IL-10* (-592 A/C) genes in relation to these cytokines level and pulmonary tuberculosis treatment responsiveness. A total of 87 patients and 100 healthy individuals were included in this study. According to drug sensitivity test using polymerase chain reaction (PCR), patients divided into two groups, including 67 drug-sensitive (DS-TB) and 20 drug-resistant (DR-TB). Genomic DNA was genotyped for two SNPs by PCR-based methods. Serum cytokine levels were measured using ELISA.
**Results**: In both patients and controls, the *IL-10* -592A allele and AC genotype were presented in a greater proportion, but these differences were only significant between DR-TB and controls ($p< 0.05$). TB patients showed higher IL-10 serum levels in comparison to healthy individuals ($p< 0.05$). Although, no significant differences were observed for allele and genotype frequencies in *IFN-γ* +874, the serum levels of IFN-γ was higher in DS-TB patient compared with DR-TB and healthy individuals.
**Conclusion**: Thus, our findings suggest that association of the *IL-10* (-592 A/C) polymorphism with susceptibility to DR-TB in the studied population.

**Keywords:** Tuberculosis, Polymorphism, IFN-γ, IL-10, Drug resistance tuberculosis

ICIA2018\Immunology of Mycobacterium Tuberculosis\Poster Discussion\8070
**8070**

## Association of P2X7 Gene Common Polymorphisms with Pulmonary Tuberculosis in Lur Population of Iran

Farhad Shahsavar [1], Toomaj Sabooteh [2*], Seyyed Amir Yasin Ahmadi [3], Ali Amiri [4], Alireza Azargoon [4]

1. *Department of Immunology, Lorestan University of Medical Sciences, Khorramabad, Iran*
2. *Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran*
3. *Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran*
4. *Department of Internal Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran*

**Background:** Different genetic and environmental factors are associated with susceptibility to pulmonary tuberculosis (TB) in different individuals of different populations. Based on previous studies role of P2X7 gene common polymorphisms in susceptibility to pulmonary TB was associated with ethnicities. Aim: We intend to perform this study on genetic reservoir (gene pool) of Lur population of western Iran.
**Methods:** For the present case-control study, 100 unrelated pulmonary TB patients and 100 unrelated controls were enrolled through convenient sampling. TB confirmation was through smear and culture of sputum. Polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP) was used for molecular assay. This study has been approved in the ethic committee of Lorestan University of Medical Sciences with registration number LUMS.REC.1396.253.
**Results:** Among the genotypes of polymorphism 1513A/C, AA genotype was associated with susceptibility to pulmonary TB (P=0.0001; OR=4.750) whereas AC genotype was a protecting factor (P=0.0001; OR=0.192). Higher genetic reservoir of A allele was associated with more susceptibility to pulmonary TB (P=0.0001; OR=2.879) whereas C allele was a protecting factor (P=0.0001; OR=0.347). No significant result was found for -762T/C polymorphism.
**Conclusion:** In Lur population of Iran, 1513A/C polymorphism of P2X7 is associated with susceptibility to pulmonary TB. It is suggested that bio-information banks should be established and developed in countries.

**Keywords:** P2X7, Pulmonary Tuberculosis, Lur population, Iran.

ICIA2018\Immunology of Mycobacterium Tuberculosis\Poster Discussion\8261
**8261**

## Cloning, Expression and Immunological Assessment of EspB Protein from *Mycobacterium tuberculosis*

Zahra Hashemi[1], Soheila Ajdary[2,] Fariborz Bahrami[2], Alireza Rafiee[1], Farhad Riazi Rad[2]

1. Department of Immunology, Faculty of Medicine, Mazandaran University of Medical Sciences
2. Department of Immunology, Pasteur Institute of Iran

**Background:** Tuberculosis (TB)is the second leading cause of mortality in the world. One third of the world's population are infected with *Mycobacterium tuberculosis* (MTB), the causative agent of the disease. The most widely used vaccine against TB is BCG; however it provides inadequate protection. Therefore, a more effective vaccine is needed to control TB and for this purpose, identifications of the virulent factors which are both immunogenic and specific for the infection, are highly in demand. The genes residing in "region of difference 1" (RD1) are involved in pathogenesis of MTB. EspB is a newly identified protein, encoded by a gene located in this region, appears to be essential for the virulence of the bacterium. The objective of the present study was to produce recombinant EspB and evaluate its immunoreactivity in humans.
**Methods:** The encoding gene for MTB was commercially synthesized which was then used as a template to produce an amplicon, flanked by appropriate restriction enzymes by PCR. The PCR product was cloned in-frame into a pET21a expression vector and transformed into *Escherichia coli BL21* (DE3) for a histidine-tagged recombinant protein production by IPTG induction. The expression and purity of the protein (rEspB) was confirmed by SDS-PAGE and Western blotting analyses using anti-His antibody. PBMCs of patients with confirmed TB and healthy donors were isolated and stimulated with rEspB. Lymphocyte proliferation and IFN-$\gamma$ production were assessed by thymidine incorporation assay and ELISA.
**Result:** EspB gene was successfully cloned and expressed in *E. coli*. PBMCs from TB patients recognized rEspB and proliferated in response to this protein. IFN- $\gamma$ was produced by PBMCs from the patients.
**Conclusion:** Being a virulent factor of MTB, EspB is a target for anti-mycobacterial immune responses in humans and can be considered as a vaccine candidate against TB.

**Keywords**: *Mycobacterium tuberculosis,* EspB, PBMC, Immune response.

ICIA2018\Immunology of Mycobacterium Tuberculosis\Poster Discussion\8334
**8334**

## Interferon-γ Gene Polymorphisms in Pulmonary Tuberculosis Patients

Ali Pourmohammad[1], Mohammad Javad Nasiri[1]

*1. Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Upon infection with *Mycobacterium tuberculosis*, the IFN-γ plays an essential role for the elimination of pathogen. Subtle variations in IFN-γ gene may influence susceptibility to *M. tuberculosis*. This study investigates the relationship of the single base change polymorphic variants in interferon-γ and tuberculosis (TB) susceptibility.
**Methods:** We studied a population of 100 patients with culture-proven pulmonary TB and 90 healthy tuberculin-negative control subjects. Polymorphic variants in IFN-γ gene were assessed using PCR-RFLP.
**Results:** Statistical analyses revealed that IFN-γ 2109 A/G polymorphism was marginally associated with the risk of TB (P=0.04).
**Conclusions:** The present results revealed that certain genetic variants in IFN-γ gene may be associated with TB development, which may be useful preliminary data for future investigation.

**Keywords:** Susceptibility, Tuberculosis, Interferon-gamma, Polymorphisms

ICIA2018\Immunology of Mycobacterium Tuberculosis\Poster Discussion\8386
**8386**

# Evaluation and Comparison of *IFN-γ* and *IL-2* Gene Expression in Peripheral Blood of Patients with Positive and Negative Quantiferon-TB Test

Mahsa Jafari[1], Dr. Reza mansouri[2]

*1. Shahid sadoughi university of medical sciences*
*2. Reza Mansouri MD-PhD Assistant Professor Department of Immunology Faculty of Medicine Shahid Sadoughi University of Medical Sciences Safaieye, Bo-Ali street Yazd/Iran*

**Background:** Tuberculosis (TB) is a leading cause of mortality and morbidity worldwide. This infection is a major public health problem. TB control is largely dependent to early diagnosis and managements of suspected cases. Today several bacteriologic and immunologic methods such as microscopic study of Smear microscopy and sputum Culture. And tuberculin skin test is used for laboratory diagnosis of TB infection, but they were not as successful in decreasing TB as expected. In order to modify mentioned shortcomings, in recent years, IGRA, an immunologic test, is recommended for TB patients as a diagnostic test, and meanwhile releasing IFN-γ from lymphocytes is studied. Immunologic methods applied for diagnosing TB are IGRA and TST. These measuring are tentative. They also cannot make distinction between recent TB infection and the current one. IFN-γ has a key role in removing mycobacterium TB from the body. Considering this matter that 10% of patients with latent TB are subject to active TB and studding the amount of solution IFN-γ alone cannot be helpful in diagnosing latent TB and determining different stages of disease. Thus it is possible that other cytokines such as IL-2 can help in diagnosing latent TB. Because of the importance of screening and controlling latent TB infection in restricting the disease, besides IFN-γ studding gene-expression of relevant biomarkers such as IL-2 may have an important role in diagnosing latent TB and preventing disease progression. In this study expression of two genes, *IFN-γ* and *IL-2*, in patients with positive or negative Quantiferon-TB test are done. **Methods:** In this research 25 subjects with positive result in Quantiferon-TB test (13 females and 12 males with average age of 58.56 +_ 19.296) As the target group and 25 subjects with negative result in Quantiferon-TB test ( 13 females and 12 males with average age of 58.56 +_ 19.296) as witness group were chosen. In this study expression of two genes, *IFN-γ* and *IL-2*, in patients with positive or negative Quantiferon-TB test are done. After RNA extraction from the present blood in Quantiferon tube for mentioned two groups, cDNA syntheses was done and eventually gene-expression by means of real-time PSR technic was studied. Then amount of gene expression of these genes, their relations together and with IFN-γ release in Quantiferon-TB test were analyzed statistically. **Results:** In this study with two target and witness groups, findings indicated that *IFN-γ* gene-expression rate has significant correlation with becoming positive in Quantiferon-TB test. But between *IL-2* gene-expression rate and becoming positive in Quantiferon-TB test is not significant correlation. There is no significant correlation between *IFN-γ* and *IL-2* gene-expression rate with IFN-γ release in Quantiferon-TB test. **Conclusion:** By knowing the results of current study, *IFN-γ* gene-expression in patient's blood with positive Quantiferon-TB test can only considered as one of Para clinical indexes in diagnosing mycobacterium TB infection.

**Keywords:** mycobacterium tuberculosis, Tuberculin Skin test, Interferon Gamma Release Assay, Interleukin 2, Interferon Gamma

ICIA2018\Immunology of Mycobacterium Tuberculosis\Poster Discussion\9870
**9870**

## Study of The Exosomal miRNAs in Patient with Active Pulmonary Tuberculosis

Shamila D. Alipoor[1,2,3], Esmaeil Mortaz[1], Payam Tabarsi[1], Johan Garssen[4,5], Mohammad Varahram[6], Ian M. Adcock[7, 8], Ali Akbar Velayati[6]

*1. Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Institute of Medical Biotechnology, Molecular Medicine Department, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran*
*4. Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University; Utrecht, Netherlands*
*5. Nutricia Research Centre for Specialized Nutrition, Utrecht, Netherlands*
*6. Mycobacteriology Research Centre, National Research Institute of Tuberculosis and Lung Disease (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*7. Priority Research Centre for Healthy Lungs, Hunter Medical Research Institute, The University of Newcastle, Newcastle, NSW, Australia*

**Background:** Tuberculosis (TB) remains a major threat to human health. Due to the limited accuracy of the current TB diagnostic tests it is critical to determine novel biomarkers for this disease**.** Circulating exosomes have been used as diagnostic biomarkers in various human diseases.
**Methods:** serum-derived exosomes were isolated from TB patients and matched control subjects. The expression of miR-1224, -1293, -484, -425 and -96 were examined by RT-qPCR. Receiver operating characteristic (ROC) curve was performed to evaluate the diagnostic potency of each individual serum exosomal miRNA.
**Results:** only miR-484, -425 and -96 were significantly increased in the exosomes of TB patients as determined and significantly correlated with the TB infection level in patients. ROC curve analysis showed the diagnostic potency of each individual serum exosomal miRNA with an area under the curve (AUC) =0.72 for miR-484 ($p<0.05$), =0.66 for miR-425 ($p<0.05$) and =0.62 for miR-96 ($p<0.05$). The predictive value of the individual miRNAs was greatest in the patients with high bacterial burden particularly for miR-484 (AUC=0.82) and miR-425 (AUC=0.868) in 3+ patients. ROC curves using combined miRNA expression levels gave greater AUC values with the combination of all 3 being more predictive of TB per se (AUC=0.78).
**Conclusion:** These results demonstrate that exosomal miRNAs have diagnostic potential in active tuberculosis. The diagnostic power may be improved when combined with conventional diagnostic markers.

**Key words:** Exosomes, Tuberculosis, miRNA

ICIA2018\Immunology of Mycobacterium Tuberculosis\Poster Discussion\9872
**9872**

**A Computational Study on the Roles of Serum Exosomal miRNAs in Patient with Active Tuberculosis.**

Shamila D. Alipoor[1], Esmaeil Mortaz[2], Payam Tabarsi[2], Johan Garssen[3], Ian M. Adcock[4], Mohammad Varahram[5], Neda Dezfuli[2], Ali Akbar Velayati[5]

*1. Institute of Medical Biotechnology, Molecular Medicine Department, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran*
*2. Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University; Utrecht, Netherlands*
*4. Airways Disease Section, National Heart & Lung Institute, Imperial College London, London, UK*
*5. Mycobacterilogy Research Center (MRC) National Research Institute of Tuberculosis and lung diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Tuberculosis (TB) remains a major threat to human health. Due to the limited accuracy of the current TB diagnostic tests it is critical to determine novel biomarkers for this disease**.** Circulating exosomes have been used as diagnostic biomarkers in various human diseases. In this study a miRNAs network analysis was perform on the changed miRNAs after BCG infection**.**
**Method:** MicroRNA target genes were determined using miRTarBase and microT-CDs algorithms. Pathway analysis was performing using EnrichR and the KEGG pathway database. Protein–protein interaction (PPI) networks were constructed and analyzed by STRING online server with an annotation score ≥0.5 as the cut-off and visualized and analysis by cytoscape v.3.4.0. The highly interconnected regions (clusters) was extracted using MCODE plugin in cytoscape .Topological analysis of this network was perform to determine properties of nodes including score and connectivity degree (k) parameters.
**Result:** The extended network consist of 295 node connected via 2552 edges. A total of three clusters were detected by MODE plugin implemented in cytoscape. The result showed highest score is belonged to cluster A with score 20.83. The number of nodes in clusters A, B, C was 56, 16, 9 with scores: 20.83; 9.6 and 8.75 for clusters A, B and C respectively. Pathway analysis showed that all the clusters contain nodes that involved in the immunological and metabolism pathways.
**Conclusion**: Our results confirmed that infection with mycobacterium affected exosomal miRNAs which may reflect the intracellular status and pathology of the cells. These data illustrated the potential role of exosomal miRNAs as a biomarker for TB diagnosis.

**Keywords**: Exosome, Tuberculosis, Computational study

# Immunology of Rheumatic Diseases

# Poster Discussion

ICIA2018\Immunology of Rheumatic Diseases\Poster Discussion\7506
**7506**

# Nanocurcumin Impacts on Aberrant T cells Related MicroRNAs Profile Expression in Ankylosing Spondylitis Patients

Yousef Daneshmand pour [1, 2], Sanam Dolati [1, 3], Mehdi Yousefi[1, 3]

1. *Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Department of medical Genetics , Faculty of Medicine, Tabriz University of Medical sciences*
3. *Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Ankylosing spondylitis (AS) is a chronic, progressive inflammatory rheumatic disease that sacroiliac joints and the axial skeleton are involved and back pain and progressive stiffness of the spine are main clinical features. Oligoarthritis of the hips and shoulders, enthesopathy, and anterior uveitis are common but, involvement of the heart and lungs is rare. The aim of current study was to evaluate the effect of Nanocurcumin on expression level of significant miRNAs associated with Th17 and Treg function.
**Methods:** 24 AS patients were divided in two groups. One group received 80 mg nanocurcumin every day for 16 weeks and 12 other patients received placebo as control group. The peripheral blood mononuclear cells (PBMCs) were collected from blood samples of patients and then, expression level of significant miRNAs was evaluated by Real time PCR (mir-141, mir-155 and mir-200, mir-27, mir-17 and mir-146a).
**Results:** Our study showed in case of Th17 related miRNAs, expression level of mir-141, mir-155 and mir-200 (P value=0.04, 0.02 and <0.0001, respectively) were significantly decreased in case group. In Treg related miRNAs, mir-17 and mir-27 were significantly decreased while mir-146a was significantly increased (p value=0.0026 and p value=0.0003, p value=0.02 respectively).
**Conclusion:** Our data indicates significance of Th17 and Treg function in AS and well therapeutic effect of Nanocurcumin on these patients. Also, our results shows designing therapeutic strategies based on changing miRNA expression in Treg and Th17 can be possible treatment for AS patients in future.

**Keywords:** Ankylosing spondylitis, Nanocurcumin, miRNA, T Cells

ICIA2018\Immunology of Rheumatic Diseases\Poster Discussion\7563
**7563**

# The Potent Suppressive Effect of β-D-mannuronic Acid (M2000) on Molecular Expression of the TLR/ NF-kB Signaling Pathway in Ankylosing Spondylitis Patients

Maryam Roozbehkia[1], Mahdi Mahmoudi[2*], Somaye Aletaha[1], Nima Rezaei[3], Abbas Mirshafiey [1]

*1. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran)*
*2. Rheumatology Research Center, Shariati Hospital, Tehran University of Medical Science, Tehran, Iran)*
*3. Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Sheffield, UK*

**Background:** Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease characterized by the inflammation of sacroiliac joints and axial skeleton. A combination of genetic, environmental and immunological factors are involved in AS's pathogenesis. TLRs are type I transmembrane glycoproteins that play a crucial role in the innate immune responses against invading pathogens. Observational studies have demonstrated a possible association between TLR dysregulation and AS. The β-D-mannuronic acid (M2000), as a novel NSAID with immunosuppressive property, has shown an inhibitory effect on Toll-like receptor (TLR) 2, 4 signaling in HEK293 cells.
**Methods:** In the present study, we investigated the gene expression of Myd88, IKB-alpha, NF-kB and MAPK14 (genes of the TLR/NF-kB Signaling Pathway) by Real time PCR in AS patients in comparison to healthy subjects and also the effect of β-D-mannuronic acid on disease activity and mRNA expression of these molecules in affected patients.
**Results:** We showed for the first time that the gene expression level of Myd88, IKB-alpha, NF-kB and MAPK14 was higher in AS patients in comparison to healthy subjects. Moreover we confirmed that the β-D-mannuronic acid not just reduced significantly the disease activity of AS individuals compared to placebo, but also it could significantly decrease the expression level of genes associated with TLR/NF-kB Signaling Pathway in treated patients with M2000.
**Conclusion:** These results may provide a new therapeutic approach to attenuate inflammatory responses in AS patients,(Identified; IRCT 2013062213739N1).

**Key words**: Ankylosing spondylitis, M2000, TLRs, Mannuronic acid, Myd88, IKB-alpha, NF-kB, MAPK14

ICIA2018\Immunology of Rheumatic Diseases\Poster Discussion\7572.doc
**7572**

# Analysis of PD-1 and Tim-3 Expression on $CD4^+$ T Cells of Patients with Rheumatoid Arthritis; Association to Disease Severity

Zohreh Koohini[1], Hadi Hossein Nataj[1], Maryam Mobini[2], Aref Hosseinian Amiri[2], Alireza Rafiei[1], Hossein Asgarian Omran[1,3]

*1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
*2. Department of Rheumatology, Imam Khomeini Hospital, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.*
*3. Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

**Background:** T-cell immunoglobulin and mucin-domain containing-3 (Tim-3) and Programmed cell death-1 (PD-1) are inhibitory receptors which involved in various immunological functions like regulating Th1 and Th17 activity, induction of tolerance and reducing production of inflammatory cytokines. In this study, the expression of Tim-3 and PD-1 were evaluated on $CD4^+$ T cells of patients with Rheumatoid Arthritis (RA) and their association with disease severity was also addressed.
**Methods:** A total of 37 RA patients and 31 sex and age-matched healthy controls were included in this study. Disease severity of RA patients was determined by Disease Activity Score of 28 joints scoring system (DAS28). A three-color flow cytometry method was applied to determine the frequency of $Tim\text{-}3^+/PD\text{-}1^+/CD4^+$ T cells. To measure the cytokine production, PBMCs were stimulated with PMA/ionomycin. Concentrations of IL-17, IL-10, IFN-γ, and TNF-α were measured in culture supernatants by ELISA.
**Results:** The frequency of $PD\text{-}1^+/CD4^+$ and $Tim\text{-}3^+/PD\text{-}1^+/CD4^+$ T cells were significantly higher in patients with RA compared to controls (p=0.0013 and p=0.050, respectively). The percentage of $Tim\text{-}3^+/CD4^+$ T cells was similar in patients and controls (p= 0.4498). The RA patients have produced significant higher levels of TNF-α, IL-17, and IFN-γ than those of healthy controls (p= 0.0121, p= 0.0417 and p= 0.0478, respectively). Interestingly, an inverse correlation was found between the frequency of $Tim\text{-}3^+/CD4^+$ cells and disease severity of RA patients (r=-0.4696, p= 0.0493). Similarly, the percentage of $Tim\text{-}3^+/PD\text{-}1^+/CD4^+$ T cells was also revealed an inverse correlation with DAS28 score (r=-0.5268, p= 0.0493). Moreover, significant positive correlations were detected between the concentrations of TNF-α (r=0.6418, p= 0.0023) and IL-17 (r=0.4683, p=0.0373) with disease activity of RA patients.
**Conclusions**: Our results indicate that Tim-3 and PD-1 are involved in immune dys-regulation mechanisms of rheumatoid arthritis and could be considered as useful biomarkers for determination of disease severity and progression.

**Keywords:** Rheumatoid Arthritis, Tim-3, PD-1, Disease Activity Score

ICIA2018\Immunology of Rheumatic Diseases\Poster Discussion\7783
**7783**

# *Lactobacillus delbrueckii* and *Lactobacillus rhamnosus* encompass a Tolerogenic Effect on Pristane Induced Lupus Murine Model

Sahar Khorasani[1,2], Maryam Rastin[1], Mahmoud Mahmoudi[1], Fahime Lavi Arab[1], Fatemeh Mardani[1], Nafiseh Tabasi[1], Alireza Esmaeili[1,] Mahdieh Khazaee[1]

*1. Immunology Research Center, BuAli Research Institute, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*2. Faculty of Ferdows Paramdical Sciences, Birjand University of Medical Sciences, Birjand, Iran*

**Background:** It was shown that some *Lactobacillus* probiotics may induce regulatory T cells and so have beneficial role in SLE remission. The aim of this study was to evaluate the tolerogenic effect of *Lactobacillus delbrueckii* and *Lactobacillus rhamnosus* on pristane induced lupus murine model in both prophylactic and therapeutic manner.
**Methods:** Fifty-four female BALB/c mice were randomly divided into 9 groups. After SLE induction by pristane, four groups were treated from day 0 and 4 groups treated 2 months later. The treatments were as follows: *Lactobacillus rhamnosus*, *Lactobacillus delbrueckii*, mix of both probiotics and prednisolone. In addition, one group was also considered as an induction control group without any treatment. Finally we evaluated the presence of anti-nuclear antibody (indirect immunofluorescence), anti-double strand DNA and ribonucleoprotein (ELISA), renal function (proteinuria, serum level of creatinine and urea and also histological staining of kidney mice), the expression of FoxP3, IL-10, TGF-β and IL-6 (real time) and Treg number (flow cytometry).
**Results:** Our study demonstrated the probiotics therapy lead to decrease ANA, anti-dsDNA, anti-RNP, kidney dysfunction, IL-6 and increase IL-10, TGF-β, FoxP3 and also Tregs compared to induce lupus control group. In addition, the prednisolone receiving groups show decrease IL-6 and increase TGF-β more prominent than the probiotic ones.
**Conclusion:** The results indicate the role of *Lactobacillus rhamnosus* and *Lactobacillus delbrueckii* in the enhancement of Tregs number and decrease of inflammatory cytokines and disease severity. Also it seems the tolerogenic effect of probiotics (by increase Tregs, FoxP3 and IL-10) was more prominent than prednisolone receiving once, whereas the prednisolone has more anti-inflammatory effect (by decrease IL-6 and maybe Th17) compared to the *Lactobacillus*. Concerning the results, this study suggests managing SLE patients with a lower dose of prednisolone with the *Lactobacillus* supplementation; further studies will confirm the results.

**Keywords:** *Lactobacillus rhamnosus*, *Lactobacillus delbrueckii*, Lupus erythematosus, Pristane, Regulatory T cell

ICIA2018\Immunology of Rheumatic Diseases\Poster Discussion\7842
**7842**

# Foxp3 Gene Polymorphism in Rheumatoid Arthritis Patients Associated with Tregs Frequency in Iranian Population

Amir Salek Farrokhi[1], Vida Hashemi[2], Rana Ezzeddini[3], Mohammad Taher Tahoori[4]

1. *Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
2. *Department of basic science, Faculty of Medicine, Maragheh University of Medical Sciences, Maragheh, Iran*
3. *Department of Clinical biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
4. *Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

**Background:** Rheumatoid arthritis (RA) is characterized by infiltration of T lymphocytes. Regulatory T cells (Tregs) and Forkhead box P3 (Foxp3) have a crucial role in preventing autoimmunity and undesirable T cell responses. The aim of this study was to analyze the association of Foxp3 gene polymorphism and Tregs frequency in Iranian RA patients.
**Methods:** RA patients (n=240) and control subjects (n=240) were selected from Iran in order to carry out the study. Genomic DNA was genotyped for –3279 C/A Foxp3 gene SNP using the PCR-RFLP. The frequency of Tregs and serum level of IL-10, TGF-β, anti-CCP and RF were determined by flow cytometry and ELISA methods, respectively.
**Results:** The results showed a significant association of Foxp3 –3279 A allele with augmented risk of RA in Iranian patients compared to wild-type allele. The frequency of CA and AA genotypes were significantly higher in patients. RA patients with AA genotype have a significant reduction in frequency of Tregs compared to patients with CC and CA genotypes. TGF-β and IL-10 significantly diminished in patients with AA genotype compared to patients with CA and CC genotypes.
**Conclusion:** Our findings indicated that the Foxp3 polymorphism was associated with Tregs frequency and susceptibility to RA in the Iranian populations.

**Keywords:** Rheumatoid Arthritis, Foxp3, Regulatory T Cells, Polymorphism

ICIA2018\Immunology of Rheumatic Diseases\Poster Discussion\8246
**8246**

## Increased Autophagy in Peripheral Blood Mononuclear Cells of Rheumatoid Arthritis Patients

Bahareh Kardideh[1], Mahdi Taghadosi[1], Zahra Samimi[2], Hamid Reza Mohammadimotlagh[2], Mona Sadeghalvad[3]

1. *Department of immunology, School of medicine, Kermanshah University of Medical sciences, Kermanshah, Iran*
2. *Student research committee, School of medicine, Kermanshah University of Medical sciences, Kermanshah, Iran*
3. *Student research committee, School of medicine, Tehran University of Medical sciences, Tehran, Iran*

**Background:** Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that primarily affects the joints. During RA, T cells and other immune cells recruit to the synovial tissue and promote RA. Autophagy is a process in which intracellular organelles and compounds are degraded and their products are reused by cells. Autophagy as a regulator of cell homeostasis can affect immune cells activation and contribute in RA pathogenesis. The aim of this study was to evaluate the autophagy-related genes (Atgs) expression in two groups of RA patients and healthy persons.
**Methods:** Peripheral Blood was obtained from three groups of donors including 20 patients with early RA, 20 under treatment RA patients (with Methotrexate, Hydroxychloroquine and Prednisolone therapy) and 20 age- and sex-matched healthy persons. The expression of three autophagy related genes including Lc3b (microtubule associated Light chain 3b), Beclin-1 and Atg5 was investigated by Real time PCR technique.
**Results:** The expression of Atgs was significantly increased in patients with early RA compared to healthy persons. The expression of these genes in under treatment patients was significantly lower than early RA patients.
**Conclusion:** This study showed that in early RA patients, the increased expression of Atgs can promote RA pathogenesis. In the other Words our findings suggest that the decreased autophagy can reduce RA severity.

**Keywords:** Autophagy, Rheumatoid Arthritis, Autoimmune Disease, Autophagy related genes

ICIA2018\Immunology of Rheumatic Diseases\Poster Discussion\8281
**8281**

# The Effect of Disease Modifying Anti-rheumatic Drugs (DMARDS) in Combination with Prednisolone on TNF-Alfa Plasma Levels and Disease Activity in Rheumatoid Arthritis Patients

Zahra Samimi[1], Bahareh Kardideh[2], Cyrus Jalili[2], Mahdi Taghadosi[2]

*1. Student research committee, School of medicine, Kermanshah University of Medical sciences, Kermanshah, Iran*
*2. Department of immunology, School of medicine, Kermanshah University of Medical sciences, Kermanshah, Iran*

**Background:** Rheumatoid Arthritis (RA) is an inflammatory autoimmune disease that leads to destruction of joints and adjacent tissues. Several inflammatory cytokines such as tumor necrosis factor-α (TNF-α) have critical roles in RA pathogenesis. Disease Modifying Anti-Rheumatic Drugs (DMARDs) are the mainstay of therapy in the treatment of RA. The aim of this study was to assess the plasma levels of TNF-α between three groups untreated, under treatment patients and healthy subjects and also evaluate the effect of DMARDs in combination with Prednisolone on the plasma levels of TNF-α and severity of RA disease.
**Methods:** Peripheral Blood was obtained from three groups including, 30 untreated, 30 under treatment patients and 30 age- and sex-matched healthy subjects. Under treatment patients were received combinational DMARDs therapy, including Methotrexate, Hydroxychloroquine plus Prednisolone. The plasma levels of TNF-α in three groups were measured by ELISA technique. The disease severity was assessed by disease activity score (DAS-28) and patients based on DAS-28 were categorized into four different groups, including remission, low, moderate and high disease activity.
**Results:** The plasma levels of TNF-α had no significant difference between three groups ($p>0.05$) but the DAS-28 was significantly higher in the untreated group in comparison with under treatment patients ($p<0.001$). DAS-28 was significantly correlated with TNF-α level in untreated patients ($p=0.001$, $r_s=0.594$) but we could not find a significant correlation between DAS-28 and TNF-α in under treatment group ($p=0.12$, $r_s=-0.29$).
**Conclusion:** It can be concluded that the plasma levels of TNF-α could be a good predictor of disease activity in newly diagnosed untreated RA patients, but quantification of its levels in plasma is not a good biomarker for diagnosis of RA. Also we found that DMARDs combination and prednisolone therapy do not have effect on the plasma levels of TNF-α.

**Keywords:** Rheumatoid Arthritis, DMARD, Disease activity score, TNF-α

ICIA2018\Immunology of Rheumatic Diseases\Poster Discussion\8306.doc
**8306**

# Synbiotic Supplementation and the Effects on Clinical and Metabolic Responses in Patients with Rheumatoid Arthritis: a Randomized, Double-blind, Placebo-controlled Trial

Batol Zamani[1], Shima Farshbaf[2], Hamid R. Golkar[1], Fereshteh Bahmani[2], Zatollah Asemi[2]

*1. Department of Gastroenterology, Kashan University of Medical Sciences, Kashan, Iran*
*2. Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran*

**Background:** Synbiotic intake may be associated with reduced inflammation in patients with rheumatoid arthritis (RA) due to optimized inflammatory markers, oxidative stress and insulin resistance. This research was conducted to assess the effects of synbiotic supplementation on the clinical and metabolic parameters of patients with RA.
**Methods :** A total of fifty-four patients with RA were allocated into two groups to receive either a synbiotic capsule (n 27) or a placebo (n 27) for 8 weeks in this randomised, double-blind, placebo-controlled trial. Fasting blood samples were taken at baseline and week 8 of the study to quantify related markers.
**Result:** After the 8-week intervention, compared with the placebo, synbiotic supplementation resulted in a significant reduction in serum high-sensitivity C-reactive protein (hs-CRP) levels (–1427·8 (SD 3267·2) v. +2833·4 (SD 5639·7) ng/ml, P=0·001).In addition, compared with the placebo, synbiotic supplementation improved disease activity score-28 joints (DAS-28) (–1·6 (SD 0·8) v. –0·3 (SD 0·5), P<0·001) and visual analogue scales (VAS) pain (–30·4 (SD 18·7) v. –11·5 (SD 15·9), P<0·001). In addition, a significant elevation in plasma nitric oxide (NO) (+0·8 (SD 4·4) v. –2·6 (SD 4·5) μmol/l, P=0·008), and significant reductions in insulin values (–13·8 (SD 26·4) v. +4·2 (SD 28·2)pmol/l, P=0·01), homoeostasis model of assessment-estimated insulin resistance (HOMA-IR) (–0·5 (SD 1·0) v.+0·1 (SD 1·1), P=0·03) and homoeostatic model assessment-β-cell function (HOMA-B) (–9·4 (SD 17·9) v. +3·3 (SD 18·9), P=0·01) following supplementation with the synbiotic compared with the placebo. Compared with the placebo, synbiotic supplementation also resulted in a significant increase in plasma GSH (+36·6 (SD 63·5) v. –58·5 (SD 154·4) μmol/l, P=0·005).
**Conclusion:** Overall, our study demonstrated that synbiotic supplementation for 8 weeks among patients with RA had beneficial effects on hs-CRP, DAS-28, VAS, NO, insulin levels, HOMA-IR, HOMA-B and GSH levels.

**Keywords:** Synbiotics, Supplementation, Rheumatoid arthritis, Metabolic profiles

ICIA2018\Immunology of Rheumatic Diseases\Poster Discussion\8507
**8507**

# The Effect of Ginger Powder on IL2, TNFα and IL1β Cytokines Gene Expression Levels in Patients with Active Rheumatoid Arthritis

Aryaeian Naheed[1]*, Mahmoudi Mahdi[2], Shahram Farhad[2], Poursani Shiva[2], Jamshidi Fatemeh[2], Tavakoli Hajar[3]

1. *Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Tehran, Iran.*
2. *Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran.*
3 *Departments of Cellular and Molecular Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran.*

**Background:** Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects the joints and consequently leads to the destruction of cartilage and bone lesions. Ginger is a native plant to India, which traditionally has been used in treatment of osteoarthritis, joint and muscle pain, neurological diseases, and inflammation of gums, tooth pain, asthma, stroke, diabetes, and constipation. This study aimed to determine the effect of ginger on some immunological and inflammatory factors in patients with rheumatoid arthritis.
**Methods:** In this study, 66 patients with active rheumatoid arthritis were enrolled. Patients were randomly divided into two groups that were treated using ginger and placebo (wheat flour) respectively. To determine the effect of confounding factors on the findings of the study, questionnaires for nutrient intake, physical activity, medication, and determination of body mass index were filled for each participant, at the beginning and end of the study. Serum CRP and mRNA levels of IL-1β, IL-2, and TNF-α were measured by ELISA and Quantitative Real-Time PCR respectively.
**Results:** Results of the study showed ginger supplementation caused a significant decrease in CRP ($P$= 0.05), and IL-1β ($P$= 0.02). TNFα mRNA level in ginger group compared to placebo group decreased, although the difference was not significant between the two groups ($P$= 0.09). Ginger had no effects on IL2 expression.
**Conclusions:** The study showed that ginger reduces inflammatory markers, CRP, and IL-1β in patients with active RA and it seems that ginger can improve the inflammation in the patients.

**Keywords:** Ginger, Active rheumatoid arthritis, Inflammatory markers.

ICIA2018\Immunology of Rheumatic Diseases\Poster Discussion\8617
**8617**

## *Molecular analysis of ERAP1 Allelic Variations in Patients with* Ankylosing Spondylitis

Farhad Babaie[1,2], Hamed Mohammadi[1, 2], Sorayya Salimi[1, 2], Alireza Rezaiemanesh[3], Behzad Baradaran[1, 2], Zohreh Babaloo[1, 2]

1. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
3. *Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*

**Background:** Ankylosing spondylitis (AS) is a group chronic inflammatory arthritis termed seronegative spondyloarthropathies. It typically affects the joints of the spinal and axial skeleton and exhibits typical clinical features and genetic factors such as HLA-B27 and ERAP1. Among the non-HLA loci, the strongest association was observed for the ERAP1 of single nucleotide polymorphisms (SNPs).
**Methods:** In our study, we have determined the frequencies of ERAP1 allelic variants and genotypes for three non-synonymous SNPs in AS patients and healthy individuals from the Iranian population. Both the AS patients and healthy group consist of HLA-B27 positive and HLA-B27 Negative individuals. We implemented the SSP-PCR system for genotyping of 160 AS patients and 160 healthy controls from the Iranian population.
**Results:** Considerable differences in allele's frequencies within patients vs control cohort were shown for 3 SNPs including rs30187, rs2287987, and rs10050860 under investigation. 3 SNPs were associated with the risk of AS [odds ratio (OR) 0.778, 95% CI 0.516–0.968, P =0.037 for rs30187, OR 1.57, 95% CI 1.07–2.33, P =0.025 for 10050860 and OR 1.60, 95% CI 1.10–2.32, P =0.015 for rs2287987].
**Conclusion:** The ERAP1 gene polymorphism might be a risk factor in the pathogenesis of AS. In contrast, ERAP1 gene polymorphisms may serve a protective role in AS.

**Keywords:** Ankylosing spondylitis, ERAP1, single nucleotide polymorphism

# Immunomodulation and Immunoregulation

# Poster Discussion

ICIA2018\Immunomodulation and Immunoregulation\Poster Discussion\8220
**8220**

**Mesenchymal Stem Cell-Conditioned Medium Ameliorate inflammation in Mouse Model of Acute Colitis**

Sedigheh Pouya[1], Maryam Heidari[1], Kaveh Baghaei[2], Hamid Asadzadeh Aghdaei[2], Afshin Moradi[3], Saeed Namaki[1], Mohammad Reza Zali[3], Seyed Mahmoud Hashemi[1]

*1. Department of Immunology, School of medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Basic and Molecular Epidemiology of Gastrointestinal Disorder Research Center, Research Institute for Gastroenterology and Liver Disease, Shahid Beheshti University of Medical Science, Tehran, Iran*
*3. Gastroenterology and Liver Disease Research Center, Research Institute for Gastroenterology and Liver Disease, Shahid Beheshti University of Medical Science, Tehran, Iran*

**Background**: Inflammatory bowel disease (IBD) is a multifactorial disease that the imbalanced immune responses have an important role in development and progression of the disease. On the other hand, studies on mesenchymal stem cells (MSCs) and its immunomodulatory properties have shown that these cells can be effective in immune/inflammatory response regulation through their secretions. Therefore, this study was designed to investigate the effects of the mesenchymal stem cell-conditioned medium (MSC-CM) injection in mouse model of acute colitis.
**Methods:** Acute colitis was induced in C57BL/6 mice with 2.5 % dextran sulfate sodium (DSS) dissolved in drinking water. The disease was induced in 1 cycle (4 days use of water containing DSS, followed by 6 days of water). The MSC-CM injection was performed 3 times (500 µl/mouse). During the study, changes in body weight, bleeding, stool consistency, disease activity index (DAI), and mortality rate were recorded. After euthanizing the mice, weight, and length of the colon, the percentage of Treg cells, the levels of TGF-β, IL-17, and IL-10 were measured. The pathology examination of the colon was also performed.
**Results**: According to the results, MSC-CM injection inhibited body weight loss, bleeding, DAI, and mortality rates. The stool consistency improved. The percentage of Treg cells and the TGF-β production was increased while the level of IL-17 decreased. In pathological observations, it was found that after injection of MSC-CM, infiltration of inflammatory cells and epithelial destruction decreased.
**Conclusion**: The results showed that MSC-CM has the ability to modulate the immune response and decrease inflammation. Then it can be used as a cell-free therapy in the treatment of inflammatory bowel disease.

**Keywords** Inflammatory bowel disease, Mesenchymal stem cells, Conditioned Medium, Dextran Sulfate Sodium

ICIA2018\Immunomodulation and Immunoregulation\Poster Discussion\8278
**8278**

# MicroRNA-150 Targets PU.1 and Regulates Macrophage Differentiation and Function in Experimental Autoimmune Encephalomyelitis

Leila Shakerian[1, 2], Samira Ghorbani[1], Farideh Talebi[1], Farshid Noorbakhsh [1*]

1. *Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
2. *Shefa Neuroscience Research Center, Khatam Al-Anbia Hospital, Tehran, Iran*

**Background:** MicroRNAs are regulatory RNA species which are involved in various physiological and pathological processes, including central nervous system (CNS) diseases. Multiple sclerosis (MS) is an inflammatory demyelinating disease which is characterized by leukocyte infiltration and monocytoid cell activation, followed by demyelination. PU.1 is a key transcription factor which is involved in differentiation and activation of monocytoid cells. Herein, we investigated the expression of PU.1 transcription factor and its potential regulation by miRNAs in the CNS of mice affected with experimental autoimmune encephalitis (EAE) as well as in vitro cultured primary macrophages. Physical interactions between PU.1 and miRNAs as well as the effect of miRNAs on macrophage inflammatory response and polarization were also investigated.
**Methods:** Three miRNAs, i.e. miR-18a, miR-150 and miR-155, which could potentially target PU.1, were selected based on bioinformatic criteria. Gene and microRNAs expression were first analyzed in the CNS of EAE mice at different time points after disease induction as well as in primary macrophages using real-time RT-PCR. MiRNA species which showed a negative correlation with the target gene both in vivo and in vitro were selected for further analyses. as Transfection experiments in macrophages as well as luciferase assays using vectors expressing luciferase coding sequence with the 3'UTR of PU1 were performed to investigate the physical interaction of miRNA sequences with PU.1 transcripts. Following the confirmation of interaction, the effects of miRNA on macrophage response and polarization were evaluated.
**Results:** Our findings showed a significant increase in PU.1, miR-155 and miR-18a at both acute and chronic phases of EAE disease. While showing an increase at the acute phase, miRNA-150 expression was significantly reduced at the chronic phase of disease. Expression analyses on activated macrophages revealed a substantial induction of PU.1 and miR-155 following LPS stimulation. On the contrary, miR-150 showed a remarkable decrease following treatment with LPS. Luciferase assays as well as transfection experiments confirmed that PU.1 transcripts were targeted by miR-150. Overexpression of miRNA-150 modulated macrophage inflammatory response and shifted polarization toward M2 phenotype.
**Conclusion:** These findings point to the potential role of miRNA-150 in regulating inflammatory responses in CNS through targeting PU.1. Our results raise the possibility that miR-150-PU1 interaction might be a likely target for therapeutic interventions in autoimmune neuroinflammation.

**Keywords**: microRNA, miR-150, PU1, macrophage, Experimental autoimmune encephalomyelitis

ICIA2018\Immunomodulation and Immunoregulation\Poster Discussion\8279
**8279**

# miR-101a Modulates Lymphocyte Activation in Experimental Autoimmune Encephalomyelitis through targeting c-fos

Samira Ghorbani[1], Neda Heidari[1], Farideh Talebi[1], Farshid Noorbakhsh [1*]

*1. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system. MicroRNAs are small, noncoding RNA molecules which function via base-pairing with complementary sequences within mRNA molecules, usually resulting in gene silencing. Herein, we analyzed the expression of Fos family T cell transcription factors and the miRNAs which are known to target them in the spinal cord of mice affected by EAE, an animal model of MS.
**Methods:** The expression of Fos family members(c-fos, Fosb, Fosl1, Fosl2) and their potentially targrting miRNAs were measured in the spinal cords of EAE as well as activates lymphocytes by real time RT-PCR. To examine the direct interaction between miRNA and target gene, luciferase reporter assay system was used.
**Results**: Our data showed a significant increase of c-fos and Fosl2 levels in EAE mice at the acute and chronic phases of disease. Although miRNAs-targeting c-fos (miR-7b, miR-101a, miR-101b) were down-regulated in chronic phase of EAE, only down regulation of miR-101a was statistically significant .Our data also revealed a rapid upregulation of c-fos in lymphocytes at 5 minutes post activation, whereas increased levels of other members of Fos family were not observed. Activated T cells showed early reduced levels of miR-7b, miR-101a and miR-101b 5 minutes after stimulation. Cells transfected with a c-fos-3'UTR containing plasmid showed a significant reduction in the luciferase activity following transfection with miR-101a, indicating the interaction between miRNA and the 3'UTR region.
**Conclusions:** Our findings indicate that miR-101a might be involved in regulating c-fos expression level during EAE and lymphocyte activation, hence, leading to development of new therapeutic approaches for T cell mediated autoimmune disorders.

**Keywords:** MicroRNA, EAE, c-fos

ICIA2018\Immunomodulation and Immunoregulation\Poster Discussion\8310.doc
**8310**

## The Role of P21 in Different Molecules Ubiquitination n TCR Signaling Pathway

Rahman Shokri[1, 2], Dimitrios Balomenos[1]

1. *Department of Immunology and Oncology, University of Autonoma de Madrid*
2. *Current address: Pasteur Institute of Iran-Karaj*

**Background:** p21 can act as a cell cycle inhibitor, cellular senescence mediator or control DNA damage repair. Our latest data with $p21^{-/-}$ T cells revealed a novel p21 function as a negative regulator of the activation machinery in repeatedly stimulated T cells. Protein ubiquitination is an important post-translational process that can affect protein targets in different ways. In this study, we checked the role of p21 on different protein ubiquitination like p50 (data not shown) and TRAF6 in T cell receptor (TCR) signaling pathway.
**Methods:** T cell cultures were incubated with proteasome inhibitor MG132 before harvest, washed in PBS/N-ethylmaleimide (N-EM), lysed in SDS buffer by boiling (5 min), and diluted (1/10) in RIPA buffer (without SDS) supplemented with 20 µM N-EM and protease/phosphatase inhibitor cocktail. Equal amounts of protein were incubated with appropriate antibody. After SDS-PAGE, proteins were transferred to a nitrocellulose membrane and immunoblotted with an anti-Ub antibody (P4D1; Santa Cruz Biotechnology) to detect ubiquitination.
**Results:** TRAF6 ubiquitination was reduced in p21-deficient compared with wild type $CD4^+$ T cells at 30 and 60 minutes after second stimulation, which shows p21 induces TRAF6
ubiqiutination.
**Conclusion:** TRAF6 is recruited to the T cell immunological synapse and interacted with LAT to activate T cells. Also MALT1 promotes TRAF6 auto-ubiquitination, which then recruits the TAK1/TAB2 adaptor protein complex to activate IKK. Therefore, TRAF6 is an important molecule in TCR signaling pathway and it's ubiquitination by p21 leads to T cell activation and regulation. Overall, p21 is an important factor that negatively regulates $CD4^+$ T cells activation after second stimulation by different process like ubiquitination.

**Keywords:** TCR, T $CD4^+$, Activation, Ubiquitination, TRAF6, p21

ICIA2018\Immunomodulation and Immunoregulation\Poster Discussion\8459
**8459**

# Evaluation of the Immunoregulatory and Proliferative Capacities of Adipose Derived Mesenchymal Stem Cells (Ad-MSCs) following pre-treatment with Human Menopausal Gonadotropin (hMG)

Shadi Kesmati[1], Moein Farshchian[2], Sepideh Sadat Hosseini[1], Halimeh Hassanzadeh[2], Majid Momeni Moghaddam[1], Fatemeh Sadeghifar[1*], Maryam Moghaddam Matin[2,3], Hamid Reza Bidkhori[2*]

*1. Department of Biology, Hakim Sabzevari University, Sabzevar, Iran*
*2. Stem Cells and Regenerative Medicine Research Department, Academic Center for Education, Culture and Research (ACECR)-Khorasan Razavi, Mashhad, Iran*
*3. Department of Biology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran*

**Background:** The hypothesis behind the clinical application of Mesenchymal Stem/Stromal Cells (MSCs) is supported on two characteristics of them: Their proliferative capability based on their multipotential state and MSCs' trophic activity via their anti-inflammatory and immunomodulatory effect. Prolonged culture of MSCs *in vitro* reduces their proliferative ability and changes them to into mature phenotypes. Pre-treatment of the cells with some chemical agents during *in vitro* expansion before transplantation is one strategy to overcome this limitation by enhancing both aforementioned capacities. Human menopausal gonadotropin (hMG) is a natural medication used to induce ovulation in women with infertility problems. hMG consists of both LH and FSH, hormones needed to help trigger ovulation. The aim of this study is to determine whether pre-treatment of Ad-MSCs with hMG, will enhance the expression of some genes involved in proliferation and immunomodulation (c-Myc, Oas2 and TGF-β).
**Methods:** MSCs were harvested from human processed lipoaspirate. Adipose-derived MSCs were treated at passage 2 for targeted gene expression profiling. MSCs were pre-treated with 2, 4 and 8 IU hMG for 24h, 48h and 72h. QPCR experiments were performed by SYBR Green method in CFX-96 Bio-Rad Real-Time PCR. c-Myc, Oas2 and TGF-β were compared at mRNA level in both groups.
**Results:** Results show hMG significantly up-regulated the c-Myc and Oas2 gene expression compare with the control group while it decreased TGF-β expression significantly.
**Conclusion:** Pre-treatment of Ad-MSCs with hMG may allow the in vitro cultured cells to regain their proliferation and immunoregulatory capacities.

**Keywords:** Adipose-derived mesenchymal stem cells, pretreatment, hMG, c-Myc, Oas2, TGF-β, Immunomodulation.

ICIA2018\Immunomodulation and Immunoregulation\Poster Discussion\8717

**8717**

# The Role of Regulatory T cells in the Failure of Kidney Function in Patients with Autoimmune and Non-autoimmune Disorders

Farshid Fathi[1], Hossien Moteddayyen[2], Nilofar Keyhanmehr[1], Nahid Ekandari[1], Noushin Lotfi[1], Shohreh Mohammadi[3], Hossien Hafezi[4], Mehdi Raie[5]

1. *Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
2. *Department of Laboratory Medicine, Kashan University of Medical Sciences, Kashan, Iran*
3. *Khorshid Hospital, Isfahan University of Medical Sciences, Isfahan, Iran*
4. *Department of Dermatology, Isfahan University of Medical Sciences, Isfahan, Iran*
5. *Health Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*

**Background:** Autoimmune response is one of the causes of the failure in the renal function in patients with end-stage renal disease. Regulatory T cells (Tregs) are a subset of CD4+T cells that play an indispensable role in regulating immune responses and preventing the development of autoimmune diseases. Therefore, the aim of this study was to investigate the percentage of Tregs in kidney patients who need to renal transplantation due to autoimmune responses and other causes.

**Methods:** Heparinized whole blood (5 ml) was obtained from patients with end-stage renal diseases and healthy subjects. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll density centrifugation. PBMCs were stained with FITC anti-human CD4, PE anti-human CD25, and PE-cyn5 anti-human Foxp3 antibodies. The percentage of the stained cells was measured by a FACS Calibur flow cytometer and analyzed using the CellQeust software.

**Results:** Our results indicated that the percentage of Tregs in kidney patients with autoimmune disorders was significantly decreased compared to healthy individuals and patients with renal failures owing to non-autoimmune disorders($p<0.01$-$0.05$). However, there was no difference in Tregs percentage between kidney patients without autoimmune disorders and healthy individuals.

**Conclusion:** These findings suggest that the reduction of Tregs number in kidney patients can contribute to the risk of developing autoimmune responses that lead to the loss of kidney function.

**Key words:** Autoimmune disorders, Regulatory T cells, Kidney patients.

# Immunoparasitology

# Poster Discussion

ICIA2018\Immunoparasitology\Poster Discussion\7935
**7935**

# Lack of IL-17a May Be Involved in Healing of the Lesions in Patients Afflicted by Cutaneus Leishmaniasis

Mohammad Moafi[1], Hossien Rezvan[2], Roya Sherkat[3], Roya Taleban[4]

1. *Acquired Immunodeficiency Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*
2. *Department of Pathobiology, Faculty of Veterinary Science, Bu-Ali Sina University, Hamedan, Iran*
3. *Acquired Immunodeficiency Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*
4. *Acquired Immunodeficiency Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background:** Cutaneous Leishmaniasis (CL) is assumed as neglected tropical disease, which spontaneously heals less than one year. However, the lesions of CL rarely persist more than one year. The persistency of the lesions could be attributed to different factors, including pro-inflammatory cytokines. In this context, the role of IL-4 and IFN-gamma in duration of lesion healing has been evaluated in some of the previous studies; however the role of IL-17a in duration of healing lesion is not completely understood. The current study aims to assess the role of IL-17a in non-healing form of CL (NHCL).
**Methods:** This was a cross-sectional study implemented from 2015-1015 in immunodeficiency research center, Isfahan University of Medical Sciences (IUMS). This study included 10 cases afflicted by NHCL and 33 cases suffering from healing form of CL. The peripheral blood mononuclear cells (PBMCs) of the patients were isolated by Ficoll and were harvested in three different medium. The stimulators were as follow: purified protein derivative (PPD), Phytohemaglotinin (PHA), and Soluble *Leishmania* Antigen (SLA). The supernatant was isolated and the levels of IL-17a were determined using ELISA.
**Results:** The levels of IL-17a produced in PPD stimulated wells was significantly lower, compared with PHA and SLA stimulated wells ($P<0.05$). Furthermore, the level of IL-17a produced in PHA stimulated wells was significantly higher, compared with PHA stimulated wells ($P<0.05$). The level of IL-17a produced in NHCL cases was significantly lower than healing cases ($P<0.05$).
**Conclusion:** Our findings support the notion that IL-17a plays a crucial role in the duration of lesion healing in those afflicted by CL.

**Keywords:** Cutaneous Leishmaniasis, IL-17a

ICIA2018\Immunoparasitology\Poster Discussion\8011x
**8011**

## In vitro Efficacy of Rapamycin on Old World *Leishmania* Strains

Fatemeh khadir [1, 2] Ahmad Oryan [2]*, Tahereh Taheri[1], Maryam Heidari-kharaji[1]*, Sima Rafati[1]*

1. *Immunotherpay and Leishmania Vaccine Research Dept., Pasteur Institute of Iran, Tehran, Iran*
2. *Department of Pathology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran*

**Background:** Leishmaniasis is a serious health problem in different countries. There is no human vaccine and the disease is generally controlled by chemotherapy, but most of the available drugs are highly toxic or result in drug resistance. Here we investigated Rapamycin as an anti-leishmanial drug due to its mTOR (mammalian target of Rapamycin) blockage activity that affects the parasite propagation. Introducing Food and Drug Administration (FDA)-approved Rapamycin for anti-leishmanial activity may open up a new approach in the treatment of this important disease.
**Methods:** In the present study, in vitro efficacy of Rapamycin was investigated against two species of *Leishmania* including *L. major* and *L. tropica*. In vitro potency was evaluated using MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide) method on promastigote and amastigote form of *Leishmania* parasites, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were done for observing the effect of the drug on the shape and function of the host cells and parasites.
**Results:** Rapamycin is significantly effective to inhibit the growth and survival of both *L. major* and *L. tropica*, throughout different analyses using scanning and transmission electron microscopy. There are some changes in the appearance and size of the promastigote form of both species. In addition, our result in in vitro showed the interaction of host cell and amastigote form of both species were changed dramatically in the presence of Rapamycin.
**Conclusion:** Based on in vitro analysis, Rapamycin seems to be a potent anti-leishmanial agent. Rapamycin as FDA approved drug may open a new window in order to control cutaneous leishmaniasis as sole or combinational chemotherapy approach against *L. tropica* and *L. major* infection.

**Keywords:** *Leishmania major*, *Leishmania tropica*, Rapamycin, TEM, SEM.

ICIA2018\Immunoparasitology\Poster Discussion\8172x
**8172**

## A Simple Method for Evaluation of *Leishmania* Parasite Burden among Experimental Models

Mahdieh Azizi[1], Sepideh Haghdoost[1], Mostafa Haji Molla Hoseini[1], Farshid Yeganeh[1]

*1. Department of Medical Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** The model of *Leishmania* infection in mice has been widely used in various studies. A limitation dilution assay (LDA) is commonly used method for evaluating the efficacy of therapeutic methods and/or immunization effectiveness. This method suffers from the risk of contamination, loss of samples, and time consuming. Furthermore, utilizing from it requires having familiarity and access to special results analysis software. The purpose of present study was to introduce a simple and alternative method for measuring the burden of *Leishmania* among the experimental model of this infection.
**Methods:** At the first *Leishmania Major* that express Green Fluorescent Protein was produced (GFP- *Leishmania*).Then the Stationary phase of promastigotes (The standard L. major strain of MRHO/IR/75/ER) were used for infection of ten female BALB/c mice. Finally, mice with different wounds diameter were selected to compare the parasite burden using LDA method (described elsewhere) and our fluorescence microscopic technique. Briefly, in our technique, we count florescent cell in the same area of the microscopic field following by adjusting cell count.
**Results**: At least 3.14 (log of parasite/Lymph node) and the maximum of 7.85 (log of parasite/Lymph node) estimated by LDA method. In fluorescence microscope method, we calculate 0.03-33.3 infected cells per lymph node. Correlation analysis was performed between two methods and the correlation coefficient of 0.96 was obtained between the two methods ($p<0.0001$).
**Conclusion**: Our finding showed GFP- *Leishmania* is an appropriate tool for parasite burden estimation using our fluorescent microscopic technique. In addition, expressing GFP makes it possible to continuously evaluate infected mice by in-vivo imaging method during the study.

**Keywords**: GFP-Leishmania, Parasite burden, Fluorescent microscopy

ICIA2018\Immunoparasitology\Poster Discussion\8249x
**8249**

***Leishmania tropica*: Infectious Dose Affects Pathogenicity and Immunogenicity in a Mouse Model**

Mosayeb Rostamian[1], Maryam Abolghazi[2], Davood Jafari[3], Hadiseh Farahani[2], Fatemeh Doroudian[1], Hamid M. Niknam[1*]

1. *Immunology Department, Pasteur Institute of Iran, Tehran 13164, Iran*
2. *Department of Microbiology, Qom Branch of Islamic Azad University, Qom, Iran*
3. *Islamic Azad University of Borujerd, Science and Research* Unit, *Borujerd, Lorestan, Iran*

**Background:** *Leishmania (L.) tropica* is a causative agent of cutaneous leishmaniasis in humans. An experimental model is needed for this species. The effect of parasite dose on *L. tropica* infection was studied in the current paper. High and low doses of *L. tropica* were used for ear infection of naïve BALB/c mice and lesion development, parasite load, and cytokine response were assessed. *L. major* infection was used for comparison.
**Methods:** Mice were challenged in footpad by a fixed high dose of *L. tropica*. Infection with high dose *L. tropica* in contrast to low dose resulted in dissemination of parasite to spleen.
**Results:** Primary infection with high dose *L. tropica* in comparison to low dose resulted in lower lesion diameter and lower parasite load of lymph nodes after secondary *L. tropica* challenge. *L. tropica* in comparison to *L. major* results in lower lesion diameter, more growth in lymph nodes at early phases of infection, dissemination to spleen, and lower levels of interleukin-10.
**Conclusion:** Our findings show only the high dose of *L. tropica* results in visceralization of parasite and protection at the site of infection after secondary challenge of *L. tropica*. Therefore dose of infection is an important factor in experimental model of *L. tropica*.

**Keywords:** Leishmaniasis, Infectious dose, *Leishmania tropica*, *Leishmania major,* Spleen, Cytokine, BALB/c mice.

ICIA2018\Immunoparasitology\Poster Discussion\8256x
**8256**

# Haematological and Serum Biochemical Parameters in Horses with Natural Babesiosis in Urmia, Northwest of Iran

Neda Ahmadi afshar[1], Farnaz Malekifard[1,] Mousa Tavassoli[1]

1. D*epartment of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, West Azarbaijan*

**Background:** Equine piroplasmosis (EP) is a tick-borne disease caused by hemoparasites *Theileria equi* and *Babesia caballi*. This disease affects all members of the Equus genus (horses, donkeys and zebras. The aim of this study was to determine the effect of babesiosis on some hematological and biochemical parameters in horses infected with *T.equi and B.caballi* in Urmia suburb, West Azerbaijan province, Iran.
**Methods:** A total of 240 blood samples collected randomly from horses of 25 villages. The specimens were transferred to the laboratory and the blood smears stained with Geimsa. The parasitological diagnosis was confirmed using multiplex PCR analysis. As a control group, 20 clinically healthy horses reared under the same management and environmental conditions were also sampled.
**Results:** The results of the PCR assays showed 26(10.83%), 14(5.83%) and 4 (1.66%) were distinguished as *T.equi, B.caballi* and mixed infection, respectively. Compared to controls, BUN, creatinine, cholesterol, triglyceride, HDL and LDL level showed a significant increase ($P<0.05$).Significant elevation ($P < 0.05$) of total leukocyte count, number of lymphocyte, neutrophil, monocyte and eosinophil were found in infected horses. While, hemoglobin concentration (Hb), packed cell volume (PCV), red blood cells (RBCs), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) significantly decreased ($P < 0.05$) in infected horses.
**Conclusion:** Hematological and biochemical parameters changes suggesting that these indicators would indirectly promote the presence of these parasites in the horses.

**Keywords:** *Theileria equi, Babesia caballi*, hematological and biochemical parameters, Horse, Iran

ICIA2018\Immunoparasitology\Poster Discussion\8257x

**Serologic and Microscopic Studies on Piroplasmosis in Donkeys (*Equus asinus*) in the Northwest of Iran**

Neda Ahmadi afshar[1], Farnaz Malekifard[1,] Mousa Tavassoli[1]

*1. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, West Azarbaijan*

**Background:** Equine piroplasmosis is a tick-borne protozoal disease of horses, mules, donkeys and zebra; it causes economic losses to the equine industry. The aim of this study was to determine the seroprevalence of *T. equi* and *B. caballi* infection in donkeys of West Azerbaijan province in North West of Iran.
**Methods:** From April to September 2014, a total of 120 blood samples collected randomly from apparently healthy donkeys and examined by microscopy and indirect immunofluorescent antibody test.
**Results:** Microscopic observation on 120 blood smears determined 3(2.5%) and 1 (0.83%) samples were infected by *T.equi* and *B.caballi,* respectively. Antibodies against *T. equi* and *B. caballi* were found in 13(10.83%), and 5 (4.16%) serum samples, respectively.
**Conclusion:** We couldn't detect mix infection with both parasites. No significant difference was observed between the seroprevalence of piroplasm infection with risk factors such as age and gender in donkeys. This is the first report of detection of *T. equi* and *B. caballi* infection using IFAT in Iran. Our findings indicated that *T. equi* and *B. caballi* were prevalent among donkey population in Iran.

**Keywords:** *Babesia caballi,* donkey, Iran, Indirect Immunofluorescent antibody test*, Theileria equi*

ICIA2018\Immunoparasitology\Poster Discussion\8538x
**8538**

**Evaluation of NOHA, an Arginase Inhibitor, on *Leishmania tropica* on BALB/c Mice Using Reporter Genes**

Shima Nahidi[1], Elham Gholami[1], Yasaman Taslimi[1], Negar Seyed[1], Sima Habibzadeh[1], Elahe Davarpanah[1, 2], Behrooz Vaziri[3], Sima Rafati[1], Tahereh Taheri*[1]

1. *Department of Immunotherapy and Leishmania Vaccine Research, Pasteur Institute of Iran, Tehran, Iran*
2. *Department of Genetics Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran*
3. *Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran*

**Background:** Leishmaniasis is one of the five most important parasitic diseases worldwide. Among the *Leishmania* (*L.*) species, causative agent of this disease, *L. tropica* is responsible for both cutaneous and visceral leishmaniasis transmitted from human to human by sand fly. Arginase (ARG) is essential for cell growth and parasitic pathogens is where converts L-arginine into ornithine and urea. We examined the inhibitory impact of ARG using N-hydroxy-nor-arginine (nor-NOHA) as a powerful ARG inhibitor on *L. tropica* infectivity in mice model.
**Methods:** Different groups of BALB/c mice were infected subcutaneously (in footpad) or intradermally (in ear) with *L. tropica*$^{EGFP\text{-}LUC}$. Two infected groups received nor-NOHA intraperitoneally two times a week within 6 months. Two infected groups remained untreated. One group was treated/uninfected as a control. All groups were compared in respect to parasite load, *in vivo* bioluminescence imaging, footpad swelling and ARG/nitric acid activity.
**Results:** Infection rate in all groups was monitored during 6 months. In infected ear, milder swelling and inflammation was observed which subsided gradually but, in infected footpad swelling was increased up to the end of experiment. In both injected sites, parasite load correlated with swelling size or redness. Also, *in vivo* bioluminescence monitoring of infected footpad mice was clearly observable increasing of parasite burden in infected footpad with *L. tropica*$^{EGFP\text{-}LUC}$. The comparison between different groups (untreated and treated) did not show any significant difference in footpad size. Also, there was observable not significant decreasing of parasite burden and ARG activity in treated and untreated groups).
**Conclusion:** Our results first demonstrated that *L. tropica*$^{EGFP\text{-}LUC}$ could be used to follow parasitemia progression in infected footpads of mice. Second arginase inhibition partially controls *L. tropica* infection reflected in parasite burden and ARG activity decrement. Furthermore *in vivo* imaging is practically more applicable in the study of metabolic pathways in *L. tropica*.

**Keywords**: *Leishmania tropica*, Arginase, nor-NOHA, nitric oxide, *in vivo* imaging.

ICIA2018\Immunoparasitology\Poster Discussion\9748x

**Achievement Amastigotes of *Leishmania infantum* and Investigation of Pathological Changes in the Tissues of Infected Golden Hamsters**

*Sajad Rashidi[1], Kurosh Kalantar[2], Gholamreza Hatam[3]*

1. *Department of Parasitology and Mycology, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran*
3. *Basic Sciences in Infectious Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** *Leishmania (L) infantum* is identified as a causative agent of visceral leishmaniasis (VL). Amastigote form is used for investigations on vaccines, treatment, and diagnosis. This study aims to achieve the *L. infantum* amastigotes in the tissue of infected golden hamsters and J774 macrophages and investigate the pathological changes which occur in the liver and spleen of the hamsters with VL.
**Methods:** Four male golden hamsters were infected with promastigote (intra-peritoneal ($10^8$) and intra-cordial ($2\times10^7$)) of *L. infantum*. After 5 months, the hamsters were euthanized and touch and pathology smears were prepared from the livers and spleens. The tissues were homogenized and centrifuged at 100× g. Supernatants were collected and centrifuged at 2000× g and the pellet was collected. Also, J774 macrophages were infected with *L. infantum* promastigotes (1macrophage/10 promastigotes) and the infected macrophages were ruptured. Then, centrifuge stages were done the same as the previous part.
**Results:** The amastigotes were observed in the touch and pathology smears of both livers and spleens. The amastigote load in the livers was more than the spleens in the touch and pathology smears. Although the structure of the livers underwent the pathological changes, the spleens were unchanged. The macrophage infectivity ratio was 95%.
**Conclusion:** This study presents a simple and accessible way to achieve lots of pure and real *L. infantum* amastigotes. In addition, it seems that the pathological changes in the liver of the hamsters with VL are different from the pathological changes in their spleen which might be due to the genetic and immune process. Also, experimental surveys in vivo cannot be an appropriate way for the investigation into VL pathological changes because the results in experimental and natural infected animals vary. It is recommended that we should rely on the results of human experiments in this regard.

**Keywords:** *L. infantum*, Amastigote, J774 macrophage, Golden hamster, Pathological change

ICIA2018\Immunoparasitology\Poster Discussion\9962x

## *Toxocara canis* Infection in Children with Hypereosinophilia (2–15 Years Old) Referred to Health Centers of Lorestan Province, Iran

Pardis Bayat[1], Maryam Moslehi Baharanchi[1], Nadereh Taee[2], Hossein Mahmoudvand[3,*]

1. *Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran.*
2. *Department of Pediatric, Lorestan University of Medical Sciences, Khorramabad, Iran*
3. *Department of Medical Laboratory Sciences, School of Allied Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran*

**Background**: The present study aims to assess the seroprevalence of *Toxocara canis* infection in children with hypereosinophilia (2–15 years old) referred to health centers of Lorestan province, Iran.
**Methods**: This cross-sectional study was performed from August 2016 to March 2017 on 73 children with hypereosinophilia (eosinophilia>10%) (2–15 years old) referred to health centers of Lorestan province, Iran. All serum samples were tested using the commercially available anti IgG-Toxocara kit (IBL, Germany) according to manufacturer's instructions. The variables used to evaluate association between risk factors and status of anti-*T. canis* IgG antibodies were age, gender, area of residence, contact with dogs, and consumption of raw or unwashed vegetables and fruits.
**Results**: Of the 73 children with hypereosiniphilia, 3 (4.1%) tested seropositive for anti-*T. canis* IgG antibody. Risk factors that were significantly related to *T. canis* seropositivity included living in rural regions ($p<0.05$) and contact with dogs ($p<0.05$). However, other demographic and risk factors did not demonstrate any association with *T. canis* seropositivity.
**Conclusion**: We found that *T. canis* infection is prevalent among children with hypereosinophilia referred to health centers of Lorestan province, Iran with an overall seroprevalence rate of 4.1%. These findings suggested that *T. canis* infection can be considered as one of the reasons for hypereosinophilia in children from Lorestan Province Iran.

**Keywords**: Toxocariasis; ELISA; eosinophilic; children

ICIA2018\Immunoparasitology\Poster Discussion\9967x

**Relationship between Helminthic Infections and Hyper eosinophilia in Children Referred to Health Centers of Lorestan Province, Iran**

Maryam Moslehi Baharanchi [1], Pardis Bayat[1], Nadereh Taee[2], Hossein Mahmoudvand[3,*]

1. *Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran.*
2. *Department of Pediatric, Lorestan University of Medical Sciences, Khorramabad, Iran*
3. *Department of Medical Laboratory Sciences, School of Allied Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran*

**Background**: Intestinal helminthic infections are well-known as one of the most important socioeconomic and health problems around the world. The present study aims to evaluate the prevalence of the helminthic infections in children with hyper eosinophilia referred to Health Centers of Lorestan Province, Iran.
**Methods**: This cross-sectional study was performed from August 2016 to March 2017 on 73 children with hyper eosinophilia (eosinophilia>10%) referred to Health Centers of Lorestan Province, Iran. The microscopic analysis was accomplished on 73 stools by means of the direct smear, and formol-ether methods.
**Results**: Out of the 73 children with hypereosiniphilia, 5 (6.8%) children were infected with at least one or more intestinal parasites including *Enterobius vermicularis, Hymenolepis nana, Trichostrongylus* spp, and *Ascaris lumbricoides*. Statistical analysis showed that some risk factors were significantly associated to the prevalence intestinal helminthic parasites included gender ($p<0.02$), living in rural regions ($p<0.001$), hands washing habit ($p<0.001$) and consumed raw or unwashed vegetables and fruits ($p<0.001$).
**Conclusion**: These findings suggested that intestinal helminthic parasites can be considered as one of the reasons for hyper eosinophilia in children from Lorestan Province Iran.

**Keywords**: Intestinal parasites, stool, eosinophilia, children

# Immunopharmacology and Medicinal Plants

# Poster Discussion

ICIA2018\Immunopharmacology and Medicinal Plants\Poster Discussion\7387
**7387**

# The Effect of Mesenchymal Stem Cells Treated with Caffeine in Alleviating the Rheumatoid Arthritis Induced in Wistar rat

Elham Moases Ghafari[1], Seyyed Meysam Abtahi Froushani[1]

*1. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Previous documents indicated that Caffeine treated mesenchymal stem cells (MSCs) augment the instruction of anti-inflammatory macrophages. This study was done to investigate the effect of Caffeine treatment on the potential of MSC in alleviating the animal model of Rheumatoid Arthritis (RA).
**Methods:** Bone marrow-derived MSCs were co-cultured with 0.5 mM of Caffeine for 24 h. RA was induced by injection of complete Freund's adjuvant into the footpad of Wistar rats. One week after immunization, rats were divided into 3 groups: treated with MSCs alone, treated with MSCs co-cultured with Caffeine and untreated group. The change in the diameter of wrists and ankles of each rat was recorded every 5 days until 33 days after induction. At the end, nitric oxide production by Griess method, the ability of yeast opsonized phagocytosis and intensity of respiratory burst (NBT Test) in the splenocytes population were analyzed.
**Results:** The edema and swelling of ankles of RA rats treated with Caffeine pulsed MSCs were significantly regressed compared to RA rats received MSCs alone. Nitric oxide, respiratory burst, and phagocytosis rate of neutral red in RA rats received MSCs exhibited significant decrease compared to RA rats without treatment. Albeit, these reductions were more prominent in RA rats treated with MSCs pulsed with Caffeine compared to RA rats received MSCs alone.
**Conclusion:** Caffeine pulsed MSCs caused more favorable outcomes in RA model compared to un-treated MSCs.

**Keywords:** Caffeine, Mesenchymal stem cell, Rheumatoid arthritis, Immunity Responses

ICIA2018\Immunopharmacology and Medicinal Plants\Poster Discussion\7414
**7414**

# Role of 13-Cis Retinoic Acid in Mycobacterium tuberculosis Infection Control by Human U937 Macrophage

Bahareh Abd Nikfarjam[1, 2], Marjan Nassiri Asl[1, 3], Mehri Hajiaghayi[1, 2],
Taghi Naserpour Farivar[1, 4]

*1. Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran*
*2. Department of Immunology, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran*
*3. Department of Pharmacology, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran*
*4. Department of Microbiology, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran*

**Background:** Mycobacterium tuberculosis survives and proliferates within the main cells of the innate immune system, macrophages. The goal of our study was to investigate the immunostimulatory effects of 13-Cis retinoic acid in PMA-induced human U937 macrophages against Mtb R37Ra infection by evaluating its potential role in the cell surface expression of HLA-DR, CD14 molecules and NO production as well as prevention of the Mtb growth within macrophages.
**Methods:** In this study, we investigated the immunostimulatory effects of 13-Cis retinoic acid on Mycobacterium tuberculosis infected macrophages using Flowcytometry and Griess methods.
**Results:** 13-Cis retinoic acid enhanced the cell surface expression of HLA-DR and CD14 molecules on U937 macrophages and prevented the growth of Mtb within macrophages suggesting an activated phenotype for 13-Cis retinoic acid treated U937 cells. Also, 13-Cis RA, has significantly increased NO generation compared to untreated control macrophages ($P<0.001$).13-Cis retinoic acid has a significant inhibitory effect on Mycobacterium growth ($P<0.05$). Moreover, 13-Cis RA is potent in stimulation of surface markers expression on infected macrophages ($P<0.05$).
**Conclusion:** The results of our study showed that infected U937 macrophages treated with 13-Cis retinoic acid represented significant increases in NO production, CD14 and HLA-DR expression and also prevents intracellular survival of Mtb. Therefore, 13-Cis retinoic acid may have a significant therapeutic approach in the control of Mtb infection.

**Keywords:** Mycobacterium, Tuberculosis, 13-Cis retinoic acid, U937 Macrophages

ICIA2018\Immunopharmacology and Medicinal Plants\poster discussion\7417
**7417**

## *In vitro* Assessment of G2013 (guluronic acid) on microRNA-155 in TLR4 Signaling Pathway

Seyed Shahabeddin Mortazavi Jahromi[1, 2], Mehdi Malek Jamshidi[1, 2], Mehdi Yousefi[3], Farzaneh Tofighi Zavareh[4], Abbas Mirshafiey[4]

1. *Department of Cellular and Molecular Biology, Kish International Campus, University of Tehran, Kish, Iran*
2. *School of Biology, University College of Science, University of Tehran, Tehran, Iran*
3. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

4. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** microRNAs are considered one of the main epigenetic mechanisms in regulating gene expression, and it has been shown that impairment in their expression causes autoimmune diseases including rheumatoid arthritis and multiple sclerosis. The present research intended to investigate the effects of G2013 (guluronic acid) on the gene expression level of microRNA-155 in the HEK-Blue TLR4 cell line.
**Methods:** Total RNA was extracted, and cDNA was synthesized for microRNAs and gene expression level of microRNA-155 was evaluated using the Real-Time PCR technique.
**Results:** It was found that this medication could not significantly reduce the gene expression level of microRNA-155 at low and high doses in HEK-Blue hTLR4 compared to the control group ($p > 0.05$). It was also observed that when used lipopolysaccharide (LPS) to HEK-Blue hTLR4 cells led to a significant increase in gene expression level of microRNA-155 while the addition of LPS four hours after treating the cells with guluronic acid could not increase the gene expression level of microRNA-155 ($p < 0.05$).
**Conclusion:** This study revealed that guluronic acid, as a novel non-steroidal anti-inflammatory drug, is able to reduce gene expression level of microRNA-155 after stimulation by LPS.

**Keywords:** G2013, Guluronic acid, microRNA-155, TLR4

ICIA2018\Immunopharmacology and Medicinal Plants\Poster Discussion\7418
**7418**

# The Evaluation of the Effect of β-D-mannuronic Acid (M2000) on MicroRNA-146a and Related Molecules at the Level of Gene Expression in HEK Blue hTLR2 Cell Line

Seyed Shahabeddin Mortazavi Jahromi[1,2], Mehdi Malek Jamshidi[1,2], Ali Farazmand[1,2], Mehdi Yousefi[3], Zahra Aghazadeh[4], Abbas Mirshafiey[4]

1. *Department of Cellular and Molecular Biology, Kish International Campus, University of Tehran, Kish, Iran*
2. *School of Biology, University College of Science, University of Tehran, Tehran, Iran*
3. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
4. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** microRNA-146a is a post-transcriptional negative regulator of immune cell signaling through TLRs (TLR2, TLR4). Changes in the gene expression of microRNA-146a were reported in the pathogenesis of many autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus, and psoriasis. This research aimed to study the effects of β-D-mannuronic acid (M2000) on the expression of microRNA-146a and target molecules (IRAK1, TRAF6) in the HEK-BluehTLR2 cell line.
**Methods:** M2000 cytotoxicity was assessed by the MTT assay. The expression levels of the mentioned genes were detected by quantitative real-time PCR method.
**Results:** The MTT findings showed that M2000 (< 500 µg/ml) has no cytotoxic effect on HEK-Blue hTLR2 cells. According to our findings, low and high doses of M2000 could significantly reduce gene expression level of microRNA-146a compared to control group ($p < 0.05$). It was also revealed that two doses of this medicine could significantly decrease the gene expression level of IRAK1 and TRAF6 compare to control group ($p < 0.05$).
**Conclusion:** This research showed that M2000, as novel NSAID with immunosuppressive properties, was able to moderate TLR2 signaling and minimize the probability of inflammatory reactions by inhibiting microRNA-146a, IRAK1, and TRAF6.

**Keyword:** β-D-mannuronic acid, M2000, microRNA-146a, Target molecules (IRAK1 and TRAF6)

ICIA2018\Immunopharmacology and Medicinal Plants\Poster Discussion\7732
**7732**

**Thymol enhances the *in vivo* Generation of Foxp3+ regulatory T cells in Mice**

Haideh Namdari[1], Maryam Izad[2], Zahra Amirghofran[1, 3*]

*1.Department of Immunology, Medical School, Shiraz University of Medical Sciences, Shiraz*
*2.Department of Immunology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran*
*3. Autoimmune Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background**: Regulatory T cells (Tregs) play a critical role to maintain tolerance and prevent autoimmunity, thus selective *in vivo* increase of Tregs has wide therapeutic implications for autoimmune and inflammatory diseases. We investigated the effects of thymol, a main component of thyme on Tregs generation in mice.
**Methods:** Splenocytes from non-immunized and ovalbumin (Ova)-immunized mice treated and untreated with thymol were isolated for enumeration of Treg cells by flow cytometry. Isolated cells were cultured in the presence of Ova to measure *ex vivo* expression of Tregs and their suppression activity using CFSE labeling.
**Results:** We demonstrated that *in vivo* treatment of Ova-immunized mice with thymol increased the number of Tregs to more than 8% compared to the untreated mice ($P<0.01$). *Ex vivo* Ova challenge of splenocytes from Ova-immunized mice treated with thymol showed reduced proliferation of stimulated naïve T cells to <30% of control ($p<0.01$). The percentage of $CD4^+Foxp3^+$T cells in *ex vivo* culture of splenocytes from thymol-treated Ova-immunized mice increased to 11% compared to those from untreated Ova-immunized mice ($p<0.01$). Intracellular staining of cells for TGF-β demonstrated an increased level of this cytokine in $CD4^+Foxp3^+$ Treg cells ($48.1\pm5.9\%$, $p<0.05$).
**Conclusion:** These data showed the ability of thymol to improve Tregs generation and their suppressor activity by increasing TGF-β production and decreasing stimulated naïve T cells proliferation. These findings suggest thymol as a helpful natural immunosuppressive compound for treating autoimmune and inflammatory diseases.

**Keywords**: Thymol; immunomodulation; $CD4^+$ T cells; regulatory T cells; ovalbumin

ICIA2018\Immunopharmacology and Medicinal Plants\Poster Discussion\8283
**8283**

# The Cytotoxic Effect of Kombucha, a Fermented Microbial Product, on the 4T1, Mouse Breast Cancer Cell Line

Somayeh Ahmadiafshar[1], Mahsa Alem[1], Norouz Delirezh[1]

*1. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Kombucha has long been used in traditional medicine to treat a variety of ailments. It has also been seen that Kombucha ceases to grow cancerous cells. It can renew the chemical and ionic balance in the cell membrane so prevent many disorders. This study was done to the evaluation of the cytotoxic and apoptotic effects of the Kombucha on 4T1 as mouse breast cancer cell line.
**Methods:** For this purpose, after cell line culture, cells were divided into different groups such as negative control, treatment with different doses of Kombucha such as 50, 100, 150 and 200 µl/ml, for 24 h. The cell morphology of all groups was studied via invert microscope. In order to evaluation of cell vitality and viability tetrazolium dye (MTT) assay and natural red (NR) respectively were tested. Finally, propidium iodide (PI) and acridine orange (AO) staining was done to assess apoptosis and necrosis situation of the cancerous cells.
**Results:** The NR assay test showed that administration of Kombucha could statistically significant induces changes in the cell membrane viability. Moreover, MTT reduction assay test showed that Kombucha could decrease vitality and biological activity of Kombucha treated mouse breast cancer cell line compared to control group. PI and AO staining manifested that Kombucha induced apoptosis and necrosis in treated 4T1 cell line, but not in control cells.
**Conclusion:** The administration of Kombucha could improve the cytotoxic effects on breast cancer and could alter the phenotype of these cells.

**Keywords:** Kombucha, 4T1, Mouse breast cancer cell line.

ICIA2018\Immunopharmacology and Medicinal Plants\Poster Discussion\8621
**8621**

# Evaluation of the immunomodulatory effects of *Mentha longifolia* main compound on proliferation and cytokine levels in human lymphocytes and Jurkat cell line

Maryam Bayat[1], Zahra Amirghofran[1, 2]

*1. Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran*
*2. Autoimmune Disease Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** *Mentha longifolia* is a medicinal plant used in traditional medicine for various purposes. The present study assessed the immunomodulatory effects of *M. longifolia* main compound (MLMC), on peripheral blood mononuclear cells (PBMCs) and Jurkat T cell line.
**Methods:** PBMCs and Jurkat cell line were treated by different concentrations of MLMC and the proliferation inhibition was measured by BrdU and MTT colorimetric assays. Analysis of cell viability was performed by propidium iodide (PI) staining using flow cytometry. Cytokine levels were measured by enzyme-linked immunosorbant assay (ELISA).
**Results:** Our results showed that MLMC decreased the proliferation of PBMCs and Jurkat cell line dose and time-dependently; at the highest concentration (800 µg/ml), 43.9 ± 17% and 53.5 ± 10.8% of Jurkat cells were inhibited after 24 and 48 h of treatment, respectively. Corresponding rate of proliferation inhibition in PBMCs in the presence of the same concentration of MLMC was 96.37±0.27.
Analysis of cell viability using PI staining showed no significant cytotoxic effects at all concentrations used in both Jurkat and PBMCs. Measuring cytokine levels by ELISA indicated a decreased dose dependent IFN-γ secretion in treated PBMCs; The highest concentration (800µg/ml) of the compound reduced this cytokine level to 18.3 ± 2.4 pg/ml compared to the control (141 ± 20 pg/ml). The compound had no effects on IL-4 production by PBMCs.
**Conclusion:** These data showed the inhibitory effects of MLMC from *Mentha longifolia* on the proliferation and cytokine levels in PBMCs and Jurkat cells which imply the immunomodulatory effects of this compound.

**Keywords:** Immunomodulatory effect, *Mentha longifolia*, PBMCs, Jurkat cell line

ICIA2018\Immunopharmacology and Medicinal Plants\Poster Discussion\8629
**8629**

# Influences of the Ginger Extract on the Expression of Chemokines CCL20 and CCL22 and Chemokine Receptors CCR6 and CCR4 in Experimental Autoimmune Encephalomyelitis

Abdollah Jafarzadeh[*1], Zahra Arabi[2], Rayhaneh Ahangar Parvin[2], Marziyeh Mohammadi Kordkhayli[1], Zahra Taghpour[3], Maryam Nemati[4]

*1. Department of Immunology, Medical School, Kerman University of Medical Sciences, Kerman, Iran*
*2. Department of Immunology, Medical School, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
*3. Department of Histology, Medical School, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
*4. Department of Laboratory Sciences, Para-Medicine School, Kerman University of Medical Sciences, Kerman, Iran*

**Background:** Chemokines play a central role in the leukocytes infiltration into the central nervous system (CNS) which is an essential step in the pathogenesis of multiple sclerosis. Ginger possesses a wide range of immunoregulatory and anti-inflammatory properties. This study aimed to evaluate the effects of ginger extract on the expression of CCL20 and CCL22 and CCR6 and CCR4 in experimental autoimmune encephalomyelitis (EAE).
**Methods:** EAE induction was induced in female C57BL/6 mice by immunization with myelin oligodendroglial glycoprotein. Then, the EAE mice were treated with PBS or ginger extract, from day +3 to +30. At day 31, mice were scarified and the expression of aforementioned markers measured in the spinal cord measured using real time-PCR.
**Results:** The gene expression of CCL20, CCL22 and CCR4 in the spinal cord of PBS-administrated EAE mice was significantly increased as compared with healthy control group ($P<0.04$, $P<0.05$ and $P<0.02$, respectively). Treatment of EAE mice with 200- and 300 mg/kg ginger extracts reduced the expression of CCL20, CCL22 and CCR4 in comparison with PBS-administrated EAE group ($P<0.04$, $P<0.01$ and $P<0.002$ for 200 mg/kg ginger and $P<0.01$, $P<0.005$ and $P<0.004$ for 300 mg/kg ginger, respectively). The CCR6 expression in EAE mice treated with 200- or 300 mg/kg ginger extracts was lower than PBS-administrated EAE mice ($P<0.01$ and $P=0.07$, respectively).
**Conclusion:** Ginger extract down-regulates the expression of CCL20 and CCL22 and their receptors in EAE mice. The possible therapeutic potential of ginger for treatment of MS can need to be considered in more investigations.

**Keywords:** Experimental autoimmune encephalomyelitis, ginger extract, CCL20, CCL22, CCR4, CCR6

ICIA2018\Immunopharmacology and Medicinal Plants\Poster Discussion\9787
**9787**

# Immunomodulatory Effect of APS on the Interaction of Peripheral Blood Mononuclear Cells with Cervical Cancer Cells

Elham Shokati[1], Elahe Safari [1], Kobra Entezami[1], Samaeh Khorami[1]

*1. Immunology Department, School of Medicine, Iran University of Medical Sciences*

**Background:** Immunomodulator components from traditional medicinal plants are explored as a beneficial therapeutic approach to improve the effectiveness of common therapy because of the severe side effects of common cancer therapies. Astragalus membranaceus is one of the important medicinal herbs. Polysaccharides are the main constituents of Astragalus membranaceus. Astragalus polysaccharides (APS) have been shown different immunostimulatory effects like anti-inflammatory, anti-oxidative and anti-cancer properties. In this study, the potential effect of APS on the proliferation of PBMC and induction of MDSCs in co-culture with cervical cancer cell line was evaluated.
**Methods:** The proliferation characteristics of PBMCs in co-culture with cell lines were performed with CFSE dilution assay. Cell viability of PBMCs and HeLa cell line was assessed with MTT test. The frequencies of MDSCs in the PBMCs of healthy individuals after co-culture between PBMCs and HeLa cell line was examined by using FACS analysis.
**Results:** The result showed that APS could significantly promote the viability of treated PBMCs co-cultured with HeLa cancer cell line ($p<0.05$). APS could improve proliferation of treated PBMCs co-cultured with HeLa cancer cell line ($p<0.01$). Moreover, no significant inhibition was observed on MDSCs expansion in treated PBMCs co-cultured with HeLa cell line.
**Conclusion:** Our results indicate that APS can improve immune response in vitro by increasing PBMCs proliferation. However, no effect was observed on rate of MDSC. But, it seems that APS as an immune modulator can enhance the anti-tumor immune response.

**Keywords**: APS, Immunomodulation, Cancer

ICIA2018\Immunopharmacology and Medicinal Plants\Poster Discussion\9886.doc
**9886**

# Effect of Ganoderma. Lucidum (*G.Lucidum*) on Specific Immunoglobulin Isotype against Tetanus Vaccine

Sajjad Ahmadi Renani[1], Kazem Ahmadi[2]

1. *Tehran University of Medical Science, Tehran, Iran*
2. *Department of Immunology, Faculty of medicine, Baqiyatallah University of Medical Science, Tehran, IrIran*

**Background:** The immuno-modulating effects of Ganoderma. Lucidum (*G.Lucidum*) polysaccharides have been widely studied, including promoting the function of antigen-presenting cells, humoral and cellular immunity. Previous studies have also shown its effect on cytokine production by peritoneal macrophages and enhancement of CD40/CD86 marker on human peripheral blood mononuclear cells. The aim of this study was evaluation of its effect on mice antibody isotype.

**Methods:** Twenty four mice were dived in 4 groups each of six. The first six mice were kept as control. The second group was orally treaded with 40 µg/day/mouse *G.Lucidum* in drinking water for 30 days. The third group was subjected to three intra dermal administration of tetanus toxoid as vaccine without *G.Lucidum*. The last six mice in addition to *G.Lucidum* in drinking water, subjected to three intera dermal injection of tetanus toxoid as vaccination. All mice were anaesthetized and blood samples were collected at 45 days of first vaccination. Sera were separated and specific antibody isotype against tetanus toxoid were measured by ELISA.

**Results**: The results showed that *G.Lucidum* enhanced specific antibody level comparing with group having vaccine without *G.Lucidum* ($p<0.001$). It has also been shown that the amount of IgG antibody against tetanus toxoid is sharply higher in group having both vaccine and *G.Lucidum* ($p<0.001$).

**Conclusion:** The data presented are in agreement with previous research indicating the higher expression of CD40/CD86 marker on human blood mononuclear cells and also higher cytokine release in response to *G.Lucidum* treatment. Taken together, it seems that *G.Lucidum* could be a good immune modulator both in humoral and cellular immunity.

**Keyword:** *G.Lucidum,* tetanus vaccine, antibody isotype.




# Innate Immunity and Inflammation

# Poster Discussion

ICIA2018\Innate Immunity and Inflammation\Poster Discussion\7348
**7348**

# Decrease in Activated Tregs is accompanied by Their IL-17 Expression in the Peripheral Blood of Patients with Atherosclerosis

Mohammad Reza Yazdani[1], Shahdad Khosropanah[2], Mehrnoosh Doroudchi[1]

1. *Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Department of Cardiology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Human atherosclerotic plaques contain 1-5% Treg cells compared to other chronically inflamed tissues (25%). A protective role is suggested for CD4+CD25+Foxp3+ natural Tregs (nTreg), while the role of Th17 cells in the atherosclerosis is controversial. In this study we enumerated nTreg subsets as well as IL-17 producing Tregs in the peripheral blood of patients with atherosclerosis.
**Methods:** Fifteen ml heparinized blood was obtained from each of the 13 non-diabetic patients and 13 control individuals with normal/insignificant coronary artery disease confirmed by angiography. Peripheral Blood Mononuclear Cells were separated by Ficoll. Flowcytometry was performed in both group without stimulation, for detection of CD4/CD25/CD45RO/Foxp3 markers, and after stimulation (PMA/ION: 50/250 ng/ml) for detection of CD4/ CD45RO/ IL-17/FoxP3. The data were analyzed with the FlowJo software and analyzed using Mann-Whitney test by SPSS.
**Results:** Higher frequencies of resting nTregs (CD45RO-CD25+Foxp3$^{lo}$) and activated nTregs (CD45RO+CD25$^{hi}$Foxp3$^{hi}$) were found in controls. The frequency of effector/memory T-cells (CD4+CD25+CD45RO+Foxp3-) was higher in patients. In CD4+CD25+ and CD4+CD25+Foxp3+ populations, Foxp3-MFI was higher in controls. CD4+IL-17+Foxp3+ and CD4+IL-17+Foxp3- subsets showed higher frequencies in patients before and after stimulation. In CD4+ population, the MFI of IL-17 was increased in controls compared with patients both before and after stimulation. After stimulation, CD4+CD45RO+Foxp3+IL-17- T-cells (aTregs) increased in controls, while Th17 (CD4+CD45RO-IL-17+Foxp3-) and CD4+CD45RO+IL-17+Foxp3- T-cells were more frequent in patients. The frequency of effector/memory T-cells remained higher in patients while the percentage of nTregs was higher in controls after stimulation. The frequency of intermediate cells (CD4+CD45RO+IL-17+Foxp3+) with a high IL-17-MFI was higher in patients.
**Conclusion:** We showed that in patients with atherosclerosis, the frequencies of aTregs and nTregs decrease and the frequencies of Th17 and effector T-cells increase compared to controls. IL-17 production by an intermediate population with an aTreg phenotype suggests a population shift from FoxP3+ to FoxP3+IL-17+ and FoxP3- cells in atherosclerosis.

**Keywords**: IL-17, Treg, Atherosclerosis, FoxP3

ICIA2018\Innate Immunity and Inflammation\Poster Discussion\7484
**7484**

# Effects of Mannuronic Acid, as an Anti-inflammatory Drug, by targeting SHIP Signaling Pathway in Inflammation Process

Fatemeh Pourgholi [1,2], Mahsa Hajivalili [1,2], Zohreh Hekmati [2], Mehdi Yousefi [1,2]

1. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** B-D-mannuronic acid (M۲۰۰۰) is a newly designed nonsteroidal anti-inflammatory drug (NSAID) that also exhibits immunosuppressive and antioxidant properties in various experimental models. Src Homology-2 domain containing inositol-5'-phosphatase (SHIP) is an adaptor protein in downstream TLR2 signaling pathway. SHIP has a vital role in controlling inflammation functions via its ability to down regulate PI3K signaling pathway. The aim of this research was evaluate M2000 effects on expression levels of SHIP in Lipopolysaccharide (LPS) induced inflammation in HEK-TLR2 cell line.
**Methods:** Optimum dose of M2000 was estimated by MTT assay and HEK-TLR2 cell line was treated by different concentrations of M2000 and LPS (1µg/ml). After 24 hours incubation, total RNA was extracted using TRIZOL in all of conditions. Then cDNA was synthesized according to instructions and then expression level of SHIP was assessed by quantitative Real time PCR method.
**Results:** Our MTT results shown that optimum doses of M2000 was up to 25µg/ml ($p<0.05$). According to MTT results we considered 5 µg/ml and 25 µg/ml as low and high doses.
Our findings demonstrated that expression levels of SHIP in 5 and 25 µg/ml doses of M2000 were significantly up regulated to 1.68 (1.68 ±0.1207, p=0.01) and 1.89 (1.89±0.04,p=0.004) fold, respectively, in comparison to control group. SHIP expression level after treatment by LPS (1µg/ml), 2.33 fold decreased (2.33±0.1635 p=0.03). After co treatment of LPS and M2000 in low and high doses, expression levels of SHIP 1.53(1.53±0.0346, p=0.04) and 1.85 fold increased (1.85±0.0206 p=0.04) according to control group.
**Conclusion:** M2000 can be used as a safe NSAID in LPS induced inflammation and decrease Inflammatory cytokine productions by targeting SHIP in different inflammatory diseases. Also M2000 can be a good candidate in treatment of autoimmunity and inflammatory disorders.

**Keywords:** M2000, SHIP, inflammation, NSAID, HEK-TLR2

ICIA2018\Innate Immunity and Inflammation\Poster Discussion\7582
**7582**

# Evaluation of Gene Expression of NLRP3 and NLRC4 as Inflammasome Receptors in Peripheral Blood of SM Injured Patient by Real-Time PCR

Sholeh Etehad asnaf[1],

*1. Department of Biology School of Science University of Islamic Azad North Tehran Branch*

**Background:** Mustard gas is one of the most widely used chemical warfare agents used during the Iraqi wars against Iranian warriors. Some mechanisms of chronic pulmonary damage caused by mustard gas such as oxidative stress, protease-anti protease imbalance and inflammation have been studied. In the context of inflammation, the role of inflammasome and the genes involved in it, especially the receptors of this inflammatory complex, is important. Therefore, the present study evaluated the expression of NLRP3 and NLRC4 genes as inflammatory receptors in peripheral blood of veterans of pulmonary chemo using Real-Time PCR.
**Methods:** The present study was conducted as a cross-sectional analytical study on 15 chemical warfare victims exposed to mustard gas, 15 COPD patients and 15 healthy individuals as controls that were referred to the lung clinic of Baqiyatallah Hospital (AS). After extracting RNA from the blood sample and synthesizing cDNA, expression of the two NLRP3 and NLRC4 genes was evaluated using Real Time PCR. Finally, the data were analyzed by SPSS version 20.
**Results:** The two NLRP3 and NLRC4 genes did not change significantly in veterans and people with COPD compared to healthy subjects. Although the expression of these two genes was higher in COPD patients than veterans (0.17 to 0.05 and 0.15 to 0.04, respectively), there was no statistically significant relationship.
**Conclusion:** According to the results of the present study, it has been found that two NLRP3 and NLRC4 genes have the potential to be involved in inflammation and chronic obstructive pulmonary embolism. As a result, it is hoped that with the full knowledge of the process of chronic damage to the lungs caused by mustard gas, one can use it to create a pattern of diagnosis and prevent its further complications.

**Keywords:** Mustard gas, chemical veterans, inflammasome, NLRP3 gene expression, NLRC4 gene expression

ICIA2018\Innate Immunity and Inflammation\Poster Discussion\7719
**7719**

# Effect of Aqueous Extract and Saponin Fraction of *Tribulus terrestris L.* on the Expression of ICAM1 and VCAM1 in the Human Endothelial Cell Lines in Vitro

Elahe Amirinezhad Fard[1], Zahra Fereydouni[1], Ali Mostafaie[1]

*1. Department of immunology, Kermanshah University of Medical sciences, Kermanshah*

**Background:** Atherosclerosis is a common chronic inflammation which during of it low density cholesterol and lipid deposition on the wall of medium- and large-sized arteries are formed, known as fibrotic-lipid plaques (atheroma). In this study we aimed at investigating the effect of aqueous extract and saponin fraction of *Tribulus terrestris L.* on the expression of Intracellular Adhesion Molecule-1 (ICAM1) and Vascular Cell Adhesion Molecule-1 (VCAM1) in the Human Umbilical Vein Endothelial Cell (HUVEC) and Human Bone Marrow Endothelial Cell (HBMEC) lines in vitro during normal and LPS-induced conditions.
**Methods:** HUVEC and HBMEC lines were cultured and induced with LPS. Afterwards, the effect of aqueous extract and saponin fraction of *T. terrestris* on the expression pattern of endothelial proteins especially VCAM-1 and ICAM-1 in the HUVEC and HBMEC lines in vitro during normal and LPS-induced conditions was characterized by SDS-PAGE and blotting.
**Results:** Our findings showed that the aqueous extract and saponin fraction of *T. terrestris* decreased the expression of adhesion molecules (ICAM-1 and VCAM-1) in both LPS-induced HUVEC and HBMEC lines.
**Conclusion:** Aqueous extract and saponin fraction of *T. terrestris* clearly affected protein pattern of HUVEC and HBMEC lines, so that the expression of proteins with molecular weight of more than 90 and 65 KDa decreased. In addition, the extract and saponin fraction of *T. terrestris* down regulate the expression of ICAM-1 and VCAM-1. Anti-inflammatory activity of the aqueous extract is more than the effect of saponin fraction of *T. terrestris.*

**Keywords:** Atherosclerosis, ICAM1, VCAM1, *Tribulus terrestris L.*

ICIA2018\Innate Immunity and Inflammation\Poster Discussion\7937
**7937**

# The Inhibitory Role of M2000 (Beta-D-Mannuronic acid) on the Expression of Toll-like Receptor 2 and 4 in HT29 cells

Laleh Sharifi[1], Mona Moshiri[2], Mohammad Mehdi Soltan Dallal[2], Saied Bokaie[3], Zahra Norouzbabaie [4], Abbas Mirshafiey[5]

1. *Uro-Oncology Research Center, Tehran University of Medical Sciences, Tehran, Iran*
2. *Department of Microbiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
3. *Department of Epidemiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
4. *Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
5. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Anti-inflammatory agents play main roles in controlling inflammatory diseases such as inflammatory bowel disease (IBD); but due to the vast side effects their use are restricted. M2000 (Beta-D-Mannuronic acid) is a new immunomodulatory drug. According to the key role of TLRs in inflammatory responses, in this study we aimed to evaluate the role of M2000 on the expression of TLR2 and TLR4 in HT29 cell line as colonic epithelial cells.
**Methods:** MTT assay was carried out to determining the viability rate of cells after M2000 treatment. HT29 cells were cultured and treated with low (5µg/ml) and high (25µg/ml) doses of M2000. Total RNA was extracted and cDNA synthesized, quantitative real-time PCR was done to quantify the TLR2 and TLR4 mRNA synthesis.
**Results:** We found that M2000 at concentration of ≤ 1000 µg/ml had no obvious cytotoxicity outcome. The results of quantitative real-time PCR showed that the expression of TLR2 mRNA in HT29 cells in the treatment group of low-dose M2000 were decreased significantly compared with untreated control group, 1.00±0.16 *vs* 0.32±0.03 (p=0.02). Also we showed that high dose of M2000 decrease the expression of TLR2 significantly 1.00±0.16 *vs* 0.05±0.03 (p=0.01) Treating the cells with low-dose M2000 led to significant reduction in TLR4 compared to control cells, 1.00±0.09 *vs* 0.52±0.08 (p=0.01); similarly high dose of M2000 decreased gene expression of TLR4, 1.00±0.09 *vs* 0.16±0.03 (p=0.008).
**Conclusion:** The results of present study in a model of colonic epithelial cells indicate that M2000 can be consider as a new treatment of inflammation in IBD. However more studies are required to recognize the comprehensive molecular mechanism of M2000 and evaluate its therapeutic effects.

**Keywords:** M2000, Beta-D-Mannuronic acid, IBD, TLR2, TLR4

ICIA2018\Innate Immunity and Inflammation\Poster Discussion\7979
**7979**

# Amelioration of High-Glucose Induced Inflammation by Quercetin Through Macrophage Polarization in RAW624.7 Cell Line

Reyhaneh Babaei Khorzoughi[1], Maryam Shabani[*1]

*1. Department of Clinical Biochemistry, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Macrophages are divided into two phenotypes (M1 and M2) based on their surface markers. M1 markers include CD11, iNOS, etc. and M2 markers include CD206, CD163, etc. These markerś activations are affected by several mechanisms such as ER stress, hypoxia, lipotoxicity, ROS production and NF-kB activation. High glucose concentrations give rise to lipotoxicity and ROS products that result in M2 to M1 shift and lead to inflammation. Pro-inflammatory cytokines are released by M1 macrophages and M2 macrophages release anti-inflammatory cytokines. Furthermore, the balance between M1/M2 has an important role in inflammation mechanisms. Many studies have been shown polyphenols have anti-inflammatory potential and safety effects. Quercetin is a flavonol, one of the six subclasses of flavonoid compounds. This polyphenol possesses strong anti-inflammatory capacities; through an increase of anti-oxidative activities, reduction of lipogenesis and macrophage polarization regulation.
**Methods:** This study has surveyed the glucose (53mM) modulation of RAW 264.7 macrophages activation and effects of Quercetin (25µM) on high glucose-induced lipotoxicity and macrophage polarization. We have measured high glucose-induced lipogenesis by oil red O staining. For investigating macrophage polarization, we assess M2 marker, CD206, as an anti-inflammatory factor and M1 marker, CD11c, as an inflammatory factor via flow cytometry.
**Results:** Our results suggest that high glucose induce RAW264.7 lipogenesis and modify RAW264.7 morphology. Flow cytometry analyses showed that high glucose increase M1 marker, CD11c, significantly (about 80%) in vitro. RAW264.7 were treated with Quercetin strongly reduce lipid droplet in oil red O staining and decrease M1 marker, CD11c to approximately 20%. Our data have shown quercetin Caused a very slight increase in M2 marker, CD206 (4%) that isn't signed.
**Conclusion**: These results show that Quercetin produces a potential anti-inflammatory effect by modulating macrophage polarization and attenuate high glucose-induced lipogenesis in vitro. Decreasing M1 phenotype involved in the anti-inflammatory property of Quercetin.

**Keyword**: Inflammation, Quercetin, High Glucose, Macrophage polarization

ICIA2018\Innate Immunity and Inflammation\Poster Discussion\8043
**8043**

# Increased Interleukin-1β and Caspase 1 Production through Activation of Inflammasome Contributes to the Neuroinflamation and Memory Impairment in a STZ-Induced Model of Alzheimer Disease

Mahdiye Saadi [1, 2], Ahmad Karkhah[1, 2], Fereshteh Pourabdolhossein [2] , Hamid Reza Nouri [2]

*1. Student Research Committee, Babol University of Medical Sciences, Babol, I.R. Iran*
*2. Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, I.R. Iran*

**Background:** Alzheimer's disease (AD) is an inflammatory neurodegenerative disease considered by neuronal cell death and progressive dementia. Recent evidence demonstrated that IL-1β plays a critical role in the pathogenesis of AD. Thus, the present study was conducted to investigate which inflammasome pathway contribute in IL-1β production and memory impairment in Alzheimer disease.
**Methods:** In this study, Alzheimer disease was induced in male Wistar rats by two bilaterally injection of Streptozotocin 3 mg/kg into the lateral ventricles (each side 3µl) with 48 h interval. Morris Water Maze was used to evaluate learning and memory. In addition, Hematoxylin and Eosin staining was used to assess inflammatory parameters in hippocampus region. Expression level of several genes involved in inflammasome pathway including NLRP1, AIM2, NLRP3, NLRC4, ASC, IL18, IL-1β and Caspase 1 in the hippocampal tissue was measured using Real-time PCR.
**Results:** Behavioral study revealed that STZ injection in the lateral ventricles impaired learning and memory function ($p< 0.05$). Histological study using H & E staining confirmed the inflammatory response in the hippocampus region of STZ treated animals. Gene expression studies demonstrated a significant ($p< 0.05$) increase in NLRC4 mRNA expression in STZ treated group when compared to control group. The expression level of ASC, IL-18 and Caspase 1 in the hippocampus of STZ treated group was more than control, but there was no statistically significant difference between the two groups. In addition, mRNA expression level of NLRP1, AIM2 decreased in STZ treated group as compared with control group, however this decrease was not statistically significant**.**
**Conclusion:** We concluded that increased interleukin-1β and caspase 1 production through activation of NLRC4 inflammasome may be involved in neuroinflammation and memory impairment in Alzheimer disease which can introduce promising targets for treatment of Alzheimer disease.

**Keywords:** Alzheimer, interleukin-1β, Caspase 1, NLRC4, Neuroinflamation, Memory impairment.

ICIA2018\Innate Immunity and Inflammation\Poster Discussion\8064
**8064**

## Development of Experimental Fibrotic Liver Diseases Animal Model by Carbon Tetrachloride

Atoosa Gitiara[1], Samaneh Tokhanbigli[1], Sogol Mazhari[1], Kaveh Baghaei[1*], Behzad Hatami[2], Seyed Mahmoud Hashemi[3], Ali Asadi Rad[3], Afshin Moradi[2], Nakisa Zarrabi Ahrabi[4], Hamid Asadzadeh Aghdaei[1], Mohammad Reza Zali[2]

*1. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
*2. Gastroenterology and Liver Diseases Research center, Research institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
*3. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*4. Department of Biology Central Tehran Branch, Islamic Azad University, Tehran, Iran*

**Background:** Liver fibrosis is a result of inflammation and liver injury caused by wound healing responses which ultimately lead to liver failure. Consequently, after liver fibrosis, the progression will be continued to liver cirrhosis and at the end stage hepatocellular carcinoma (HCC). Many studies have demonstrated that one of the most important causes of liver fibrosis is Non-alcoholic steatohepatitis (NASH). Fibrotic Liver is affected by an excessive accumulation of extracellular matrix (ECM) proteins like collagen and α-SMA.
**Methods:** in two different experiment, male Vistar, and Sprague Dawley Rat models ranging from 200±60, corresponding to an age of approximately 10 weeks were utilized in order to induce CCL4 treated liver fibrosis.
**Results:** After 6 weeks of CCL4 injection, different tests have been carried out to verify the liver fibrosis including serum markers such as Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT), molecular tests containing, laminin and α-SMA and also pathological observation by Hematoxylin and eosin staining in both fibrosis and control group.
**Conclusion:** The results of Pathology and Real-time PCR showed that fibrosis was induced much more effective in Sprague Dawley rat model in compare with Wistar rats.

**Keywords:** Liver Fibrosis, CCL4, Animal Model.

ICIA2018\Innate Immunity and Inflammation\Poster Discussion\8169
**8169**

## The Effects of Marhame-Mafasel in IL-1β ،IL-6 ،TNFα Expression in Animal Induced Arthritis

Mohammad Majidi[1, 2], Roya Yaraee[1*], Shahin Bonakdar[2], Mohsen Naseri[1], Mohammadreza Jalali[3], Fatemeh Heidarnejad[2], Arsalan Jalili A[4] Maryam Salimi[5]

1. *Department of Immunology, Shahed University, Tehran, Islamic Republic of Iran*
2. *National Cell Bank Department, Pasteur Institute of Iran, Tehran, Iran*
3. *Department of Pathology, School of Medicine, Shahed University, Tehran, Iran*
4. *Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.*
5. *Department of Biology and Anatomical Sciences, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background**: Rheumatoid arthritis (RA) is a systemic chronic disease with synovial membrane, tendon and auricular tissue inflammation. Two fundamental criteria of RA include chronic inflammation and bone absorption. Current treatments of RA have many side effects and are so expensive. Currently, new treatments procedures and inexpensive herbal drugs are developed. Marhame-Mafasel made of 2 traditional herbs (Arnebiaeuchroma and Martricariachamomilla) and produced by pharmacology department of Shahed university of Iran. Marhame-Mafasel has been used for RA patient's treatment for many years. In this study, the effects of Marhame-Mafasel on a part of immune system were evaluated by an animal model of RA.
**Methods**: 15 male wistar rats were used in three group of Marhame-Mafasel, Paraffin (negative control) and piroxicam (positive control). Volume of hind paw was measured every other day from 0 to 19 using water changed volume approach. Gene expression of TNFα ،IL-1β and IL-6 were evaluated using Real-Time PCR and inflammation in joint was evaluated using histopathology assay.
**Results**: Hind paw swelling of Marhame-Mafasel and priroxicam groups at day 10 and 19 that are most important days in animal model of RA(10: acute inflammation, 19: chronic inflammation), was reduced. This note should be taken, reduction between each groups weren't statistically significant. Histopathology and Real-Time PCR evaluation between three groups were not statistically significant.
**Conclusion**: Although the hind paw swelling of Marhame-Mafasel and priroxicam groups was reduced, Because of the small number of animals and the lack of precise measurement instrument, results have large variance then more future studies must be carried out.

**Keywords**: Marhame-Mafasel, IL-1β ،IL-6 ،TNFα, Animal induced arthritis

ICIA2018\Innate Immunity and Inflammation\Poster Discussion\9840
**9840**

# Phagocytosis of Apoptotic Mesenchymal Stem Cells by Mouse - Peritoneal Macrophages Effects on Cytokine Production and Phagocytosis Ability

Maryam Ghahremani Piraghaj[1], Seyed Mahmoud Hashemi [1]*, Hosein Ghanbarian [2], Sara Soudi [3]

*1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti of Medical Sciences, Tehran, Iran University*
*3. Faculty of Medical Sciences, Department of Immunology, Tarbiat Modares University, Tehran, Iran*

**Background**: Macrophages are one of the most important immune cells. Macrophages can be divided into two main subgroups of classical (M1) and alternative or (M2), due to different stimuli. One of the factors that cause the macrophage to orient towards M2 is the phagocytosis of apoptotic cells (efferocytosis). The phagocytosis of mesenchymal stem cells, can be very important in cell therapy due to immunomodulatory properties and their ability to modulate macrophage function
**Methods**: Adipose-derived mesenchymal stem cells were isolated from CB57bl/6 Mice and characterized by flow cytometry as well as differentiation to *osteoblasts and adipocytes*. MSCs in the passage 2 exposed to UV light for induction of apoptosis for thirty minutes and followed by incubation for two hours. The cells were then isolated and added to macrophage in a ratio of 4 to 1. After that cells were incubated for 48 hours, after incubation time the production of TNFα and IL10 cytokines was measured by ELISA. Macrophages phagocytosis ability was also measured by yeast and apoptotic thymocytes phagocytosis in different groups.
**Results**: The phagocytosis of apoptotic mesenchymal stem cells by macrophages reduces the production of inflammatory cytokine TNFα and increases the production of inhibitory cytokine IL-10. Also after phagocytosis of mesenchymal stem cells, the ability of yeast phagocytosis in these macrophages is reduced and phagocytosis of apoptotic thymocyte was increased
**Conclusion**: The phagocytosis of apoptotic mesenchymal stem cells, induce non-inflammatory phenotype in macrophages. So, injected Mesenchymal Stem Cells maintain their immunomodulatory properties even if they apoptosis in the body.

**Keywords**: macrophage, Mesenchymal stem cell, Efferocytosis, Phagocytosis

# Monoclonal Antibody Diagnostic and Therapeutic

# Poster Discussion

ICIA2018\Monoclonal Antibody Diagnostic and Therapeutic\Poster Discussion\5240
**5240**

# The Effect of All-trans Retinoic Acid Alone and Combination with Osahexaenoic Acid on Monoclonal Antibody Production in Hybridoma Cells: an *in vitro* and *in vivo* Study

Mohsen Rokni[1], Ali Akbar Saboor Yaraghi[1] ,Ali Reza Razavi[1], Fazel Shokri[1], Tayebeh Sohrabi[2], Maryam Kargar[3], Masoome Keykha[3], Masoud haghshenas[4]

*1. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
*2. Department of Hematology and blood bank, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*3. Department of Immunology, Faculty of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran*
*4. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran*

**Background:** Hybridoma cells can produce monoclonal antibody (MAb) and the productions of these antibodies can be affected by different factors including stimulants, inhibitors, and supplements. Several types of research have been done to improve MAb production in cell culture and in animal model. However, the impact of micronutrients on the production of MAb in mouse hybridoma cells and in the peritoneum of animals is not fully understood.
**Methods:** The hybridoma cells, $M_3C_5$, were cultured and treated with different concentrations of all trans-retinoic acid (ATRA) and osahexaenoic acid (DHA), alone and in combinations and at different time of exposure, then the changes in production of Mab in culture medium were evaluated using ELISA and compared with vehicle-treated controls. The hybridoma cells, after single and combined treatment with ATRA, DHA, and vehicles were IP injected to Balb/c mice and the changes in production of Mab in ascites of mice, were determined by ELISA. The effect of these metabolites on the cloning efficiency of hybridoma cells was also explored.
**Results:** The results showed that single and combined treatment of ATRA and DHA significantly elevated the productions of MAb by $M_3C_5$ hybridoma cells, in both *in vivo* and *in vitro* conditions. The production of MAb following *in vitro* single treatment with 1µM of ATRA ($p<0.003$) and 10µM of DHA ($p<0.007$) for 2 days was significantly increased. The *in vitro* effects of ATRA on up-regulation of MAb production was obtained more than DHA however, at *in vivo* conditions, DHA was more effective in increasing the MAb production($p<0.001$).
**Conclusion:** The results of this study, for the first time, showed that ATRA and DHA are effective supplements that can increase production of MAb in mouse hybridoma cells under both *in vitro* and *in vivo* conditions.

**Keywords:** Hybridoma cells, Monoclonal antibody, osahexaenoic acid, All-trans retinoic acid, Cloning efficiencies, Ascites.

ICIA2018\Monoclonal Antibody Diagnostic and Therapeutic\Poster Discussion\7720
**7720**

# Characterization of Monoclonal and Polyclonal Antibodies Recognizing Prostate Specific Antigen: Implication for Design of a Sandwich ELISA

Sahar Raoofi Mohseni[1], Forough Golsaz Shirazi[1], Mostafa Hosseini[2], Jalal Khoshnoodi[1], Tannaz Bahadori[3], Mohammad Ali Judaki[1], Mahmoud Jeddi Tehrani [3], Fazel Shokri[1,3]

*1. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
*2. Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
*3. Monoclonal Antibody Research Center, Avicenna Research Institute, ACERCR, Tehran, Iran*

**Background:** Prostate cancer is the second common cancer in men. Prostate-specific antigen (PSA) is a tumor-associated glycoprotein with enzymatic activity which is secreted by the prostate gland. Following entry to the blood, 70-90 % of PSA forms complexes with protease inhibitors and its enzymatic activity is inhibited. The serum level of PSA is increased and the rate of free PSA (fPSA) to total PSA is decreased in prostate cancer patients. Therefore, measurement of PSA and fPSA in serum is very valuable for diagnosis and prognosis of prostate cancer.
**Methods:** In the present study, five anti PSA monoclonal antibodies (mAb) were characterized by enzyme-linked immunosorbent assay (ELISA) and immunoblotting. For design of a sandwich ELISA, epitope specificity of these antibodies was studied by a competition ELISA. Free PSA was purified by electeroelution technique from seminal plasma and used to produce polyclonal anti-fPSA antibody in rabbit. Purified Polyclonal antibody (pAb) and mAbs were conjugated with HRP enzyme and Biotin (Bio) to set up the sandwich ELISA.
**Results:** Three of the mAbs were found to recognize PSA, similarly. One of these mAbs (2G3) was paired with anti-fPSA pAb to detect fPSA in serum. Eventually serum fPSA concentration of 356 subjects was measured and compared by our designed ELISA and a commercial ELISA kit. Our results demonstrated a significant correlation ($r = 0.68$; $P < 0.001$) between the two assays. Sensitivity and specificity of our designed ELISA is 72.4% and 82.8%, respectively
**Conclusion**: These results imply suitability of our designed ELISA for detection of fPSA in patients with prostate cancer.

**Keywords:** Free PSA, prostate cancer, sandwich ELISA test, monoclonal antibodies

ICIA2018\Monoclonal Antibody Diagnostic and Therapeutic\Poster Discussion\8164
**8164**

# Evaluation of the Binding Activity of a Specific VH Antibody Fragment for *Neisseria Meningitidis* Factor H Binding Protein (fHbp) via ELISA

Hediyeh Rafighdoust[1], Fatemeh Yarian[2,3], Fattah Sotoodehnejad Nematalahi[1], Mona Salahshoor dahr[4]

*1. Department of Biology, School of Basic Sciences, Science and Research Branch, Islamic Azad University,Tehran,Iran*
*2. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Cellular &Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*4. Department of Biology, School of Basic Sciences, Islamshahr Azad University, Tehran, Iran*

**Background:** Although widespread use of whole antibodies in medicine and pharmacy is undeniable, they also have many limitations for most diagnostic and therapeutic applications. Antibody fragments have shown high potential detection of infectious agents, and studies in this area are potentially ongoing. Previously, we obtained a specific scFv antibody for *N. meningitidis* factor H binding protein (fHbp) and it's binding to the fHbp was characterized by bioinformatics tool. The bioinformatics data showed that the VH fragment of the scFv has more binding to the antigen. The main study was evaluation of the binding activity of a specific VH fragment to the recombinant fHbp antigen.
**Methods:** The T.Vector contained VH antibody fragment gene and pET28a vector were cut with NcoI, NotI enzymes. It was successfully cloned into pET28a vector, transformed into *E.coli* strain BL21 and expressed in optimal condition. The expressed protein was approved by SDS-PAGE and Western blotting. The recombinant His-tagged VH antibody was purified via affinity chromatography using $Ni^{2+}$-NTA agarose resin. Binding activity of the VH fragment antibody for recombinant factor H binding protein (fHbp) was evaluated via ELISA.
**Results:** The cloned VH fragment antibody into the pET28a (+) vector was expressed. The expressed protein (about 12 kDa) was investigated by SDS-PAGE and approved in western blotting by anti-his tag antibody. We used the ELISA technique to determine the functions of the purified VH fragment antibody.
**Conclusion:** cloning, expression and purification of the VH fragment antibody was successfully performed. ELISA was performed to determine the functions of the purified VH antibody. The results indicate that despite the small size of the antibody fragment, it was exhibited more binding activity to recombinant fHbp. The VH fragment antibody may provide the basis for the development of a diagnostic kit.

**Key words:** VH fragment antibody, Cloning, *Neisseria meningitides,* factor H binding protein

ICIA2018\Monoclonal Antibody Diagnostic and Therapeutic\Poster Discussion\8192
**8192**

# Design and Expression of a Specific Diabody for *Neisseria Meningitidis* Factor H Binding Protein (fHbp)

Mona Salahshoor Dahr[1], Fatemeh Yarian[2,3], Maryam Ghane[1], Mojgan bandehpour[2,4], Hediyeh Rafighdoust[5]

*1. Department of Biology, School of Sciences, Islamic Azad University Islamshahr Branch, Islamshahr, Iran*
*2. Cellular &Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*4. Department of Biotechnology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*5. Department of Biology, School of Basic Sciences, Science and Research Branch, Islamic Azad University,Tehran,Iran*

**Background:** The single-chain fragment variable antibody, scFv, is an antibody fragment that has shown a unique capability for binding to many antigens, such as proteins and even whole pathogens. By engineering the monovalent antibody fragments (such as Fab and scFv) into multivalent molecules as diabodies, triabodies or larger aggregates, it can be significantly increased their functional affinity and specificity to the target antigens. Previously, we obtained two specific scfvs against *N.meningitidis* factor H binding protein (fHbp). The functionality of these novel scFvs were characterized by ELISA. The main purpose of this study is design and expression of a specific diabody for this protein.
**Methods:** In this study, the nucleotide sequence of the diabody was designed and synthesized. The pGH vector contained the diabody gene and pET28a vector were cut with Nco I, Not I enzymes. The cloned diabody into the pET28a (+) vector were transformed into *E.coli* strain BL21 and expressed in optimal condition. The expressed protein was approved by SDS-PAGE and Western blotting.
**Results:** The desired gene is 1430 nucleotides. The expressed protein (about 51 kDa) was investigated by SDS-PAGE and was approved in western blotting by anti-his tag antibody.
**Conclusion:** The design antibody was successfully cloned into pET28a vector and expressed. It is expected that the designed diabody will be efficient for diagnosing of all *N. meningitis* serogroups in CSF in the future.

**Keywords:** Diabody, Cloning, factor H binding protein, *Neisseria Meningitidis*

ICIA2018\Monoclonal Antibody Diagnostic and Therapeutic\Poster Discussion\8202
**8202**

# Isolation of MKN-45 Gastric Cancer cell Recognizing Single Chain Variable Fragment (scFv) Antibodies Using Phage Display Technology

Tayebeh Mehdipour[1,2], Mohammad Reza Tohidkia[2], Ali Akbar Rahim Rahimi[2,3], Mohammad Nouri[2,4]

1. *Department of Medical Biotechnology, School of Advanced Medical Science, Tabriz University of Medical Science, Tabriz, Iran*
2. *Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran*
3. *Department of Microbiology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
4. *Department of Biochemistry, School of Medicine, Tabriz University of Medical Sciences, Iran*

**Background:** Aim of this study was to isolate recombinant antibody fragments recognizing poorly differentiated metastatic gastric cancer (GC) cell line, MKN-45, to direct discovery of potential tumor markers related to diffuse type adenocarcinoma.
**Methods:** A semi-synthetic human scFv library (Tomlinson I) was used for identification of novel antibody fragments recognizing MKN-45 GC cell line. Diverse antibody phage-scFv libraries were prepared from *E.coli* transformants infected with two different helper phages, KM13 and hyperphage, in parallel. Overall four rounds of subtractive selection were carried out, each consisting of three pre-absorptions using NIH 3T3 and AGS cell lines prior to positive selection on MKN-45 cells. Characterization and specificity analysis of the selected scFv antibodies were determined through whole cell ELISA and flow cytometry.
**Results:** ELISA-based screening of 192 phage-displayed scFv clones indicated that 2 and 3 scFv candidates rescued by KM13 and hyperphage respectively, produced strong binding activity against MKN-45 and low reactivity to negative AGS and NIH 3T3 control cells. Further analysis by flow cytometry illustrated that B12 clone from KM13 and G1clone from hyperphage had the most binding activity and specificity for MKN-45 GC cell line.
**Conclusion:** The selected scFvs may be potential immunereagents in diagnosis and even novel therapeutic agents of metastatic GC. Additionally, efficient and sit-specific delivery of the drugs and nanoparticles can be done with the aim of these antibody fragments.

**Keywords:** Phage display, Whole cell panning, Gastric cancer, Diffuse adenocarcinoma

ICIA2018\Monoclonal Antibody Diagnostic and Therapeutic\Poster Discussion\8332
**8332**

# Optimization of Refolding Procedures for Novel Humanized Immunotoxin Produced in the *E. coli*

Bahman akbari[1], Safar farajnia[2]

*1. Department of Medical Biotechnology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*2. Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** High-level expression of many recombinant proteins in *E. coli* leads to formation of inclusion bodies, highly aggregated proteins; they can also form in mammalian, insect, and yeast cells. Over expression of a humanized immunotoxin (IT) against epidermal growth factor receptor (EGFR) led to the production of inclusion bodies. The aim of this study was optimization of refolding process for recombinant humanized anti-EGFR IT produced in the *E. coli.*
**Methods**: The BL21 (Plys S) cells containing a pET-22b- humanized anti-EGFR IT construct -constructed and characterized in previous work- were induced by 0.25mmol/l IPTG at 25 C° for 4 h and the amount of expression was checked by SDS-PAGE, even though the majority of expressed proteins appeared as inclusion bodies. These inclusion bodies were individually solubilized in 8 M urea and 6 M guanidine hydrochloride and then purified by Ni-NTA affinity chromatography which was seen as a single band in SDS-PAGE analysis.
**Results**: The proper refolding humanized IT achieved by stepwise dialysis method. Reactivity assessment of humanized IT obtained from the urea approach with A431 carcinoma cells by relative ELISA test revealed that the refolded humanized IT had a high reactivity, indicating suitable folding of purified antibody. The 50% binding activity of humanized IT achieved from urea and guanidine hydrochloride approaches were 0.5 and 1.75 µg/ml concentrations, respectively.
**Conclusion**: The results of this study revealed that the urea approach was very effective in solubilizing and refolding of IT that expressed in bacteria cells as inclusion bodies.

**Keywords**: Guanidine hydrochloride, Inclusion body, Refolding, Urea.

ICIA2018\Monoclonal Antibody Diagnostic and Therapeutic\Poster Discussion\8335
**8335**

# An Investigation on the Structure and Function of Cetuximab Monoclonal Antibody in Quasi-physiological Conditions

Fatemeh Hasheminia[1], Razieh Farazmand [2]*, Nazanin Gholampour Faroji[2], Mona Abbasi kalabeh[1], Aliakbar Haddad Mashadrizeh[3], Farzaneh Rostami[4]

*1. Pharmaceutical Research Group, Khayyam Bioeconomy Institute, Mashhad, Iran*
*2. Structural biology and Bioinformatics Research Group, Khayyam Bioeconomy Institute, Mashhad, Iran*
*3. Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran.*
*4. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran.*

**Background**: The Cetuximab with brand Erbitux is one of the monoclonal antibodies that target epithelial growth factor-associated cancers (EGFR) and approved by FDA. So, today is using for a range of cancers such as colorectal, head and neck, lung and pancreatic carcinoma. So, achievement to the sequence and its modeling in quasi-physiology situation could be confirm the presented sequence point of view of structure and function, as well as opportunity for its optimization and development which are considered in this study.
**Methods**: In this study, the Protein Atlas database was used to determine the level and tissues expressing-EGFR. The NCBI, and RCSB provided the sequence and protein structure of the drug and the antigen. Antigen modeling and assembling the fragment sequences of drug were performed by using Modeller software by homology modeling method. ERRAT, Verify 3D and RAMPAGE programs were used to determine the quality of the 3D structures based on amino acid location. Assessment the stability of drug in quasi-physiological condition was performed with Gromacs at 37 ° C. Moreover, the functionality of this drug was evaluated via its affinity to corresponding antigen in comparison to other antigens as control. The PYMOL software was used to visualize structures and interactions.
**Results:** Assessment the expression of EGFR has led to reveal the high expression of this antigen on the surface of cancer cells of Head and neck, glioma, kidneys, skin and liver. Surveying the sequence of the Cetuximab reveal the presence of the variable light and heavy chain of antibodies with disulfide bonds, which are derived from mice and humans. Structural modeling of this drug has led to reveal the suitable quality of it based on the location of amino acid as well as its stability at 37 ° C during 20 nanosecond after assembling. Moreover, affinity of this drug to EGFR showed significantly difference in comparison to other antigens.
**Conclusion**: In conclusion, the obtained results of this study confirm the structure and function of the model of the Cetuximab sequence which is available in database. Moreover these results provide opportunity for Cetuximab optimization as well as immunotoxin development based on fusion to cytotoxic domains which is agenda of our group.

**Keywords**: Cancer, monoclonal antibody, Cetuximab, EGFR, *in-silico* biology

ICIA2018\Monoclonal Antibody Diagnostic and Therapeutic\Poster Discussion\8565
**8565**

# Expression and Optimization of Anti-HER2 scFv in *E.coli*

Farzaneh Farshdari[1], Elham Mohit[2], Maryam Ahmadzadeh[2]

*1. Department of Biotechnology, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic azad University, Tehran- Iran (IAUPS)*
*2. Department of Pharmaceutical Biotechnology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background**: The human epidermal growth factor receptor (HER) family plays an important role in many different kinds of cancer. Relationship between expression of human epidermal growth factor 2 (HER2) and malignity of breast tumors has been resulted in the production of monoclonal antibodies targeting $HER2^+$ tumors. Single chain variable fragment (scFv), the smallest functional region for antigen binding, has improved ability to penetrate into tumors while retaining specific affinity and having low immunogenicity. Particularly, anti-HER2 scFv can efficiently target the therapeutic proteins, drugs, and nanoparticles toward $HER2^+$ cancer cells. The main aim of this study is to express anti-HER2 scFv protein in prokaryotic system, to determine protein solubility and to evaluate the effect of host type, induction temperature, and inducting conditions on the amount and solubility of the anti- HER2 scFv protein.
**Methods:** The synthesis of scFv against HER2 gene was cloned in expression vector pET22 b (+) and its expression was investigated in the BL21 (DE3) and Origami. Furthermore, different concentrations of IPTG, induction period and induction temperature were examined in the BL21 (DE3). The effect of temperature on the solubility of this protein was also considered. Finally, the expression of anti HER2-scFv protein was evaluated using Western blot analysis.
**Results:** Western blot analysis confirmed the correct expression of anti HER2-scFv protein. Strain Bl21, was used for optimizing anti HER2-scFv protein. Totally, the optimum condition for the expression of anti HER2-scFv protein in the BL21 (DE3) was 37°C, 0.5 mM IPTG and 24 hours of induction
**Conclusion:** In this study anti HER2-scFv of Trastuzumab was expressed and optimized for the first time in BL21 (DE3) successfully.

**Keywords**: Breast cancer, scFv, Human Epidermal Growth receptor 2 (HER2)

ICIA2018\Monoclonal Antibody Diagnostic and Therapeutic\Poster Discussion\8713
**8713**

## In-silico Assay of the Structure, Function and Modeling of ONTAK in Real-like Condition

Mona Abbasi kalabe[1], Nazanin Gholampour Farogi [*2], Behrouz Jalali Ghassam[1], Aliakbar Haddad Mashadrizeh[3], Ali Eshaghi[4]

*1. Pharmaceutical Research Group, Khayyam Bioeconomy Institute, Mashhad, Iran*
*2. Structural biology and Bioinformatics Research Group, Khayyam Bioeconomy Institute, Mashhad, Iran*
*3. Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran*
*4. Department of Biology, Islamic Azad University, Khorasan Razavi, Mashhad, Iran*

**Background:** Today, the prevalence and fatality of cancer have led to develop smart drugs including immunotoxins. In 1999, ONTAK was introduced to the world market as the first immunotoxin which approved by the FDA, and presently it is utilized to treat the lymphoma disease. Hence, recognizing the properties of this drug in a quasi-physiological circumstance allows enhancing the function of it, which is on the agenda.
**Methods:** The sequences of drug were retrieved from the pharmaceutical database as well as the relevant patent. Then, its structural features were examined by computational methods. Subsequently, modeling and assembling of fragments were achieved by using Modeller software based on homology modeling method. In addition, evaluating the structure quality of the models were determined using ERRAT and RAMPAGE tests. Moreover, the structural simulation of the drug model was performed to confirm its stability in pseudo-physiological conditions. AutoDuck and IEDB software were used for analyzing of affinity and immunogenicity of model, respectively.
**Results:** The obtained results from molecular investigation of desired drug demonstrated the presence of a short length form of diphtheria toxin and human IL-2 as fatal part and fusion domain of this immunotoxin, respectively. Moreover, it also revealed the flexible glycine-rich linker in its structure. In addition, assembling of these fragments was resulted in providing an optimal quality model from the point of view of the amino acids location in the proper position as well as structural stability and immunogenicity at real-like condition at 37 ° C for 20 k ps. Furthermore, evaluation of drug affinity to IL-2R as target antigen showed a high binding affinity related to negative control.
**Conclusion:** Generally, the results of this study revealed a model of ONTAK with an optimal structure and function, which could be an appropriate choice for synthesis of this drug as well as opportunity for its optimization.

**Keywords:** Cancer, Smart drugs, Immunotoxin, ONTAK

ICIA2018\Monoclonal Antibody Diagnostic and Therapeutic\Poster Discussion\9743
**9743**

# Monoclonal Antibody Production Against Human CD105 (endoglin)

Esmaeil Jarrahi[1], Mohammad Reza Gharaati[2], Mehdi Alikhani[3], Masoud Nafari[4]

*1. Department of Molecular and cellular Biology Faculty of Basic Sciences and Advanced Technologies in biology University of Science and Culture, Tehran, Iran*
*2. Department of Stem Cells at Royan Institute*
*3. Department of Stem Cells at Royan Institute*
*4. Department of Molecular and cellular Biology Faculty of Basic Sciences and Advanced Technologies in biology University of Science and Culture, Tehran, Iran*

**Background:** CD105 is one of the most important angiogenesis factors. Pervious investigations revealed that CD105 protein had considerable expression during metastasis tumors angiogenesis in comparison with before. Also, CD105 is considered as the main marker in mesenchymal stem cells. Mesenchymal stem cells are one of the most basic cells and have differentiation capability to other cells. Therefore, detecting this protein plays an important role in research and treatment. Producing and applying monoclonal antibodies is significant in detecting and treating.
**Methods and Results:** In order to produce monoclonal antibody against human CD105, firstly the sequences being capable of using as epitope were extracted by bioinformatics, then entered expressive system as a gene construct. Expressed protein was injected to Balb-c mouse as timetable. Following, the spleen of mouse was extracted. Fusion process was carried out by mouse myeloma cell hybridation and extracted cells from mouse spleen using polyethylene glycol. For creating monoclonal characteristics, limiting dilution was carried out make the developed cells have equal lineage. Finally, the validity of this valuable molecule was proved by conducting molecule confirmation tests.

**Keywords:** Monoclonal antibodies, CD105, Mesenchymal stem cell

# Mucosal and Gastrointestinal Immunology

# Poster Discussion

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster Discussion\5273
**5273**

# IRF4-dependent Upregulation of Interleukin 9 Protein is Associated with Ulcerative Colitis Pathogenesis

Mojtaba Shohan[1], Hedayatollah Shirzad[2]

*1. Department of Microbiology and Immunology, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran*
*2. Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran*

**Background:** Ulcerative colitis is a type of inflammatory bowel diseases histologically characterized by chronic inflammation in the large intestine. Disease occurrence is dependent on the combination of environmental factors and genetical predisposition. Secretion of inflammatory mediators and immune system hyperactivity leads to sustained inflammation in the colon and it can be life threatening if remains untreated. Our aim was to measure the mRNA expression of IRF4 and protein level of IL-9 by means of real time PCR and ELISA in patients with UC, respectively.
**Methods:** 20 patients with ulcerative colitis and 21 healthy subjects were enrolled in the study. mRNA expression of IRF4 gene was determined by sybr green master mix detection method and real-time polymerase chain reaction (RT-PCR). Also protein contents of tissues were extracted and interleukin 9 protein level was measured by enzyme linked immunosorbent assay.
**Results:** Quantitative measurement of IL-9 protein and mRNA expression of IRF4 showed notable increases in patient's samples in comparison to healthy subjects.
**Conclusion:** Interleukin 9 is a multifunctional protein, which has been recently reported to be involved in the pathogenesis of autoimmune diseases and also experimental models of UC. Interferon regulatory factor 4 is a key regulator transcription factor of IL-9 production, which also targeted recently in various autoimmunities. As our data demonstrates, interleukin 9 protein is significantly upregulated in the inflamed lesions of UC patients so it may be a remarkable point of research in the novel therapeutic approaches for IBD.

**Keywords:** Ulcerative colitis, IL-9, IRF4

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster Discussion\8252
**8252**

# The Association Study of TNFα Gene Polymorphism -1031 C>T, -376 G>A with Susceptibility to Celiac Disease in Iranian population

Zohreh Nasiri[1], Abdolrahim Nikzamir[1], Majid Sirati Sabet[1], Vahid Chaleshi[3], Elham Aghamohammadi[2], Mohammad Reza Zali[3], Mohammad Rostami Nejad[3]

*1. Biochemistry Department, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
*3. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** TNFα is an important cytokine in inflammatory responses and plays an important role in the pathogenesis of autoimmune disorders such as celiac disease (CD). Regarding the position of TNFα gene in the original set of tissue constructs and in view of the role of TNFα in the pathogenesis of celiac disease, our goal in this study was to determine the relationship between -1031 C / T and -376 G / A TNFα polymorphisms with susceptibility to celiac disease in correlation with its serum level in this population compare with healthy people.
**Methods:** 104 patients with celiac disease whom their disorder confirmed by serological and pathological test and 102 control people were included in this study. DNA of both groups were extracted and gene polymorphisms was determined using PCR-RFLP technique. Also serum concentration of TNFα was evaluated ELISA method was used to determine the.
**Results:** The-1031 C / T and -376 G / A polymorphisms TNFα gene were not significantly correlated with BMI, age and sex. No significant difference was observed between CD patients and healthy controls for -1031 C / T (p=0.0566 and p=0.608 respectively) and -376 G / A gene polymorphisms (p= 0.8256 and p=0.806 respectively). Also no significant difference in serum concentration of TNFα was detected between celiac patients and healthy subjects (p= 0.189).
**Conclusion:** 1031C/T and -376G/A were not associated with CD nset in the Iranian population. This study reinforces the importance of other variants of TNFα in unraveling the pathogenic mechanisms of CD, therefore further genetic studies with larger number of patients are needed.

**Keywords:** Celiac disease, PCR-RFLP, TNFα gene polymorphism, autoimmune and inflammatory reaction

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster Discussion\8272
**8272**

# The Correlation between Serum Level of IL-15 and Abnormal Histopathology Degrees in Iranian Patients with Celiac Disease

Elham Aghamohamadi[1] , Parviz Kokhaei[1] , Fateme pak[1], Kamran Rostami[2], Afshin Moradi[3], Mohamad Amin Pourhoseingholi[3], Vahid Chaleshi[3], Mohammad Reza Zali[4], Mohammad Rostami Nejad[3]

*1. Immunology Department, School of Medicine, Semnan University of Medical Sciences, Semnan-Iran Milton University Hospital, UK*
*2. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
*3. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Celiac disease (CD) is a intestinal *autoimmune disorder* caused by the ingestion of wheat, barley and rye (gluten) in *people* who are *genetically predisposed*. Different studies showed that IL-15 is a key regulator in celiac disease immunopathology. Therefore, in this study we investigated the correlation between serum level of IL-15 and abnormal histopathology degrees in Iranian patients with celiac disease.
**Methods:** Serum samples were collected from 51 confirmed CD patients including 12 patients with Marsh I, 14 with Marsh II and 25 with Marsh III during 2014. Serum concentrations of IL-15 determined by enzyme-linked immunosorbent assay (eBioscience, Affymetrix, Inc). Demographic and clinical data were collected by questionnaire and the result were analyzed using SPSS version 21.
**Results:** The mean age of subjects in this study was 38.16 years. The mean serum concentration of interleukin 15 in patients with Marsh I was 102.738, March II 482.526 and in March III 344.288. The serum concentration of IL-15 showed the greatest increase in March II but were not significantly different between three groups. The serum concentration of IL-15 showed the greatest increase in March II but were not significantly different between three groups.
**Conclusion:** Our results indicate that there are no significant correlate between serum concentration of IL-15 and abnormal histopathology degrees. These findings provide new insights into the IL-15 signaling pathway and the pathogenesis of celiac disease.

**Keywords**: Celiac disease, IL-15, Marsh classification

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster Discussion\8352
**8352**

# Comparison of Dextran Sodium Sulfate Different Doses effects in the Induction of Chronic Colitis in C57BL /6 Mice

Maryam Heidari [1], Seyed Mahmoud Hashemi[1,4], Sedigheh Pouya[1], Kaveh Baghaei[2], Mohammad Reza Zali[3]

*1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Basic and Molecular Epidemiology of Gastrointestinal Disorder Research Center, Research Institute for Gastroenterology and Liver Disease, Shahid Beheshti University of Medical Science, Tehran, Iran*
*3. Gastroenterology and Liver Disease Research Center, Research Institute for Gastroenterology and Liver Disease, Shahid Beheshti University of Medical Science, Tehran,Iran*
*4. Department of Applied Cell Sciences, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background**: inflammatory bowel disease (IBD) is a multifactorial disease in two general title of Crohn's disease and ulcerative colitis. Animal models have provided extensive tools for identifying the etiology of the disease. Several methods, including the use of chemical compounds, have been introduced to induce experimental colitis. In this study, in order to find an optimal dose of dextran sodium sulfate (DSS), C57BL /6 mice strain were used to induce colitis and the severity of clinical sign and survival, caused by different doses of 1.5, 2 and 3% DSS, were evaluated.
**Methods**: In order to induce chronic colitis, the DSS solution administered in drinking water of mice for 4 days and continued with 7 days of normal water intake. This process repeated in 3 cycles. Clinical symptoms of colitis in mice included, body weight loss, bleeding, diarrhea and stool consistency were daily monitored. The mice sacrificed on day 34 and the colon changes were analyzed.
**Results**: In 3% DSS receiving-group, a high incidence of mortality was observed with severe clinical symptoms, but no significant changes were recorded in the group which received 1.5% DSS in comparison to healthy control. Using 2%, DSS resulted in induction of colitis with moderate symptoms and no mortality in mice.
**Conclusion**: According to the results, using 2% DSS, can be suggested as an optimal dose for induction of colitis in mice.

**Keywords:** Inflammatory bowel disease, Animal model, Experimental colitis, Dextran sodium sulfate.

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster Discussion\8363
**8363**

# Association of TNFα and IL1β Serum Levels, the Presence of Helicobacter and Peptic Ulcer in the Population of Northern Iran

Mehdi Tourani, Maryam Habibzadeh, Javad Shokri Shirvani, Hamidreza Nouri

*1. Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran*
*2. Department of Internal Medicine, Babol University of Medical Sciences, Babol, Iran*
*3. Department of Immunology, School of Medicine, Babol University of Medical Sciences, Babol, Iran*

**Background**: Peptic ulcer is a major cause of death all around the globe. Inflammatory cytokine such as TNFα and IL1β is one of the most important immune response to gastric infection. Regarding the significance of these cytokines in peptic ulcer and high prevalence of the disease in northern Iran, the relationship of TNF-α and IL-1β with peptic ulcer and presence of H. pylori was conducted by measuring the serum level of said cytokines.
**Methods:** Subjects were divided into two groups of 50 people with ulcers and without ulcers. Blood and biopsy samples were taken from each person. Each group were measured primarily in terms of the presence of H.Pylori by measuring the serum level and the presence in tissue. Then both groups were compared in terms of serum levels of IL1β and TNFα using standard ELISA kit.
**Results**: 56% of the subjects were infected with H. pylori. The infection was significantly higher in PUDs in comparison to NPUDs. The mean serum level of TNF-α in PUDs and H.Pylori+ subjects was higher than that of NPUDs and H.Pylori-, respectively. However, no significant difference of IL1β serum level was observed between PUDs and NPUDs as well as between H.Pylori positive and negative.
**Conclusion**: the findings of this research indicate that increase of the TNF-α cytokine is an important risk factor in intensification of peptic ulcer.

**Keywords:** TNF-a, IL1β, Helicobacter Pylori

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster Discussion\8505
**8505**

## Evaluation of the Expression of CXCR2 and CXCR6 in Animal Models of Ulcerative Colitis Induced by Acetic Acid

Poorya foroutan pajoohian[1*], Parinaz mahdavi[1], Mohammad Amin Boshagh[1], Ali Jalili[2], Shohreh Fakhari[2]

1. *Student Research Committee, Kurdistan University of Medical Sciences, Sanandaj, Iran*
2. *Department of Immunology, Faculty of medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran*

**Background:** The IBD (Inflammatory Bowel Disease) is classified into two types, which include Ulcerative Colitis (UC) and Crohn's Disease (CD). The pathology of these diseases suggests the prevalence of neutrophil infiltration in ulcerative colitis and the dominant infiltration of mononuclear cells in Crohn's disease. CXCRs are a series of chemokine receptors which are expressed on the surface of leukocytes. Various types of these receptors have been identified, which are categorized nearly from CXCR1 to CXCR7, Each is attached to a specific chemokine and goes to the inflammation site in a specific tissue. Therefore, the aim of this study was to evaluate the expression of CXCR2 and CXCR6 in animal models of ulcerative colitis induced by acetic acid.
**Methods:** In this experimental study, 6 male Wistar rats were randomly divided into two groups: control and treated groups. In the treated group, colitis was induced by rectal injection of acetic acid. After 48hours, the animal was anesthetized and then the mucosa samples of its Colon were separated in order to consider the histopathological indices and Polymerase Chain Reaction.
**Results:** based on PCR results the expression of these chemokine receptors in the treated group has changed in comparison with the control group. CXCR2 up regulated but CXCR6 down regulated in treated group in comparison with the control group.
**Conclusion:** According to the information obtained from this study, the likely expression of these receptors in the animal model of the ulcerative colitis has changed. Given the fact that this study is part of my thesis, the final confirmation, and publication of exact information, require more samples, as well as quantitative techniques such as Real-Time PCR, as well as verification methods such as Immunohistochemistry. Finally, in the current study, we intend to evaluate the expression of the whole CXCRs in this model.

**Keywords**: Inflammatory bowel disease, Ulcerative colitis, Chemokine receptors, Acetic acid

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster Discussion\9925
**9925**

## Evaluation of miRNA-146a and miRNA-193a Expression in Gastric Cancer cell lines

Amir Baghbanzadeh[1,2], Elham Baghbani[2], Saeed noorolyai[1,2], Ahad Mokhtarzadeh[2,1], Behzad Baradaran*[2]

1. *Department of Biochemistry, Higher Education Institute of Rab-Rashid, Tabriz, Iran*
2. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Gastric cancer is the second most popular reason of malignancy dependent death through the world. MicroRNA is short non-coding RNA with 18 to 22 bp. Many studies evidenced that miRNAs such as miRNA-146a & miRNA-193a, have an important role in gastric cancer development. The miRNA-146a and miRNA-193a act as tumor suppressor in gastric cells and the evidence demonstrated that these miRNAs act as oncomir in gastric cancer cells. In this study, we evaluated the expression of miRNA-146a & miRNA-193a in three cell lines: KATO III, MKN45 and AGS.
**Methods:** in this study, KATO III, MKN45 and AGS cell lines were cultured in RPMI 1640 with 10% FBS and 5% of $CO_2$ condition. Total RNA was extracted by Trizol kit and cDNA was synthesized. Real-time PCR was used to measure changes in the expression of miRNA-146a & miRNA-193a genes. We used U6-miRNA as an internal control.
**Results:** Real-time PCR data indicated that the expression level of both miRNA-146a & 193a in KATO III is less than two other cell lines. The expression of miRNA-146a in AGS cell line is less than MKN45. The expression of miRNA-193a in MKN45 cell line was shown low level compared to AGS.
**Conclusion:** according to our finding, the expression of following miRNAs in KATO III is less than other cell lines.

**Keywords**: gastric cancer, microRNA, migration, replacement therapy

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster Discussion\9930
**9930**

# Evaluation of TLR 8 mRNA Expression in Peripheral Blood Mononuclear Cells and Association with Clinical Outcome of IBD Patients

Vahid Chaleshi[1], Mahyar Nourian[1], Hamid Asadzadeh Aghdaei[1], Shabnam Shahrokh[1], Mohammad Reza Zali[2].

*1. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Ulcerative colitis (UC) and Crohn's disease (CD) are two major type of inflammatory bowel disease (IBD). Toll-like receptors (TLRs) play a critical role in the innate immune response to invading pathogens by sensing microorganism. Toll like receptors family are a member of immune system, which could identify a wide range of microorganism. The aim of present study was to evaluate expression of TLR8 between both groups of patients in different phases called remission and flare up and comparison of these by control group.
**Methods:** The present study 42 patients with UC, 6 patients with CD and 20 controls were included. RNA extraction from PBMC and reverse transcriptase PCR used. To evaluate the expression of target gene, qRT-PCR method was applied. Statistical analysis was performed using One-way ANOVA, Graph the chi-square test.
**Results:** The assessment of expression of TLR8 in CD and UC patients and control groups exposed no difference (P=0.4936). In addition, comparison of flare-up and remission groups with controls illustrate no significant change (P=0.5419). Likewise, no correlation exists in expression of TLR8 and five different groups of drugs which involved in the present study (P=0.3703).
**Conclusion:** Expression of TLR8 gene in IBD has not any significant relationship with control, so we cannot consider this as a biomarker for prognosis of IBD.

**Keywords:** Inflammatory Bowel Diseases, Crohn Disease, Colitis, Ulcerative, Gene Expression, Toll-Like Receptors.

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster Discussion\9942
**9942**

## Evaluation of KCNQ1 Level in CRC Tumors and Its Association with Clinical Features

Elaheh azizi[1], Sedigheh Arbabian[2,] Ehsan Nazemolhosseini Mojarad[3]

*1. Department of cellular and molecular, North Tehran Branch, Islamic Azad University, Tehran, Iran*
*2. Department of cellular and molecular, North Tehran Branch, Islamic Azad University, Tehran, Iran*
*3. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Colorectal cancer (CRC) is the third known cancer worldwide that has been mostly diagnosed at advanced stages. Therefore, finding new biomarkers with marked sensitivity and specificity is matter of urgent. Studies show that alteration ofKCNQ1expression pattern is effective in the initiation and progression of tumorigenesis. Therefore, we evaluated the level of this lncRNA within the CRC patients.
**Methods:** 52 tumor tissue samples and 10 normal controls were collected along with from the cases that had been diagnosed and approved by the Taleghani Hospital, Tehran, Iran. Samples were subjected to RNA isolation and then reverse transcript. The obtained cDNAs were amplified by Real time-PCR method. Relative expression abundances of KCNQ1were measured by normalizing to Ribosomal 18S RNA using the $2^{-\Delta\Delta CT}$ method.
**Results:** The data showed a in the expression pattern of KCNQ1within the tumor samples compared to the controls. These observations were statistically associated with patient TNM stage, tumor location and differentiation.
**Conclusion:** Taking together, increasing of the KCNQ1provokes tumorigenesis in CRC patients. So, this lncRNA could be applied as diagnosis biomarker for cancer detection.

**Keywords**: KCNQ1, Colorectal cancer, Diagnosis biomarker, Tumorigenesis

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster Discussion\9958
**9958**

# The Relationship between the Expression of PTENP1 LncRNA in People with Colorectal Cancer and its Clinical Relevance

Niloofar Sadat Ebrahimi[1], Ehsan Nazemalhosseini Mojarad[2]

1. *Tehran shomal University,Tehran,Iran*
2. *Department of Pathology, Shohada Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
3. *Gastroenterology and Liver Disease Research center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
4. *Tehran shomal university,Tehran,Iran*

**Background:** Colorectal Cancer (CRC) is the third most frequent neoplasm worldwide, that being more frequent in developed countries than in developing countries. Long non-coding RNAs (lncRNAs) are defined as transcripts longer than 200 nucleotides that are not translated into protein. Among them, PTENP1 is considered as a marked tumor suppressor, which decrease cancer progression by increasing PTEN stability and PI3K/Akt signaling pathway depression.
**Methods:** 52 fresh tumor tissues plus 10 normal controls were obtained from the Taleghani Hospital, Tehran, Iran. The RNA were isolated/purified and reverse transcribed. The yielded cDNAs were assessed using qPCR technology. Relative expression abundances of targets were determined by normalizing to Ribosomal 18S RNA using the $2^{-\Delta\Delta CT}$ method.
**Results:** The data showed that PTENP1 was markedly downregulated in tumors comparing to normal samples. This decrement was statistically associated with patient TNM stage, tumor location and differentiation.
**Conclusion:** The alteration in the expression pattern of PTENP1 is assessable during CRC development, and therefore could be used as diagnosis biomarker for cancer detection.

**Keywords:** Colorectal cancer, Long none coding RNAs, PTENP1, Tumor suppressor

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster Discussion\9996
**9996**

## Evaluation of *lncRNA IFNG-AS1* Expression Level with Clinical Outcome of IBD Patients

Fariba Rashid[1], Vahid Chaleshi[2], Gholamreza Javadi[1], Hamid Asadzadeh Aghdaei[2]

*1. Department of Biology, Islamic Azad University, Science and Research Branch, Tehran, Iran*
*2. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background**: Inflammatory bowel disease (IBD) causes chronic and relapsing gastrointestinal inflammation that classified into Crohn's disease and ulcerative colitis. lncRNAs that can affect the expression of gene products. LncRNAs are a family of non-coding RNAs that dysregulation in their expression involves the pathogenesis of IBD. In this study, the expression of *lncRNA IFNG-AS1* was examined.
**Methods**: In this case-control study, 15 Crohn's patients, 20 ulcerative colitis and 20 controls who referred to the Liver and Gastro intestinal Research Institute RNA isolated from tissue samples using by AllPrep DNA/RNA/miRNA Universal kit (QIAGEN). cDNA was synthesized using by Thermo Scientific RevertAid RT Kit. Quantitative Real Time PCR (qRT-PCR) was used to evaluate the expression of the lncRNA *IFNG-AS1* gene *(*BioFact™ 2X Real-Time PCR Smart mix Sybergreen-Rotator gene Q MDX QIAGEN).
**Results**: Based on the findings, there was a significant difference between the CD, UC and control groups (P value = 0.0017). Also, significant difference between *IFNG-AS1* gene expression and phases of disease (Remission, Flare up) and control groups was absorbed (p value = 0.0028). However, expression level of IFNG-AS1 in male and female groups have been assessed and there were no significant differences (P>0.05).
**Conclusion**: According to our result level of *IFNG-AS1* gene expression may be considered as a prognostic factor in the susceptibility to inflammatory bowel disease and also may be as a biomarker to distinguish between the CD and UC group.

**Keywords :**Inflammatory bowel disease (IBD), *IFNG-AS1*, Crohn's disease, ulcerative colitis, LncRNA

# Nanoimmunology

# Poster Discussion

ICIA2018\Nanoimmunology\Poster Discussion\6321

**6321**

# Synthesis and Characterization of Novel Nickel-Salen Supported Paramagnetic Nanoparticles for 6×His-tag Recombinant Protein Affinity Purification

Zahra Rashid[1], Ramin Ghahremanzadeh[1], Amir Hassan Zarnani[2, 3]

1. *Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran*
2. *Iran.Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*
3. *Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran*

**Background:** Affinity separations based on affinity tags that exploit the unique property of extremely specific biological interactions to achieve separation and purification is theoretically capable of giving robust purification, even from complex mixtures in a single process. In this research, a simple, efficient, inexpensive, rapid and high yield method for the purification of 6×histidine-tagged recombinant protein was developed.
**Methods:** Manganese ferrite magnetic nanoparticles (MNPs) were synthesized through a co-precipitation method and then they were conveniently surface modified with tetraethyl orthosilicate (TEOS) in order to prevent oxidation and form high density of hydroxyl groups. The salen ligand was then prepared from condensation reaction of salicylaldehyde and 3-aminopropyl (trimethoxy) silane (APTMS) in 1:1 molar ratio; followed by complexation with Ni (OAc) 2.4H2O. Finally, the prepared Ni (II)-salen complex conjugated to silica-coated MNPs and $MnFe_2O_4$@$SiO_2$@Ni-Salen complex nanoparticles were obtained. The prepared MNPs were used purification of 6×His-tag recombinant proteins.
**Results:** The functionalized nanoparticles were spherical with an average diameter around 70 nm. The obtained MNPs had a saturation magnetization about 54 emu/g and had super paramagnetic character. These MNPs were used efficiently to enrich recombinant histidine-tagged (His-tagged) protein-A from bacterial cell lysate. In about 45 minutes, highly pure His-tagged recombinant protein was obtained, as judged by SDS-PAGE analysis and silver staining. The amount of target protein in flow through and washing fractions was minimal denoting the high efficiency of purification process. The average capacity of the matrix was found to be high and about 180±15 mg. $g^{-1}$ (protein/ $MnFe_2O_4$@$SiO_2$@Ni-Salen complex).
**Conclusion:** Purification process with $MnFe_2O_4$@$SiO_2$@Ni-Salen complex nanoparticles is rapid, efficient, selective and whole purification can be carried out in only a single tube without the need for expensive systems.

**Keywords:** Magnetic nanoparticles, Protein purification, Immobilized metal affinity chromatography (IMAC), Histidine-tagged protein

ICIA2018\Nanoimmunology\Poster Discussion\6323
**6321**

# Fabrication of Anti-CD4 Monoclonal Antibody-conjugated Magnetic Nanoparticles for Positive Selection of Peripheral Blood T CD4+ Lymphocytes

Zahra Rashid[1], Fazel Shokri[1], Mahmood Jeddi Tehrani[2], Amir Hassan Zarnani[3,4]

1. *Department of Immunology, School of Public Health. Tehran University of Medical Sciences, Tehran, Iran*
2. *Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*
3. *Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*
4. *Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran*

**Background:** Magnetic-activated cell sorting (MACS) is a gentle and fast method for separation of various viable and functionally active cells by magnetic labeling. The present study aimed at synthesis of a novel MACS-based system for isolation of peripheral blood T CD+ lymphocytes.
**Methods:** Magnetic nanoparticles were first synthesized through a co-precipitation method from ferrous and ferric iron solutions and then SiO2 shell was coated on the magnetic core with tetraethyl orthosilicate (TEOS) through a silanization reaction to prevent oxidation, agglomeration and expansion of the density of OH groups on the surface of $Fe_3O_4$. Subsequently, $Fe_3O_4$@$SiO_2$@PMIDA magnetic nanoparticles were obtained as a result of the reaction between N-(phosphonomethyl) iminodiacetic acid (PMIDA) and $Fe_3O_4$@$SiO_2$. Anti-CD4 monoclonal antibody was conjugated to the surface of $Fe_3O_4$@$SiO_2$@PMIDA magnetic nanoparticles through covalent binding between carboxylic group activated with 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide)/ N-ydroxysuccinimide (EDC/NHS) on the particle surface and primary amine groups of the antibodies. The fabricated immunomagnetic beads were then utilized for isolation of CD4+ lymphocytes from peripheral blood.
**Results:** The generated immunomagnetic particles efficiently separated CD4+ lymphocytes from whole blood with purity of about 93±3 %, comparable to that of commercial MACS system.
**Conclusion:** The presented approach for fabrication of anti-CD4 magnetic nanoparticles is inexpensive, easy and efficient for isolation of T CD4+ lymphocytes. Based on the consistency of results, this platform might be applicable for separation of various cell population.

**Keywords:** MACS, Purification, Positive selection, Magnetic Nanoparticles, CD4

ICIA2018\Nanoimmunology\Poster Discussion\7813
**7813**

# A Nanobody-based chimeric antigen Receptor Can Function as an Anti-angiogenesis by Targeting a VEGFR2 in Solid Tumor

Fatemeh Hajari Taheri[1], Mahmoud Hassani[1, 2], Arash Arashkia[3], Zahra Sharifzadeh[1], Mehdi Behdani[4], Mohsen Abolhassani[1]

1. *Department of Immunology, Hybridoma lab, Pasteur Institute of Iran, Tehran, Iran*
2. *Department of Molecular Medicine, Tehran University of Medical Sciences (TUMS), Tehran, Iran*
3. *Department of Virology, Pasteur Institute of Iran,Tehran, Iran*
4. *Department of Molecular Medicine, Pasteur Institute of Iran, Tehran, Iran*

**Background**: Solid tumors account for more than 85% of cancer mortality. Since cancer cells able to form new blood vessels (angiogenesis), many attempts were made to prevent angiogenesis. VEGFR2 is a protein over expressed on the surface of metastatic cancer cells and is a target for immunotherapy of solid tumors. Chimeric antigen receptor (CAR)-T cells has shown promising results on solid tumors. In this study, we have developed a 3rd generation nanobody based CAR T cell targeting tumor cells.
**Methods**: The CAR-T cell was developed using VHH against VEGFR2 that is linked to IgG1 Fc domain as spacer, CD28 and CD3 ζ signaling domains, and co-stimulatory domain of OX-40. T cells were isolated from healthy donor blood and activated with CD3/CD28 beads in the presence of rIL-2 (20U/ml). Activated T cells were electroporated with the vector encoding the VEGFR2-CAR and then biological activity were evaluated *in vitro*.
**Results**: The surface expression of activation markers, CD69 and CD25 were measured upon coculturing with VEGFR2 expressing 293-KDR tumor cells (50% and 40% respectively), as compared with non-expressing HEK-293 as negative control. IL-2 and IFN-γ productions in response to VEGFR2-expressing target cells were 1200 and 900 pg/ml respectively. Cell surface expression of degranulation marker, CD107a, was evaluated on engineered T cells upon coculturing with VEGFR2 expressing 293-KDR cells (32% versus 6% of negative cells). Although the CD107a assay does not directly measured target cell lysis, it does provide an indication of the cytotoxic potential of the responding CD8+ T cells.
**Conclusion**: For the first time we constructed a nanobody-based anti-VEGFR2-CAR T cells. VHH is preferred to scFv because of its small size, low immunogenicity, enhanced stability, and penetration into the dense solid tumors. Therefore, this engineered VEGFR2-CAR T cells may be developed for the adoptive T-cell immunotherapy of solid tumors.

**Keywords**: CAR T cells, VEGFR2, Angiogenesis, Nanobody, VHH, Adoptive transfer

ICIA2018\Nanoimmunology\Poster Discussion\8516
**8516**

# Encapsulation of Tumor cell Lysate in PLGA Nanoparticles and Evaluation of Their Proliferative Activity in Mice

Sanaz Sheikhzadeh[1], Nowruz Delirezh[1], Rahim Hobbenaghi[2]

1. *Department of Microbiology, Faculty of Veterinary Medicine, Urmia University*
2. *Department of Pathology, Faculty of Veterinary Medicine, Urmia University*

**Background:** Breast cancer is the second leading cause of cancer death in women following lung cancer. Nanoparticles-based delivery systems can be used to facilitate the cancer therapy targeting immune system by enhancing the humoral and cellular immune responses. Encapsulation of tumor antigens in biodegradable nanoparticles increases its immunostimulatory capacity than when tumor antigens are given in the soluble form. Poly (lactic-co-glycolic acid) (PLGA) is one of the most developed biodegradable polymers that use for preparation of nanocapsule drugs. In this study, we assessed Nanoparticles (NPs) size, polydispersity index and capacity of PLGA NP-based delivery system on cellular proliferation in mouse model.
**Methods:** Tumor cell lysate was obtained from Four cycles of freeze-thaw of murine 4T1 mammary tumor cells and then encapsulated in to PLGA copolymers (50% lactide: 50% glycolide) by double-emulsion solvent evaporation technique. Nanoparticles were characterized in terms of average particle size and size distribution using dynamic light scattering (DLS). The mice were challenged subcutaneously with 4T1 cells. After tumor growth, mice in the experimental groups were vaccinated with free or nano-encapsulated forms of breast tumor cell lysate and animals in the control groups received PBS. One week after the last immunotherapy, mice spleens were removed for MTT assay test.
**Results:** The tumor cell lysate loaded nanoparticles size was in the range of 200-300 nm with a polydispersity index of 0.3 (n=3). A significant increase in the numbers of immunocyte was found in mice that received PLGA nano-encapsulated form of tumor cell lysate in comparison with mice that received free tumor cell lysate.
**Conclusion:** Our results demonstrated that tumor cell lysate loaded PLGA nanoparticles promote proliferation of immune cells and could be used as a potential therapeutic approach for the breast cancer treatment.

**Keywords**: PLGA nanoparticles, Cellular proliferation, Tumor cell lysate, Breast cancer

ICIA2018\Nanoimmunology\Poster Discussion\8521
**8521**

# Targeting of HMGA2 by siRNA-loaded Nanoliposome Induces Apoptosis and Metastasis in Gastrointestinal Cancer Cells

Ali Mohammadi[1, 2], Behzad Mansoori[1, 2], Behzad Baradaran[1]

1. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Considering the complex nature of gastrointestinal cancer, different methods including surgery, radiotherapy, and chemotherapy are considered for the treatment; however the explained methods couldn't effective in these patients the novel strategies including silencing of oncogenes by the safe delivery method could consider as a novel therapy in colorectal cancer. The aim of this study is to understand the silencing effect of nano-liposomes loaded HMGA2 siRNA on gastrointestinal cancers.
**Methods:** The small interfering RNA-lipoplexes prepared with DOTAP/Chol/DOPE and dissolved separately in butyl alcohol then the aqueous sucrose and siRNA solution prepared and added to butyl alcohol solution to form a monophase solution. The size, polydispersity index (PDI), and zeta potential of nano-liposomes evaluated by Zetasizer analyzer. The silencing of the HMGA2 in cancer cells were evaluated by the qRT-PCR method. The effect of liposomes on cell cytotoxicity was evaluated by MTT assay. The migratory effect of the liposomes was evaluated by wound healing assay. To evaluate if this liposome could induce apoptosis the annexin/PI assay was performed.
**Results:** The result of size, polydispersity index (PDI), and zeta potential of nano-liposomes showed the liposome has less than 350nm size and 0.67 PDI and -79 zeta potential. The result of gene silencing showed the optimum condition of HMGA2 silencing was 80 pmolHMGA2 concentration and 48 hr after treatment in each cancer cell lines. The result of MTT assay showed silencing of HMGA2 in optimal condition could reduce the viability of the cancer cell in the three cell lines. The result of apoptosis assay showed more than 50% of the cell deaths are related to the apoptosis in all three cells. The gene expression evaluation confirms the apoptosis induced by inner pathway by inducing Caspase 3 and Caspase 9 expression. Also reduction in bcl2 expression confirms the activation apoptosis pathway in the treated cancer cells. The wound healing assay showed the suppression of cancer cell migration after treatment of this nano-liposome.
**Conclusion:** The result of this study showed the nano-liposomes loaded HMGA2 siRNA could have effects in the treatment of gastrointestinal cancers and it might have potential have more study in order to develop the new agent for the treatment of gastrointestinal patient base on target therapy.

**Keywords:** HMGA2, Nano-liposome, Gastrointestinal cancer

ICIA2018\Nanoimmunology\Poster Discussion\9871
**9871**

# Preparation and Characterization of a Novel Nanobody against T-cell Immunoglobulin and Mucin-3 (TIM-3)

Vida Homayouni [1], Mazdak Ganjalikhani-hakemi [2] , Abbas Rezaei [2], Hossein Khanahmad [3], Mahdi Behdani [4], Fatemeh Kazemi Lomedasht [4]

1. *Acquired immunodeficiency research center, Isfahan University of Medical Sciences. Isfahan, Iran*
2. *Immunology Department, Faculty of Medicine, Isfahan University of Medical Sciences. Isfahan, Iran*
3. *Genetic Department, Faculty of Medicine, Isfahan University of Medical Sciences. Isfahan, Iran*
4. *Biotechnology Research Center, Biotechnology Department, Venom & Bio-therapeutics Molecules Lab, Pasteur Institute of Iran, Tehran, Iran*

**Background:** As T-cell immunoglobulin and mucin domain 3 (TIM-3) is an immune regulatory molecule. Based on the unique features of Nanobodies, we aimed to construct an anti-TIM-3 Nanobody as an appropriate tool for manipulating immune responses for future therapeutic purposes.
**Methods:** We immunized a camel with TIM-3 antigen and then, synthesized a VHH phagemid library from its B cell's transcriptome using nested PCR. Library selection against TIM-3antigen was performed in three rounds of panning. Using phage-ELISA, the most reactive colonies were selected for sub-cloning in soluble protein expression vectors. The Nanobody was purified and confirmed with a nickel-nitrilotriacetic acid (Ni-NTA) column, SDSPAGE and Western blotting.
**Results:** Specific 15kD band representing for Nanobody was observed on the gel and confirmed with Western blotting. The Nanobody showed significant specific immune-reactivity against TIM-3 with a relatively high binding affinity.
**Conclusion:** Finally, we successfully prepared a functional anti-humanTIM-3 specific Nanobody with a high affinity and an anti-proliferative activity on an AML cell line in vitro.

**Keywords:** Antibody, Nanobody, HcAbs, Phage Display, TIM-3

ICIA2018\Nanoimmunology\Poster Discussion\11071
**11071**

## The effect of Leishmania cystein peptidase A and B conjugated to PLGA on Nitric oxide production

H. Noormehr [1], A. Zavaran Hosseini [1*], S. Soudi [1]

*1. Department of Immunology, School of Medical science, University of Tarbiat Modares*

**Background:** Nanocarriers with optimized physicochemical and biological properties are taken up by cells more easily than larger molecules, so they can be successfully used as antigen delivery tool. Protein antigens have drawn a lot of attention from investigators working on leishmaniasis vaccines. Leishmania major contains many distinct cysteine peptidase genes which contain cathepsin L-like enzymes (CPA and CPB). Here, we examined the effect of CPA, CPB conjugated to PLGA nanoparticle on nitric oxide production by macrophages against L.major infection.
**Methods:** For this purpose L.major RNA were extracted and coding DNA of cpa and cpb genes were amplified by PCR method. Both cpa and cpb were sub-cloned in pET28a vector and expressed in E.coli. Produced rCPB and rCPA purified from inclusion bodies by Ni–NTA agarose affinity column. rCPA, rCPB were conjugated to PLGA nanoparticle. Morphology and Conjugation efficacy rCPA/ rCPB-PLGA were assessed by SEM and FTIR, respectively. 5 microgram of protein content of rCPA,rCPB,and teir conjugated form were injected intra-peritoneal to four different BALB/c mice groups respectively. After 24hours, NO production of macrophages were measured in response to 10:1 L.major infection.
**Results:** No morphologically, difference were seen between CPB/ CPA – PLGA with non-conjugated form. CPA and CPB conjugation to PLGA were confirmed by FTIR method. Internalization of CPB/ CPA – PLGA were confirmed by FITC labeling of antigen. Both CPB/ CPA – PLGA treated groups showed a significant increase in the amount of NO production by macrophages in response to L.major compared with control groups.
**Conclusion:** Our results proposed that PLGA nanoparticles may be a promising adjuvant in the development of therapeutic leishmania vaccines. It seems that activated macrophages with nanoparticles lead to increase NO production resulting in inhibiting of leishmania major growth.

**Keywords:** L.major, cysteine peptidase A, B, PLGA, NO.

# Oral Immunology

# Poster Discussion

ICIA2018\Oral Immunology\Poster Discussion\8178
**8178**

# Evaluation of RANKL/OPG in Chronic Periodontitis Patients after Nonsurgical Therapy

Abbas Azadmehr[1], Zahra Alizade Tabari[2], Niloufar Sadat Miri[1], Mohammad Amir Alizade Tabrizi[2]

1. *Department of Immunology, Babol University of Medical Sciences, Babol, Iran*
2. *Department of Periodontics, Dental School, Qazvin University of Medical Sciences, Qazvin, Iran*

**Background**: Periodontitis (PD) is an inflammatory disease that involves progressive loss of the alveolar bone around the teeth. The receptor activator of nuclear factor kappa B (RANK)/RANK ligand (RANKL)/ osteoprotegerin (OPG) system signaling pathway is implicated in bone resorption through its key function in osteoclast differentiation and inflammatory response. The aim of the present study was to determine the alternation salivary concentration of sRANKL /OPG ratio in response to nonsurgical therapy.
**Methods**: Twenty-five patients with chronic periodontitis were selected based on clinical evaluation and twenty-five practically healthy volunteers selected as control group. Saliva samples were collected from patients and control group at first session and then the samples were recollected in periodontitis group four weeks after non-surgical periodontal therapy in order to measure the level of sRANKL and OPG by ELISA method.
**Results**: There was a significant difference between sRANKL and sRANKL/OPG concentration in patients before treatment and healthy group (Respectively, P=0.004, P=0.001). Also, the concentration of sRANKL and sRANKL/OPG in patients was significantly reduced as a result of treatment (Respectively, P=0.012, P=0.011). However, there was no significant difference between OPG concentration in healthy and patient groups (P=0.455).
**Conclusion**: As the ratio of sRANKL/OPG in saliva has a diagnostic value for chronic periodontitis, it seems to be clinically a good predictor of successful treatment outcomes. However, to validate these results, future studies with more samples are required.

**Keywords**: Saliva, Chronic Periodontitis, sRANKL , OPG

ICIA2018\Oral Immunology\Poster Discussion\8311
**8311**

## Expression of NLRP3 in Aggressive Periodontitis

Shideh Mehrmofakham[1]*, Mandana Sattari[1], Mohammadreza Talebi Ardakani[1], Sarira Shahnavaz[1], Aram Mohammmadi[1]

*1. Shahid Beheshti University of Medical Sciences, Tehran, IRAN.*

**Background:** Periodontal diseases are poly microbial infections resulting in bone destruction and tooth loss. Host's response to these infections includes both mechanisms of innate immune responses and acquired immune responses. Bacteria and especially gram-negative bacteria play an important role in prevalence of periodontal diseases. Also, it is determined that some periodontopathic bacteria make inflammatory cells to express NLRP3 which is one of the most important parts of inflammasome. According to this, the current research tries to survey the expression of NLRP3 in generalized aggressive periodontitis.
**Methods:** Gingiva tissue samples were collected from 20 people having clinically healthy gingiva and 25 people having aggressive periodontitis. After RNA extraction and cDNA preparation from each sample the amount of expression of NLRP3 was surveyed by the Real-time PCR technique.
**Results:** Expression of NLRP3 in tissues with aggressive periodontitis was significantly higher than the expression of NLRP3 in healthy gingiva (P≈ 0.034), but we could not find any significant correlation between expression of NLRP3 and clinical parameters.
**Conclusion:** It is concluded that in aggressive periodontitis the increased expression and production of NLRP3 is observed which results in inducing release of inflammatory cytokines and aggravating inflammation in the periodontal tissues.

**Keywords**: NLRP3, Aggressive Periodontitis, Poly Microbial Infections, Healthy Gingiva

ICIA2018\Oral Immunology\Poster Discussion\8312
**8312**

# Immunohistochemical Analysis of Visfatin Expression in Gingival Tissue of Periodontitis Patients

Niloufar Sadat Miri[1], Abbas Azadmehr[1], Zahra Alizadeh Tabari[2], Baharak Qa'edi[2], Forouz Keshani[3], Marzieh Nejati Fard[2]

1. *Department of Immunology, Babol University of Medical Sciences, Babol, Iran.*
2. *Periodontics Department, Dental School, Qazvin University of Medical Sciences, Qazvin, Iran.*
3. *Torabinejad Dental Research Center, Department of Oral and Maxillofacial Pathology, School of Dentistry, Isfahan University of Medical Sciences, Iran*

**Background:** Visfatin is a pro-inflammatory cytokine that has been associated with several immunomodulation processes and there is little clinical information on the relationship between periodontopathogens and visfatin. However, studies on visfatin as a potential therapeutic target in periodontal diseases are scarce. In the present study, we analyzed the visfatin in gingival tissue of patients with generalized aggressive periodontitis.
**Methods:** 13 patients with aggressive periodontitis and 20 periodontal healthy individuals enrolled in this study based on the inclusion and exclusion criteria. Tissue samples were obtained in both groups during surgery. The degree of inflammation infiltration and visfatin expression were determined by H&E staining and Immunohistochemistry (IHC) methods respectively. The data was analyzed by SPSS software and $P<0.05$ was considered statistically significant.
**Results:** Visfatin was detectable in all samples. Visfatin levels were higher in patients with aggressive periodontitis compared to those of healthy subjects ($P= 0.00$, $P=0.037$). The relationship between inflammatory, cell infiltration and visfatin expression in patients group were statistically significant, positive and relatively strong ($P= 0.025$, $R=0.617$).
**Conclusion:** Visfatin expression increased in gingival tissue of periodontitis patients group. These results demonstrated that the high expression level of visfatin in periodontitis tissues were correlated with the degree of tissue inflammation. However, more studies with larger sample sizes are necessary to validate these findings.

**Keywords:** Aggressive periodontitis, Inflammatory Cytokine,Visfatin, Immunohistochemistry

ICIA2018\Oral Immunology\Poster Discussion\8322
**8322**

# Assessment of Non-surgical Periodontal Therapy on IL22 and S100A12 Concentration in Gingival Crevicular Fluid of Periodontal Patient

Ferial Taleghani[1], Mandana Sattari[2], Rokhsareh Sadeghi[3], Razieh Nejadi[4]

1. *Department of Periodontics, Dental School, Shahed University, Tehran,Iran.*
2. *Department of Immunology, Shahid Beheshti University of medical sciences, Tehran,Iran*
3. *Department of Periodontics, Dental School, Shahed University, Tehran,Iran.*
4. *Corresponding Author, Dental School, Shahed University, Tehran,Iran*

**Background:** Periodontitis is a destructive and progressive inflammatory condition of tooth-supporting tissues, the pathogenesis of which is attributed to several risk factors, including bacteria, the host immune responses and genetics. The present study was undertaken to evaluate the effect of phaseI periodontal treatment on the concentrations of IL-22 and S100A12 in patients with chronic periodontitis.
**Methods:** In this study, 22 patients (14 female and 8 male), with a mean age of 39 years,and moderate to severe periodontitis were sampled. phaseI periodontal treatment was rendered. The subjects were recalled after 4 weeks for collecting samples.Wilcoxon's signed-rank test was used to analyze concentration of S100 and IL22 before and after treatment with CAL and PD.
**Results:** There was an inverse correlations between the mean PD and S100 before treatment ($P<0.05$). In relation to CAL, although an inverse correlation was expected between CAL and S100 concentration, no significant correlation was found between them ($P\approx0.079$).
**Conclusion:** The periodontal tissues are weak cellular sources for the secretion of IL-22 and S100.Given the inverse correlation between the concentration of S100 and the mean PD before treatment and also the inverse and significant correlation between the concentration of S100 and the decrease in CAL and PD after treatment, a possible protective role might be considered for S100 for the incidence of periodontitis and success of phase I treatment.

**Keywords:** Chronic Periodontitis – Periodontal Phase I Treatment - S100A12 – IL22

ICIA2018\Oral Immunology\Poster Discussion\8323
**8323**

## Visfatin as an Inflammatory Marker in Periodontitis Patients

Maryam Mehri[1], Abbas Azadmehr[1], Niloufar Sadat Miri[1], Zahra Alizadeh Tabari[2], Baharak Qa'edi[2], Ali Nohekhan[2]

1. *Department of Immunology, Babol University of Medical Sciences, Babol, Iran*
2. *Periodontics Department, Dental School, Qazvin University of Medical Sciences, Qazvin, Iran*

**Background**: Visfatin is an adipocytokine that suggested as one of the periodontal inflammation regulators that increased in periodontitis and can affect patient's systemic condition .It is indicated that serum/plasma level of visfatin increase in a number of inflammatory disorders. The present study aim to comparison the salivary visfatin concentration in chronic periodontitis patients and healthy individuals.
**Methods**: saliva samples were collected at first session from 20 patients with chronic periodontitis (mean age: 38.45±9.98) and 20 healthy individuals (mean age 33.85±6.84) as control group which selected based on clinical evaluation. Then four weeks after non-surgical therapy, saliva sample were recollected from patients group in order to assay the visfatin by enzyme linked immunosorbent assay method (ELISA).
**Results:** our results showed, visfatin concentration mean in patients with chronic periodontitis before non-surgical therapy (33.43ng/ml±15.72ng/ml)were significantly higher than control group (23.38ng/ml±7.85ng/ml) (p=0.015) and decrease after therapy (23.00ng/ml±9.75ng/ml) (p=0.003).The level of visfatin in patient and control groups were same after non-surgical therapy.
**Conclusions:** Our findings indicated that salivary visfatin concentration decrease after non-surgical periodontal therapy. Also visfatin value may be consider as an "inflammatory marker "and can be detect in future as a potential therapeutic target in treatment of chronic periodontitis.

**Keywords:** Visfatin, Chronic Periodontitis, Non-surgical periodontal therapy, Biomarker, Saliva

ICIA2018\Oral Immunology\Poster Discussion\8360
**8360**

# Association between gene expression of TRPV1 (Transient receptor potential vanilloid1) and IL8 (CXCL8) in pulp with superficial caries

Shaghayegh Pezeshki Naraghi[1], Mandana Sattari[1]

*1. Department of Immunology, School of medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** In view of the importance of pulpitis pathobiology and in order to assist innovation in its treatment methods, the present study focuses on investigating the role of superficial caries by expressing TRPV1(Transient Receptor Potential Vanilloid1) and IL-8 (CXCL8) genes.
**Methods:** The research is carried out by designing a cohort study on the pulp of 50 extracted premolar teeth divided into 2 groups as healthy (control) and surface caries. After extracting pulp and homogenizing the samples through quantitative real time PCR, the extent of gene expression for TRPV1 and IL-8 is separately calculated. Afterwards data gathered from each group is compared with case groups and with each other in pairs.
**Results and Conclusion:** Carrying out ANOVA test on 2 study groups in view of CXCL8 gene we obtain significant results (P≈0.001). Upon conducting Post Hoc Tukey test it becomes evident that there is a significant difference between healthy teeth and superficial caries groups (P≈0.001) and moderate caries & deeply complicated caries (P≈0.012) in such a way that in both cases the highest expression of CXCL8 gene is related to caries with involvement of the pulp.

**Keywords**: CXCL-8, TRPV-1, superficial caries

ICIA2018\Oral Immunology\Poster Discussion\8397
**8397**

# RANKL Expression and Gingivitis

Mandana Sattari[1], Niusha Amiri[1], Nikoo Hossein Khan Nazer[1]* Mahdi Samani[1], Shideh Mehrmofakham[1]

*1. Department of Immunology, School of medicine, University of Shahid Beheshti*

**Background:** The tissue destruction found in periodontal disease results, for the most part, from actions of the immune system and related effector mechanisms. Although periodontal disease is initiated and sustained by microorganisms, especially bacteria in dental plaque, the progression and severity of tissue destruction are mostly caused by immune host defense mechanisms to infecting bacteria. Immune responses have a crucial role in the pathogenesis and tissue destruction associated with infectious disease. In spite of the existing evidence for the association of periodontitis with RANKL/OPG ratio, reports of the roles of RANKL in the pathogenesis of this disease are still controversial. So the present study was undertaken to assess the expression of RANKL in gingival tissue of patients with gingivitis.
**Methods:** Gingival biopsies were obtained from patients with gingivitis (n=17), as well as healthy subjects (n=15). RANKL expression were determined by using Real- time PCR (SYBR green Assay).GAPDH gene was used as housekeeping gene.
**Results and Conclusion:** We found that RANKL expression was significantly higher in the inflamed periodontal tissues (Gingivitis) compared to healthy controls. Furthermore, there was a positive correlation between RANKL and clinical attachment loss ($P<0.05$).

**Keywords:** Gingivitis, RANKL

ICIA2018\Oral Immunology\Poster Discussion\8555
**8555**

# The Effectiveness of Acupuncture on Addiction Severity Index, a single case experimental design in a Case of Methamphetamine Abuser Patient with Trismus Syndrome

Bijan Pirnia[1], Dr Kambiz Pirnia[2], Zohreh Mortezaei[3]

1. *Department of Psychology, Faculty of Humanities, University of Science and Culture, Tehran, Iran*
2. *Internal disease specialist, Technical Assistant in Bijan Center for Substance Abuse Treatment, Tehran, Iran*
3. *Payam Noor University of Dubai, UAE.*

**Background:** Trismus is one of the side effects of methamphetamine using, which is associated with contractions of the jaw muscles. Acupuncture is a traditional and complementary treatment that is effective on pain and psychological indices. The purpose of this study was to investigate the effectiveness of the acupuncture in a case of trismus caused by methamphetamine abusing.
**Methods:** The patient was a 31-year-old man with a history of chronic methamphetamine use, reported to have dependence and severe jaw pain. In a single case study and in an ABAB design with multi baselines, we used acupuncture for three weeks to reduce pain and addiction severity index. Data were analyzed through generalized estimating equation.
**Results:** The results showed that there was no significant correlation between severity of addiction and pain ($\alpha ll > 0.05$).
**Conclusion:** Due to damage in the process of production of tyrosine hydroxylase and mitogen-activated protein kinase and the role of these precursors in the production of dopamine as an effective factor in the acupuncture, the effectiveness of this treatment can be limited.

**Keywords**: Acupuncture, Stimulus, Trismus, Addiction

ICIA2018\Oral Immunology\Poster Discussion\8562
**8562**

## Does TLR4 and Its Co-receptor Have Important Role in Pathogenesis of Aggressive Periodontitis?

Hediyeh Ghalikhani[1], Sarira Shahnavaz[1], Aram Mohammadi[1], Mandana Sattari[1], Shideh Mehrmofakham[1]

*1. Department of Immunology School of medicine Shahid Beheshti University of medical sciences*

**Background:** Periodontal diseases are poly-microbial infections that cause bone destruction and tooth loss. Host responses to these infections involving both innate and adoptive immune responses. Moesin plays role in the attachment of cytoskeleton to the cell membrane. This protein has a variety of tasks, such as a new co-receptor for TLR4. It was also shown that in the case of moesin deficiency, the incidence of inflammatory responses to LPS fell three times. Regarding to the role of moesin in recognition of LPS by TLR4 and since there is not enough information about the role of moesin in periodontal disease, the aim of this study was to evaluate the relationship between moesin and TLR4 expression and aggressive periodontitis disease.
**Methods:** 25 samples for aggressive and 25 samples for healthy group 20 samples were collected (totally 50 gingival samples). After extracting the RNA, cDNA prepared from all of samples and for determining of the quality of the cDNA, PCR amplification was performed. Then Beta actin (Housekeeping gene) and Moesin and TLR4 gene expression was investigated by Real-time PCR technique.
**Results:** There was a significant lower expression of moesin in aggressive periodontitis compared to control group (P≈0.025). There was also a significant lower expression of TLR4 in aggressive periodontitis group compared to control group (P≈0.013).
**Conclusion**: It is concluded that recognition of periopathogens by TLR4 and moesin does not have an important role in aggressive periodontitis.

**Keywords:** Aggressive Periodontitis, TLR4, Moesin

# Psychoneuroimmunology and Immunoendocrinology

# Poster Discussion

ICIA2018\Psychoneuroimmunology and Immunoendocrinology\Poster Discussion\7619
**7619**

## Evaluation of Jak1, T-bet and Foxp3 Gene Expression in Major Depression Disorder

Marzie Askari [1,2], Mohammad Haghighi [3], Iraj Salehi [4], Zohreh Zareighane [1], Reza Shahbazi [3], Alireza Zamani [1], Ghasem Solgi [1], Mahdi Alahgholi Hajibehzad [1,*]

1. *Department of Immunology, Hamadan University of Medical Sciences, Hamadan, Iran*
2. *Student Research Committee, Hamadan University of Medical Sciences, Hamadan, Iran*
3. *Research Center for Behavioral Disorders and Substance Abuse, Hamadan University of Medical Sciences, Hamadan, Iran*
4. *Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran*

**Background:** Major depression disorder (MDD) is a psychiatric illness that is associated with the immune system. The inflammation play a major role in development of MDD. The purpose of this study was the evaluation of Jak1, T-bet and Foxp3 gene expression in patients with MDD compare to healthy control subsets (HC).
**Methods:** Blood samples were collected from 40 MDD patients (mean age 38.5±13.2) and 30 (40.5±12) HCs. Twenty patients were taken Selective Serotonin Reuptake Inhibitor ($SSRI^+$) as a medication and 20 of MDD patients were new case ($SSRI^-$). Total RNA was isolated from PBMC (Peripheral blood mononuclear cells) of both patients and HCs and cDNA was synthesized using reverse transcriptase kit. Expression of genes was performed with the SYBR green Real Time PCR method. The relative genes expression level for each sample were calculated by ΔΔCt method.
**Results:** The relative level of Foxp3 was significantly increased in $SSRI^+$ compared to $SSRI^-$ group, whereas there was no significant difference in the Jak1 and T-bet expression. No significant difference was observed between HCs and $SSRI^+$ / $SSRI^-$ groups separately in the expression level of T-bet, JAK1 and Foxp3. T-bet gene expression was slightly decreased in patients compered to HCs, whereas there were no significant changes in Foxp3 and Jak1 expression.
**Conclusion:** This result show a changed level of T-bet, JAK1 and Foxp3 expression in MDD, which could contribute to the pathogenesis of the disease.

**Keywords:** MDD, Jak1, T-bet, Foxp3

ICIA2018\Psychoneuroimmunology and Immunoendocrinology\Poster Discussion\7905
**7905**

# Distribution of Dendritic Cells Subpopulations (Myeloid and Plasmacytoid) in Peripheral Blood of Hyperprolactinemic Women Compared to Normal Subjects

Fatemeh Raeesi Nejad[1] , Mohammad Mahdi Mohammadi [2], Mojgan Sanjari[3], Reza Malk poor[4] , Elham Jafari[5],Mahbubeh Zolala[6] , Mohammad Javad Abasszadeh Afshar[7]

1. *Department of Immunology, School of Medicine, Jiroft University of Medical Sciences*
2. *Department of Immunology, School of Medicine, kerman University of Medical Sciences.*
3. *Department of Endocrinology, School of Medicine, kerman University of Medical Sciences*
4. *Department of Pathology, School of Medicine, kerman University of Medical Sciences*
5. *Department of Pathology, School of Medicine, kerman University of Medical Sciences*
6. *Department of Immunology, School of Medicine, kerman University of Medical Sciences.*
7. *Department of Parasitology, School of Public Haelth,Tehran University of Medical Sciences*

**Background**: Interaction between immune and endocrine system is an inextricable two-way communication. In recent years, due to possible role of prolactin in the pathogenesis of autoimmune disorders, this hormone and its relationship with the immune system has attracted much attention. High prolactin levels in different autoimmune diseases as well as increased levels of autoantibodies in hyperprolactinemic patients are evidences for this claim. Dendritic cells subpopulations and their balance play an important role in the pathogenesis of many autoimmune diseases. The aim of the present study was to evaluate the effect of hyperprolactimenia on distribution of DCs subpopulations (myeloid and plasmacytoid) and helping to clarify the complex relationship between PRL and autoimmunity.
**Methods**: This case-control study was conducted on 70 women, including 35 hyperprolactinemic patients as case group and 35 matched healthy controls. The patients had been admitted into shahid Bahonar hospital affiliated to Kerman University of Medical Sciences. After obtaining written informed consent from all individuals, blood samples for the cytometric analysis of peripheral blood myeloid and plasmacytoid DCs subtypes were collected. Statistical analyses were carried out using SPSS v18.
**Results**: The population of myeloid and plasmacytoid DCs in hyperprolactimenia patients were decreased compared to control group. This difference was statistically significant (PpDCs=0.002, PmDCs=0.005). Also, the study found that hyperprolactimenia does not have significant effect on mDC / pDC balance in peripheral blood (P=0.928).
**Conclusion**: According to the results of this study it can be stated that increased serum prolactin reduces the number of myeloid and plasmacytoid dendritic cells in peripheral blood but does not affect their proportion and balance.
Research on the effects of this hormone on the maturity and function of these cells, as well as its effect on autoimmune diseases, seems necessary.

**Keywords**: Prolactin, Hyperprolactinemia, Dendritic Cells, Myeloid, Plasmacytoid, Flow Cytometry

ICIA2018\Psychoneuroimmunology and Immunoendocrinology\Poster Discussion\9875
**9875**

## Serum Levels of CCL5 in Type 2 Diabetic Patients with and without Nephropathy

Vahid Bagheri[1], Gholamhossein Hassanshahi [1], Hossein Khorramdelazad [1]

*1. Molecular Medicine Research Center, Rafsanjan University of Medical Sciences.*

**Background:** Type 2 diabetes (T2D) is a chronic metabolic disorder and its prevalence is increasing globally. A number of genetic and environmental factors play a role in the development of T2D. T2D is associated with microvascular complications (e.g. nephropathy) and macrovascular complications. Diabetic nephropathy is one of the main causes of chronic kidney disease and affects about 40% of diabetic patients. CCL5 (RANTES) is a member of the CC chemokine subfamily and actively participates in recruitment of leukocytes to the site of inflammation. The aim of this study was to evaluate the serum level of CCL5 in type 2 diabetic patients with and without nephropathy.
**Methods:** Peripheral blood samples were collected from 100 patients with T2D, 100 T2D patients with nephropathy, and 100 healthy controls. Ethical approval was obtained from the Ethics Committee at Rafsanjan University of Medical Sciences. Exclusion criteria included presence of inflammatory diseases, infections, and allergic conditions. Demographic and clinical features of patients and controls were also collected. CCL5 serum levels were detected using ELISA (eBioscience) in both groups immediately after blood collection.
**Results:** Our results showed that serum levels of CCL5 were significantly elevated in patients with T2D (586.37 ± 19.7 pg/ml) compared with healthy controls (502.14 ± 15.35 pg/ml) ($P < 0.004$). However, there was no significant difference in CCL5 levels between nephropathic group (547.11 ± 21.34 pg/ml) and control group ($P = 0.228$).
**Conclusion:** The results suggest that CCL5 may be involved in the pathogenesis of T2D. Moreover, CCL5 does not seem to be associated with diabetic nephropathy in patients with T2D.

**Keywords:** CCL5, Type 2 diabetes, Chemokine, Nephropathy

# Reproductive Immunology

# Poster Discussion

ICIA2018\Reproductive Immunology\Poster Discussion\6336
**6336**

# Immunomodulatory Effects of Human Amniotic Epithelial Cells on Naïve $CD_4^+$ T cells Differentiation from Women with Unexplained Recurrent Spontaneous Abortion

Hossein Motedayyen[1*], Nader Tajik[2], Amir Hassan Zarnani[2], Abbas Rezaei[1]

*1. Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
*2. Immunology Research Center (IRC), Iran University of Medical Sciences, Tehran, Iran*

**Background:** Unexplained Recurrent spontaneous abortion (URSA) is a common disorder in 1%–5% of women of reproductive age that is largely associated with the failure of feto-maternal immunologic tolerance. As human amniotic epithelial cells (hAECs) have immunomodulatory properties, we investigated immunomodulatory effects of hAECs on differentiation of naïve T cells from USRA patients.
**Methods:** Naïve $CD_4^+$ T cells were isolated from 10 patients with URSA using immunomagnetic separation method. hAECs were isolated from the amnion delivered by healthy women with uncomplicated pregnancies during elective cesarean deliveries. hAECs were co-cultured at different ratios(2:1, 1:1, 1:2, 1:5, 1:10) with naïve $CD_4^+$ T cells ($4\times 10^5$) in 24-well plates and stimulated with anti-CD3/CD28. After 6 days, the cells were collected and stained with PE anti-human CD25, PE/Cy5 anti-human CD4 and anti-human Foxp3 antibodies. Data was acquired using a FACS Calibur flow cytometer. In this experiment, naïve $CD_4^+$ T cultured alone and stimulated with anti-CD3/CD28 were considered as control.
**Results:** Our data showed that in the presence of hAECs at all ratios, there was a significant differentiation toward Tregs after 3 and 6 days of co-culture($p<0.0001$). The effect of hAECs on production of Treg cells was in a dose-dependent manner.
**Conclusion**: hAECs have the ability to induce differentiation of naïve T cells from URSA patients into T regs *in vitro*. Based on these findings, hAECs may be considered as potential candidate in immunotherapy of URSA women. However, future studies using animal model and more information are required to decide whether hAECs have clinical applications in URSA.

**Keywords:** Amniotic Epithelial Cells, Naïve T cells, Recurrent Spontaneous Abortion, Immunomodulatory effects.

ICIA2018\Reproductive Immunology\Poster Discussion\6338
**6338**

# Human Amniotic Epithelial Cells Inhibit Activation and Pro-Inflammatory Cytokines Production of Naive CD4+ T cells from Women with Unexplained Recurrent spontaneous Abortion

Hossein Motedayyen [1*], Abbas Rezaei [1], Amir-Hassan Zarnani [2], Nader Tajik [2]

1. *Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
2. *Immunology Research Center (IRC), Iran University of Medical Sciences, Tehran, Iran*

**Background:** Unexplained recurrent spontaneous abortion (URSA) has been assumed to be caused by a defect in maternal immunological tolerance to the fetus. Human amniotic epithelial cells (hAECs) have pregnancy-friendly features and the ability to modulate the innate and adoptive immune responses. This study aimed to investigate whether hAECs have immunomodulatory effects on naive CD4 + T cells from URSA patients.
**Methods:** hAECs were obtained from 15 healthy pregnant women and phenotypic profile of hAECs was determined by flow cytometry. Naive CD4+ T cells were isolated from 25 URSA patients using an immunomagnetic separation method. Naive T cells were stimulated with anti-CD3/anti-CD28 antibodies and co-cultured with different numbers of hAECs for 3 and 6 days. Immunomodulatory effect of hAECs on activation of stimulated T cell was assessed by flow cytometry and Enzyme-linked immunoasorbent assay (ELISA). The hAECs effect on pro-inflammatory cytokines production of activated T cells was also measured by ELISA.
**Results:** Our results indicated that hAECs significantly inhibited the activation of naive T cells in a dose-dependent manner ($p<0.05$-$0.0001$). They significantly reduced the production of transforming growth factor-beta1 (TGF-β1) of stimulated CD4+ T cells ($p<0.05$-$0.001$). Moreover, hAECs had potent immunomodulatory effects on the production of interferon-gama (IFN-γ) and interleukin-17A (IL-17A) of activated T cells ($p<0.01$-$0.0001$). **Conclusion:** These findings suggest that hAECs may be a suitable cell source to modulate abnormal immune responses in women with URSA.

**Keywords:** Immunomodulatory Effects, Human Amniotic Epithelial Cells, Naive T cells, Unexplained Recurrent Spontaneous Abortion

ICIA2018\Reproductive Immunology\Poster Discussion\7520
**7520**

## The Immune Profile of Peripheral Blood of Recurrent Implantation Failure Patients

Mahnaz Ghaebi [1, 2], Majid Ahmadi[1, 2], Mehdi Yousefi[1, 2]

1. *Department of Immunology, School of Medicine, Tabriz University of Medical Sciences*
2. *Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences*

**Background:** Recurrent implantation failure (RIF) is a major cause of failure of in vitro fertilization. It is defined when a woman experience failure of good quality embryos to implant following a number of IVF cycles. Immunological abnormalities may be one of the main causes of reproductive failure in RIF.
**Methods:** 20 women with RIF and 20 healthy women participated in the study. We evaluated the pre-conception level of regulatory T cells and Th17 in peripheral blood of women with RIF using flowcytometery. The expression level of genes involved in T cell responses including GATA-3, GITR, IRF-4 and T-bet was evaluated by quantitative real time PCR.
**Results:** The results showed that Treg cells were decreased in women with RIF compared with healthy women. As expected, a high level of Th17 cells was observed in patients. Expression of GATA-3 and GITR which regulate Th2 cell differentiation and are involved in Treg function, respectively, were significantly downregulated in RIFs compared with normal women. Expression of IRF-4 and T-bet were higher in women experiencing IVF failure than normal women.
**Conclusion:** The high frequency of Th17 and lower level of Tregs in peripheral blood of RIF patients compared with normal women show the critical role of Tregs in successful embryo implantation and the detrimental function of pro-inflammatory Th17 cells in this process. In addition, expression pattern of genes involved in Treg and Th2 cell responses compared with Th1 and Th17 related transcripts indicate that Th1 and Th17 cells play a detrimental role while Treg and Th2 cells are protective.

**Keywords:** Recurrent Implantation Failure, Treg, Th17.

ICIA2018\Reproductive Immunology\Poster Discussion\7527
**7527**

## Evaluation of TH17 and Treg Frequency in Preeclampsia Patients

Shadi Eghbalfard [1,2], Majid Ahmadi [1,2], Mehdi Yousefi [1,2]

1. *Department of Stem Cell and Regenerative Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background**: Preeclampsia (PE) is an immune-mediated syndrome that occur in 5-10% of all pregnancy during the second and third trimesters of pregnancy, and is generally characterized by hypertension (>140/90mmHg), proteinuria (>300mg/24 h) and maternal systemic inflammation. It has been proposed that inappropriate activation of the immune system, particularly the imbalance of Th17/Treg may be involved in the pathophysiology of PE.
**Methods:** A total of 25 pre-eclamptic patients and 25 healthy pregnant women, who were in the second or third trimesters of their pregnancies enrolled in this study. First, the peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood, and the frequency of Tregs and Th17 cells were analyzed by flowcytometery. Then, the expression level of FoxP3 and RORc genes were evaluated using real-time qPCR. In addition, ELISA was carried out to determine the levels of cytokines such as TGF-β and IL-17 in the peripheral blood of these patients.
**Results:** Results showed higher percentage of Th17 cells and lower percentage of Treg cells in patients with PE compared to healthy pregnant women ($P<0.02$ and $P<0.03$, respectively). Additionally, the expression of RORc was significantly elevated in PE patients compared with healthy group, whereas expression of FOXP3 was significantly attenuated ($P=0.02$ and $P=0.03$, respectively). As expected, a significant elevation in serum levels of IL-17 was observed in PE patients ($P<0.04$), while the opposite was the case for TGF-β ($P<0.02$).
**Conclusion:** The study indicates that a negative correlation between Treg and Th17 cells are present in women with PE, characterized by down regulation of Treg cells along with Treg cell- associated transcripts (FOXP3 and TGF-β) and up-regulation of Th17 cells and its associated factors (RORc and IL-17). It seems possible that imbalance of Th17/Treg cells and alteration in cytokines profile may break the maternal tolerance to the fetus and activate inflammatory response in PE patients.

**Keywords:** Preeclampsia, Regulatory T cell, Th17, FOXP3, Transforming growth factor-beta (TGF-beta), Interlukine-17

ICIA2018\Reproductive Immunology\Poster Discussion\7844
**7844**

# MSC Therapy Protects Fetus from Rejection in Abortion-prone Mouse Model with Involvement of Co-stimulatory and Co-inhibitory Molecules

Amir Salek Farrokhi[1], Amir Hassan Zarnani[2], Seyed Mohammad Moazzeni[1]

1. *Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
2. *Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*

**Background:** Immunological tolerance has a crucial role in the successful pregnancy since normally the maternal immune system does not reject the semi-allogeneic conceptus. Co-stimulatory and co-inhibitory molecules have essential roles in T cell activation and immune responses. Mesenchymal stem cells (MSCs) have been shown to possess broad immunoregulatory capabilities and inhibit immune cells proliferation, maturation and suppress immune reactions.
**Methods:** Abdominal fat derived MSCs from female CBA/J mice were administered intraperitoneally (i.p) to the DBA/2 mated CBA/J female mice on day 4.5 of pregnancy. On day 13.5 of pregnancy, abortion rates were calculated and CD80, CD86, CD28 and CTLA-4 gene expression in deciduas and placentas were evaluated by Real-Time PCR.
**Results:** Our results demonstrated that MSCs administration could decrease the abortion rate significantly in MSCs treated group compared to untreated group. In addition, the findings indicated a significant reduction of CD80, CD86 and CD28 as well as up-regulation of CTLA-4 gene expression following MSCs therapy in both deciduas and placenta.
**Conclusion:** Our findings demonstrated that administration of MSCs improves pregnancy outcome in abortion prone mouse model through induction of immunological tolerance by reducing the co-stimulatory molecules and increasing co-inhibitory molecules at feto-maternal interface.

**Keywords:** Mesenchymal stem cells, recurrent spontaneous abortion, Co-stimulatory molecules, Co-inhibitory molecules, Pregnancy

ICIA2018\Reproductive Immunology\Poster Discussion\7849
**7849**

# Mesenchymal Stem Cells Administration Improves Pregnancy Outcome and Induces Th2 Type Cytokines Profile in Abortion Prone Mouse Model

Amir Salek Farrokhi[1], Amir-Hassan Zarnani[2], Seyed Mohammad Moazzeni[1]

1. *Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
2. *Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*

**Background:** The imbalance of Th1/Th2 cytokines is well known in recurrent spontaneous abortion (RSA) mouse model. Mesenchymal stem cells (MSCs) possess potent immunoregulatory properties that could modulate the Th1 cytokine responses in benefit of Th2 types. In this study, we aimed to analyze the local and systemic balance of Th1/Th2 cytokines following MSCs therapy.
**Methods:** Syngeneic adipose derived MSCs were administered to abortion prone mice during the implantation window. The abortion rate was determined and IL-4, IL-6, IL-12, IL-2, IFN-γ and GM-CSF gene expression was evaluated by Real-Time-PCR in decidual and placental tissues of pregnant mice at day 13.5 of pregnancy. Splenocytes of pregnant mice were co-cultured with mitomycin C treated paternal splenocytes and IL-2, IL-4, IL-10 and IFN-γ cytokines were measured in co-cultures supernatants by ELISA method. Proliferation response of female splenocytes to paternal antigens was also evaluated using the CFSE method.
**Results:** Our results showed a significant reduction in abortion rate following MSCs administration in abortion prone mice. We also observed a significant down-regulation of IL-2 and IFN-γ as well as up-regulation of IL-4 and IL-10 production from pregnant mouse splenocytes following MSCs therapy along with a significant reduction of splenocytes proliferation against paternal antigens. Our findings revealed that MSCs therapy increased the IL-4, IL-6, IL-10 and GM-CSF and at the same time decreased the IL-12, IL-2 and IFN-γ gene expression at feto-maternal interface.
**Conclusion:** Here, we showed that MSCs therapy could modulate the systemic as well as local Th1/Th2 cytokines production along with protection of fetus from resorption in abortion prone mice. The fine balance of Th1/Th2 cytokine response could be considered as one of the possible mechanisms for fetal protection following MSCs therapy.

**Keywords:** Mesenchymal stem cells, recurrent spontaneous abortion, Th1/Th2 balance, Pregnancy

ICIA2018\Reproductive Immunology\Poster Discussion\7873
**7873**

## A Novel Sperm Protein Target in Azoospermia Men

Mohammad sadegh Soltani Zangbar[1, 2], Behrouz Gharesi fard[1, 3], Jaleh Zolghadr[3, 4]

1. *Department of Immunology, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Department of Immunology, Medical school, Tabriz University of Medical Sciences, Tabriz, Iran*
3. *Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
4. *Department of Obstetrics and Gynecology, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Infertility is one of the most important and worldwide increasing problems of couples who are in reproductive age. Male infertility has many reasons such as azoospermia, defined as complete absence of spermatozoa in the semen. Azoospermia occurs in two forms, including obstructive azoospermia (OA) and non-obstructive azoospermia. OA accounts for around 40 % of azoospermia cases. In obstructive azoospermia due to problems with the ductal system or issues with ejaculation, blood-testis barrier might breakdown and the immune system meets the sperm cells and results in producing the anti-sperm antibodies (ASA). Although the presence of the ASA is reported in 88 % of patients with OA, interestingly there is no data regarding ASA targets in OA individuals. The aim of this study was identification of sperm antibody targets in a group of obstructive azoospermic men. **Methods:** Two-dimensional gel electrophoresis (2-DE) technique was used for separation of the human sperm proteins. After separation, protein spots were transferred on PVDF membranes and blotted with pooled sera from OA patients and compared with membranes blotted with normal men sera. MALDI TOF/TOF mass spectrometry was used for identifying the desirable blotted spots. The results of mass were confirmed using RT-PCR.
**Results:** The result indicated that OA patients may produce antibody against ODF2 and other sperm proteins such as Tektin-2 and TPI1P1. Our result indicated that normal men don't produce antibody against this identified protein. The mass result of ODF2 was confirmed at RNA level by RT-PCR.
**Conclusion:** The data of the present study candidate ODF2 as a sperm protein target in OA men.

**Keywords:** Azoospermia, ODF2, 2DE, Western blot

ICIA2018\Reproductive Immunology\Poster Discussion\7973
**7973**

# Difference in Protein Expression of the Peripheral Blood CD4+ T Lymphocytes between Polycystic Ovary Syndrome (PCOS) and Healthy Women

Fatemeh Nasri[1], Fatemeh Hesam Pour[1], Behrouz Gharesi Fard[1, 2*]

1. *Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age. Although the etiopathogenesis of this syndrome is almost clear, proteome profile analysis of CD4+ T lymphocytes may represent the proteins involved in the pathogenesis of the disease. The present study aimed to compare the protein expression profiles of the peripheral blood of CD4+ T lymphocytes between PCOS patients and healthy women.
**Methods:** We used two-dimensional gel electrophoresis (2-DE) followed by mass spectrometry (MS) of selected protein spots. Moreover identified protein spots were confirmed by western blot technique**.**
**Results**: Despite the overall proteome similarities between patients and healthy women, the analysis of protein spots revealed that at least seven spots were differently expressed ($P< 0.05$). Protein identification was successfully achieved for 3 out of 7 spots by Mass technique and confirmed by western blot. All 3 identified proteins including Phosphatidyl ethanolamine-binding protein 1(PEBP1), Proteasome activator complex subunit 1(PSME1), and Triosephosphate isomerase 1(TPI) showed over-expression in PCOS patients compared with the healthy subjects. Differentially expressed proteins might be involved in oxidative processes and cardiac pathology.
**Conclusion**: In summary, this evidence highlights T lymphocytes competence as a living biosensor system to record the alteration of metabolism and gene expression and would be a good substitution for tissue biopsies in neural disorders**.**

**Keywords:** CD4+ T lymphocytes, proteomics, PCOS, mass spectrometry

ICIA2018\Reproductive Immunology\Poster Discussion\8259
**8259**

# The Effect of Mesenchymal Stem Cells Therapy on the Expression Pattern of Activating and Inhibitory Receptors on Uterine Natural killer cells in Abortion-prone Mouse Model

Fatemeh Rezaei[1], Seyed Mohammad Moazzeni[1]

*1. Immunology Department, Faculty of Medical Sciences, Tarbiat Modares Universty, Tehran, Iran*

**Background:** Uterine natural killer cells (uNKs) are the major population of immune cells in the maternal – fetal interface and play an important role in establishment and maintenance of normal pregnancy. Recurrent spontaneous abortion is one of the most common complications of pregnancy which in many cases is related to the immune cells disorders. We have shown that mesenchymal stem cells (MSCs) therapy could reduce the abortion rate in abortion prone mice. In this study we aim to evaluate the effect of MSCs therapy on the expression pattern of activating and inhibitory receptors on uNK cells.
**Methods:** MSCs were injected (IP) at day 4 of gestation to female CBA/J mice following their mating with DBA/2 male. The same mating pairs received PBS as control group and CBA/J x BALB/c mating was also used as normal pregnancy control. On day 12.5 of pregnancy embryo resorption rate was determined and decidual cells were isolated by enzymatic digestion. The expression pattern of activating and inhibitory receptors by NK cells were examined through flow cytometric analysis.
**Results:** MSCs administration dramatically decreased embryo resorption rate compared with control group. MSCs could also affect the phenotype of NK cells in uterine and changed the pattern of activating and inhibitory receptor on cell surface to a more regulatory types. **Conclusion:** This finding indicate that administration of MSCs improved the pregnancy outcome and corrected the functions and phonotype of uNK cells in abortion prone mice. However, the changes in other properties of uNK cells and other aspects of immune systems are remained to be determined and is under investigation in our laboratory.

**Keywords:** Recurrent Spontaneous Abortion, Mesenchymal stem cell, NK cells, activating and inhibitory receptors.

ICIA2018\Reproductive Immunology\Poster Discussion\8365
**8365**

**Protective Effect of Berberine against Lipopolysaccharide-Induced Abortion**

Tahereh Jalilvand[1], Hasan Namdar Ahmadabad[2], Maryam Nezafat Firizi[2]

*1. Department of Molecular Science, School of Medicine, North Khorasan University of Medical Sciences, Bojnord, Iran*
*2. Department of Pathobiology and Labratory Sciences, North Khorasan University of Medical Sciences, Bojnord , Iran*

**Background:** Berberine is an isoquinoline derivative alkaloid with anti-inflammatory activity. In present study, we investigated the protective effects of berberin in prevention of LPS-induced abortion.
**Methods:** On gestation day (GD) 9.5, the pregnant mice were injected with low, middle and high doses of berberine or with PBS. After 4 hours, berberine or PBS-pretreated mice were injected with LPS. On GD 11.5, blood samples and uterine tissues were collected from treated mice and percentage of abortion and serum levels of NO, TNF-α, IL-10 and IL12p70 determined by macroscopic examination and sandwich ELISA, respectively.
**Results:** Our findings showed that mice injected with berberine resistant to LPS-induced abortion. We also found that this treatment prevents the reduction of IL-10 and the enhancement of NO, TNF-α, and IL-12p70 in LPS-treated pregnant mice.
**Conclusion:** Taken *together, our results suggest that berberine as an anti-inflammatory agent has protective effects on LPS-induced abortion by modulation of pro- and anti-inflammatory factors.*

***Keywords:*** *Abortion, Berberine, Cytokine, Lipopolysaccharide, Nitric Oxide.*

ICIA2018\Reproductive Immunology\Poster Discussion\9905
**9905**

# Dysregulation of Fas in Endometrial, Peritoneal Fluid, and Blood Mononuclear Cells May Propose a Role for Resistance to Apoptosis in Patients with Endometriosis

Tahereh Mohammadi[1, 2], Sepide Khodaverdi[3], Nader Tajik[1, 2], Zahra Moradi[1, 2], Ali Akbar Delbandi[1, 2*]

*1. Immunology Research Center, Immunology and Infectious Disease Institute, IRAN University of Medical Sciences*
*2. Immunology Department, School of Medicine, IRAN University of Medical Sciences*
*3. Endometriosis Research Center, IRAN University of Medical Sciences*

**Background:** Endometriosis is characterized by the presence of endometrial tissues composed of stroma and glands with capacity to avoid apoptosis outside the uterine cavity. *This study aims at i*nvestigating the expression levels of Fas in *ectopic (EESCs) and eutopic (EuESCs) endometrial* stromal *cells, as well as peritoneal fluid (PFMCs) and peripheral blood mononuclear cells (PBMCs) in patients with endometriosis in comparison to non-endometriotic controls.*
***Methods**: Ectopic and eutopic endometrial tissues were taken from 29 patients with endometriosis and 10 eutopic endometrial tissues taken from 10 individuals without endometriosis. We also collected peritoneal fluid and peripheral blood from 10 endometriosis patients, as well as 10 individuals without endometriosis. Endometrial tissues were digested by enzymatic method and the endometrial stromal cells (ESCs) were characterized by flowcytometry. Peritoneal fluid and blood mononuclear cells were isolated by ficol density separation method. Gene and protein expression levels of Fas in separated cells were evaluated by Real-Time PCR and Western bloting, respectively.*
***Results**: We found that EESCs and EuESCs produced lower amounts of Fas in gene and protein levels compared to CESCs ($P<0.01$). Interestingly, the expression level of this gene was higher in PFMCs of patients than in that of the control group ($P<0.05$). In addition, the expression levels of Fas was lower in PBMCs of patients in comparison with the control group ($P<0.01$).*
***Conclusion**: It seems that lower expression of Fas in EuESCs and EESCs is one mechanisms that explain why these cells are resistant to apoptosis. Furthermore, probably the elevated expression of this gene in PFMCs of patients can describe the reasons of increasing apoptosis in the mentioned cells.*

***Keywords**: Endometriosis, Stromal cell, peritoneal fluid mononuclear cells, Fas*

ICIA2018\Reproductive Immunology\Poster Discussion\9951
**9951**

## 1, 25-Dihydroxy Vitamin D3 reduced HGF Production in Endometriosis

Sahel Heydari[1, 2], Sepideh Khodaverdi[3], Nader Tajik[1, 2], Ali Akbar Delbandi[1, 2]

*1. Immunology Research Center, Immunology and Infectious Disease Institute, IRAN University of Medical Sciences*
*2. Immunology Department, School of Medicine, IRAN University of Medical Sciences*
*3. Endometriosis Research Center, IRAN University of Medical Sciences*

**Background:** Endometriosis is an inflammatory disease characterized by the presence and growth of endometrial tissue outside the uterine cavity. HGF plays an important role in the proliferation of ectopic endometrial tissues in endometriosis. In this study, we examined the production levels of HGF by peritoneal (PFMCs) and peripheral blood (PBMCs) mononuclear cells, as well as Ectopic (EESCs) and Eutopic (EuESCs) stromal cells from patients with endometriosis compared to nonendometriotic controls. Moreover, the effects of vitamin D3 on the production of HGF in mentioned cells were investigated.
**Methods:** EESCs, EuESCs, PBMCs, and PFMCs were isolated from 10 laparoscopically-confirmed endometriotic patients. Furthermore, 10 separated CESCs, PBMCs, and PFMCs from 10 non-endometriotic women were enrolled as controls. The gene and protein expression levels of HGF were measured in all cell types by Real-time PCR and ELISA, respectively. For determining the effect of vitamin D3 on HGF expression levels, the cells were treated with the optimized concentration of 1, 25-Dihydroxy Vitamin D3. Then, the gene and protein expression levels of HGF were measured by Real-time PCR and ELISA, respectively.
**Results:** The gene and protein expression levels of HGF were higher in EESCs and PFMCs in endometriosis patients compared to nonendometriotic controls ($P<0.05$ and $P<0.01$, respectively). We found that vitamin D3 could decrease the HGF gene ($P<0.05$) and protein expression levels ($P<0.01$) in PFMCs, EuESCs and EESCs in endometriotic patients. However, this vitamin, has no effect on control group. Treatment of PBMCs with vitamin D3, caused a significant decreases in the HGF protein level in both patients and controls ($P<0.05$).
**Conclusion:** These results showed HGF is contributing to the pathogenesis of the endometriosis. In addition, these findings propose the unique therapeutic potential for 1, 25-Dihydroxy Vitamin D3 for endometriosis treatment.

**Keywords:** Endometriosis; Vitamin D3; HGF; Stromal cell

ICIA2018\Reproductive Immunology\Poster Discussion\9961
**9961**

# 1, 25-Dihydroxy Vitamin D3 Regulate Apoptosis in Endometrial Stromal and Peritoneal Fluid Cells in Endometriosis

Tahereh Mohammadi[1, 2], Sepide Khodaverdi[3], Nader Tajik[1, 2], Zahra Moradi[1,2], Ali Akbar Delbandi[1,2]

*1. Immunology Research Center, Immunology and Infectious Disease Institute, IRAN University of Medical Sciences*
*2. Immunology Department, School of Medicine, IRAN University of Medical Sciences*
*3. Endometriosis Research Center, IRAN University of Medical Sciences*

**Background:** Endometriosis is a chronic inflammatory disease characterized by the dysregulated apoptosis in ectopic endometrial cells. The objective of this study was to investigate the effects of 1, 25-Dihydroxy vitamin D3 on FasL expression in Ectopic (EESCs) and eutopic (EuESCs) endometrial stromal and peritoneal fluid mononuclear cells in patients with endometriosis compared to endometrial stromal cells (CESCs) and PFMCs from nonendometriotic controls.
**Methods:** Stromal cells were separated though enzymatic digestion of ectopic and eutopic endometrial tissues from 29 endometriotic patients and 10 nonendometriotic controls and characterized by flow cytometry. *Moreover, we collected peritoneal fluid from 10 endometriosis patients, as well as 10* non-endometriotic controls. Following cell isolation, *the gene and protein expression levels of FasL* were evaluated by Real-time PCR and Western blot*, respectively.* Also, the cells were treated with the optimum concentration of 1, 25-Dihydroxy vitamin D3 in different time manner and then the effects if this vitamin were assessed in gene and protein expression of FasL in mentioned cells.
**Results**: EESCs exhibited a significantly higher FasL gene and protein expression compared to EuESCs or CESCs ($P<0.01$). Furthermore, significantly lower gene expression levels of FasL were found in PFMCs in endometriotic patients compared to cells from nonendometriotic controls ($P<0.01$). 1, 25-Dihydroxy vitamin D3 reduced the FasL protein expression of EuESCs ($P<0.01$) and EESCs ($P<0.05$). Interestingly, this vitamin increased the gene and protein expression of FasL in PFMCs from endometriotic patients ($P<0.05$).
**Conclusion:** These findings propose that 1, 25-Dihydroxy vitamin D3 have beneficial effects on the regulation of apoptosis in patients with endometriosis.

***Keywords***: *Endometriosis; Stromal cells; FasL;* 1, 25-Dihydroxy vitamin D3

ICIA2018\Reproductive Immunology\Poster Discussion\9969
**9969**

# MSC Therapy Alters the Expression Levels of Crry and Adipsin at the Feto-maternal Interface of Abortion Prone Mice

Shahab Shahgaldi[1], Amir Salek Farrokhi[1], seyed Mohammad Moazzeni[1]

*1. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

**Background:** Recurrent spontaneous abortion, with a prevalence of 2-5% among pregnant women, is one of the most common complications of pregnancy. Today, it's well stablished that a great proportion of recurrent spontaneous abortions (RSAs) is due to immunological factors. In this regard, complement activation and regulation plays a pivotal role.
In this study, we measured the impact of MSCs on the expression levels of Crry and Adipsin at the feto-maternal interface of abortion prone mice.
**Methods:** Adipose tissue-derived mesenchymal stem cells (AT-MSCs) were isolated from the abdominal fat of CBA/J mice. On the 4.5th day of gestation, the test group received an IP injection of $1 \times 10^6$ of AT-MSCs. On the 13.5th day of gestation, tissue samples (placentae and deciduae of pregnant mice) were analyzed to measure the expression levels of Crry and Adipsin using real-time PCR.
**Results:** Following MSC therapy, the average weight of fetuses was increased. Besides, the real time PCR analysis of the expression levels of Crry and Adipsin demonstrated that Adipsin expression levels was decreased significantly upon MSC administration while the expression levels of Crry was elevated at the feto-maternal interface of abortion prone mice.
**Conclusion:** In the present study, we showed for the first time that MSCs improved fetal developmental conditions in abortion-prone mice by adjusting the expression levels of complement regulatory molecules. Our results suggested that MSCs altered the expression levels of Adipsin and Crry, which ultimately contributed to better pregnancy outcomes.

**Keywords:** Mesenchymal stem cell, recurrent spontaneous abortion, Cell therapy, Complement regulatory molecules, Complement system, Pregnancy, Adipsin, Crry.

# Stem Cells Based Immunotherapy

# Poster Discussion

ICIA2018\Stem Cells Based Immunotherapy\Poster Discussion\5302
**5302**

# The Effect of Mesenchymal Stem Cell Pulsed with 17-β Estradiol in Ameliorating Rat Model of Ulcerative Colitis

Siavash Mashhouri [1], Seyyed Meysam Abtahi Froushani [2]

*1. Graduate of Veterinary Medicine, Department of Microbiology, Faculty of Veterinary Medicine, University of Urmia*
*2. Department of Microbiology, Faculty of Veterinary Medicine, University of Urmia*

**Background**: One of the natural factors which has a proliferative effect on Mesenchymal stem cells (MSCs), is 17-β estradiol (E2). However, there is no/or limited documents about the effects of treatment of ulcerative colitis (UC) with E2-primed MSCs. Therefore, this research was set out to assess the effects of E2-primed MSCs therapy in the rat model of UC.
**Methods**: In this experimental study, after isolation of MSCs, they were co-cultured with 0 or 100nμM 17-β estradiol for 24 h. Colitis induced by acetic acid in four groups of male Wister rats; control group, MSCs treated group, co-culture MSCs by estrogen treated group and control group. MSCs ($2\times10^6$ cell) were injected into the peritoneum in different group separately. After 10 days, the rats were euthanized and evaluated for level of malondialdehyde, myeloperoxidase, nitric oxide and total protein in the homogenate of gut tissues.
**Results**: The cell therapy in the UC rats with 17-β estradiol primed MSCs showed a more desirable outcome, causing the regression of the cumulative clinical score to be more favorable than the therapy with un-treated MSCs. The levels of Myeloperoxidase, Nitric oxide and malondialdehyde were significantly decreased and conversely the total protein levels were significantly increased in UC rats received 17-β estradiol primed MSCs more pronounced than UC rats received un-treated MSCs.
**Conclusion:** 17-β estradiol treated MSCs caused more favorable regression the signs and induce better outcome in rats with UC compared to un-treated MSCs.

**Keywords:** 17-β estradiol, Mesenchymal stem cell, Ulcerative Colitis.

ICIA2018\Stem Cells Based Immunotherapy\Poster Discussion\7465
**7465**

# The Effect of LPS Treated Adipose Tissue-Derived Mesenchymal Stem Cells Conditioned Media on Nitric Oxide Production and Phagocytostic Activity of Peritoneal Macrophages

Zahra khosrowpour.[1], Seyed.Mahmoud Hashemi.[2], Sara Soudi.[3]

1. *Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences*
2. *Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences*
3. *Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University*

**Background:** Adipose tissue-derived mesenchymal stem cells (AD-MSCs) are capable of migration to inflamed site and exerts its immunomodulatory effects on tissue resident immune cells like macrophages. To investigate the effect of MSCs treated with LPS soluble factors on macrophage function, we assess nitric oxide and phagocytosis potential production of macrophages after treatment with MSCs treated with LPS conditioned media.
**Methods:** MSCs were isolated from adipose tissue of C57BL/6 mice. MSCs surface markers were analysed using flowcytometry method. MSCs were cultured and divided into two groups at passage two, first group treated with LPS for three days and the second one had no treatment. C57BL/6 peritoneal macrophages were harvested and treated for 72h with MSCs conditioned media. After treatment و macrophages were treated with yeast particle at 1:10 ratio and phagocytosis percent was measured by microscopic examination. Nitric oxide production was measured in the supernatants of treated macrophages by Griess method.
**Results:** According to obtained results we found that the level of nitric oxide production of macrophage was decreased after co-culture with MSCs treated with LPS CM compared to the control. Phagocytosis percent of yeast particles were reduced significantly in macrophages cultured with treated MSCs with LPS CM compared to the control.
**Conclusion:** In this research, we show that MSCs stimulation with LPS will change the immunomudulatory properties of macrophages by reduction of NO production, beside the decrease in phagocytosis percent.

**Keywords:** AD-MSCs, LPS, macrophage, C57BL/6

ICIA2018\Stem Cells Based Immunotherapy\Poster Discussion\7515
**7515**

# Evaluation of T-regulation of Lymphocytes and Anti-inflammatory and Inflammatory Cytokines Profile of Spleen Cells in Diabetic Mouse after Injection of Exosomes Isolated from Adipose Tissue Mesenchymal Stem Cells

Shahrzad Nojehdehi[1, 2], Seyed Mahmoud Hashemi[3, 4], Ardeshir Hesampour[5], Sara Soudi[6], Masoud Soleimani[7]

1. *Department of Biology, Islamic Azad University Central Tehran Branch, Tehran, Iran*
2. *Stem Cell Technology Research Center, 1997775555, Tehran, Iran*
3. *Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
4. *Department of Applied Cell Sciences, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
5. *Department of Biology, Islamic Azad University Central Tehran Branch, Tehran, Iran*
6. *Department of Immunology, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran.*
7. *Department of Hematology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

**Background**: Type 1 diabetes is one of the most common autoimmune diseases that are due to the activation of T cells against beta cells, resulting in degradation of them, which may have various causes, such as genetic factors and environmental stimuli, including viral infection. Continuous high blood sugar and metabolic disturbances caused by diabetes cause secondary pathophysiologic changes in many organs in the body, which can lead to many problems and costs for people with diabetes and the community. Existing evidence showed that stem cells release exosomes and nano-vesicles known as exosomes that may be used as cell-cell-linkers and potentially transmit stem cell phenotype to living cells and maintain, Facilitate differentiation, self-renewal, and repair of stem cells. In this study, the therapeutic use of exosomes as a non-cellular therapeutic regimen has advantages that can be a good justification for its replacement in conventional cell therapies. The main innovation of this project is the use of isolated exosomes from the use of soup and suppository mesenchymal stem cell culture that does not have the dangers and problems of cell therapy and also has more efficacy than the supernatant cell culture. **Methods:** First, by disease induction using STZ and based on disease symptoms, mice were separated into three groups: healthy group, control group, and treatment group. The treatment group were treated by intraperitoneal injection (2 times per week) of exosomes extracted by ultracentrifuge subtraction of mesenchymal stem cells derived from adipose tissue, Then after 8 weeks the mice were dislocated and spleen cells were isolated .the spleen lymphocytes were examined in order to evaluate their alternation in cytokine profiles by ELISA and for evaluate Regulatory T (iTreg) cell T lymphocytes (CD4 + CD25 + Foxp3 +) using flow cytometry. **Results:** The data showed, the mice that were not treated had a shorter life span and also exosomes derived from mesenchymal stem cells were able to reduce the secretion of inflammatory cytokines and increasing anti-inflammatory.Then a significant increase in the population of Treg cells in diabetic mice treated with exosomes derived from AD-MSCs in comparison to the untreated diabetic group have been reported ($P<0.05$) **Conclusion**: In this study, the therapeutic use of exosomes as a non-cellular therapy has a number of benefits that could properly explain the reason for replacing it with the common methods of cellular therapy.

**Keywords:** Exosome, Cytokines, Regulatory T Cells

ICIA2018\Stem Cells Based Immunotherapy\Poster Discussion\7552
**7552**

# Immunochemical Evaluation of Mesenchymal Stem Cells Derived from Wharton's jelly Encapsulated by Alginate, Which are Transplantation to the Peritoneal Cavity of the Rat

Saeed Azandeh[1], Masoud Moghimi[1,] Negar Varae[1]

*1. cellular and molecular research center, Ahvaz jundishapur university of medical sciences, ahvaz, iran*

**Background**: Since previous studies claimed that human umbilical cord WJ-MSCs are low immunogenic, with the assumption in this study, the evaluation of immune responses of WJ-MSCs in vitro with and without alginate was investigated.
**Methods*:*** Cellular bioavailability against B cell antibody toxicity evaluated by MTT assay and anti-MSCs antibodies were also measured by ELISA test. Also, in the evaluation of cellular immunity against MSCs, the level of IFN-γ secretion measured by ELISA method and proliferation of spleen and lymph cells against MSC was evaluated by CFSE staining and flow cytometry.
**Results*:*** In the evaluation of cellular and humoral immunity, one month after cell, scaffold with cell and without cell transplantation did not receive any immune response in the rats and control group that didn't received any graft.
**Conclusion*:*** Our result showed because of low immunogenicity of MSCs and major role of alginate with high G scaffold in immunization of component of host immunity from graft donor.

**Keywords:** alginate; cell encapsulation, immunogenicity, cellular and humoral immunity

ICIA2018\Stem Cells Based Immunotherapy\Poster Discussion\8337
**8337**

# Flow cytometry Analysis of Maturation and Costimulatory Markers of Bone Marrow Dendritic Cells Cultured by Isolated Exosomes of Mesenchymal Stem Cells

Mehri Shahir[1], Esmaeil Mortaz[1], Seyed Mahmoud Hashemi [1]

*1. Department of Medical Immunology ,School of Medicine ,Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background**: DCs orchestrate innate inflammatory responses and adaptive immunity through activation of T cells. MHCII molecules present the first classical signal in the process of antigen presentation, and co-stimulatory molecules such as CD40 represent the second signal. Since DCs are so well equipped to initiate adaptive immune responses, they are considered prime targets for modulating immune responses. MSCs have anti-inflammatory effects. Exosomes are microparticles secreted from cells and conduct cell-cell communication. In this study we aimed to investigate the effects of isolated exosomes derived from MSCs on surface expression of maturation markers of myeloid DCs.
**Methods:** Culture of C57BL/6 mouse AD-MSCs: Murine adipose-derived MSCs were isolated from C57BL/6 Mice. Extracellular matrix was digested with type I collagenase, centrifuged and the pellet was then cultured in DMEM. Isolation and purification of exosomes: Exosome was isolated by Exo-spin kit and evaluated by SEM. The protein concentration was detected by BCA kit. Preparation bone marrow derived DCs: BM mononuclear cells were prepared from C57BL/6 mouse femur and tibia and then cultured in 6-well plates containing PRMI-1640 medium supplemented with 20 ng/mL GM-CSF and 20 ng/mL IL-4. Flow cytometry: After co-culture of exosomes (100µg/mL) with DCs (1 million/ml) the surface expression of CD11c, CD83, CD86, CD40 and MHCII were analyzed in following groups: (immature DC (iDC), iDC+LPS (1µg/ml), iDC+exosome (100µg/ml), iDC+exosome (100µg/ml)+LPS (1µg/ml)) was determined in 10000 cells by Flow cytometry.
**Results:** The results showed that MSC derived exosomes could decrease the expression of the studied markers in comparison to control cells.
**Conclusion:** The current data indicates that exosomes isolated from MSCs suppress the maturation of bone marrow derived DCs. Besides, our data could suggests that exosomes might be important modulator for the immune responses induces by DCs.

**Key words:** Mesenchymal Stem Cell, Exosomes, Tolerogenic Dendritic Cell, In Vitro

ICIA2018\Stem Cells Based Immunotherapy\Poster Discussion\8377
**8377**

# The Effect of Serum Starvation on C57B6 Bone Marrow Cells

Gilda Parsamanesh[1], Amrolah Mostafazade[1], Davar Amani[2], Ali Asadirad[2], Kaveh Baghaei[3], Hamid Asadzadeh Aghdayi[3]

1. *Department of immunology, School of medicine, babol University of medical sciences.*
2. *Department of immunology, School of medicine, shahid beheshti University of medical sciences.*
3. *Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research institute for Gastroenterology and Liver Diseases, Shahid BeheshtiUniversity of Medical Sciences, Tehran, Iran.*

**Background:** Serum starvation is a condition that has an effect on cell behavior and very little is known about this mechanism. Previous study in PBMC cells has shown that the starvation (for 96 hours) leads to down-regulation of HLA class I, increasing the relative number of T-reg cells to effective T cells and also increasing the amount of TFG-β in cellular soup. Therefore, we hypothesized that serum starvation condition can be considered as an effective tool in cells transplantation. In this project, the influence of serum starvation was evaluated on the potency of starved mouse bone marrow cells for transplantation.
**Methods:** Isolated bone marrow cells from C57/B6 were cultured in serum starvation condition for 24, 48, 72, 96 hours and non-starved cells were considered as control groups. Consequently, at the end of each time point, the viability of cells was determined by flow cytometry. In order to analyze the expression of foxp3, TGF β and MHC class I Real-time PCR was carried out. Also, transplantation potency of starved cells will be investigated by one way MLR test.
**Results:** Expression of MHC class I was declined compared with non-starved cells (24, 48, 72 hours) ($P<0.000$); however, TGF β in starved cells were increased (48 hours) ($P<0.000$). In both starved and non-starved groups, foxp3 did not have significant expression.
**Conclusion:** Although serum starvation leads to down-regulation of MHC class I, increasing of TGF β in bone marrow cells, foxp3 expression was undesirably decreased too. 48 hours starvation could result in prevention of recipient immune response to transplanted bone marrow cells by down-regulation of MHC class I and increase of TGF β, nonetheless, prevention of GVHD in this condition need to be elucidated.

**Keywords:** Serum starvation/ bone marrow/ MHC class I/ TGF β/ foxp3

ICIA2018\Stem Cells Based Immunotherapy\Poster Discussion\8519
**8519**

# Enrichment of Cancer Stem Cells from Murine Colorectal Primary Tumor in order to Cancer Stem Cell-based Vaccine Studies

Leila Eini[1], Zahra Madjd[1, 2*], Roya Ghods[1, 2*], Mahmood Bozorgmehr[1]

*1. Oncopathology Research Center, Iran University of Medical Sciences, Tehran, Iran*
*2. Department of Molecular Medicine, Faculty of Advanced Technology, Iran University of Medical Sciences, Tehran, Iran*

**Background:** Cancer stem cells (CSC) are responsible for initiation, maintenance and resistance to chemo/radiation therapy. Inability of CSC targeting using immunotherapy may be a significant factor contributing to treatment failure. There are limited studies in murine CSCs isolation, therefore we aim to develop an animal model and isolate CSCs from CT-26 (colorectal cancer cell line) induced tumor in order to evaluate the effects of lysate vaccine on tumor growth and mice survival. Due to high cost and several limitations of NOD/SCID mice providing in Iran, also absence of adaptive immune responses in these mice, the use of CSCs in such syngeneic immunocompetent hosts permits the assessment of immune responses against these cells.
**Methods:** Tumor was induced in six weeks old female Balb/c mice with CT26 cells. Tumor tissues were minced and enzymatically digested. The resulting cells were seeded at a density of $1 \times 10^5$/ml in Serum-Free Medium. Culture cells were incubated for 7 days, photographed and scored for the presence of colonospheres morphologies. Flow cytometry, was employed to characterize isolated colorectal CSCs compare to parental cells.
**Results:** Results showed that, cells rounded up to form independent spheroids. Majority of colonsphere cells (85%) were positive for CD133 while 15% of parental cells stained positive for the same marker. Percentage expressions for CD166 was 34% in spheroid cells versus 4% in parental cells.
**Conclusion:** We demonstrate that CSC populations from murine colorectal primary tumor can be enriched using spheroid formation assay, this could facilitate the establishment of immunocompotent mice models for subsequent studies such as CSCs base vaccine.

**Keywords:** Cancer stem cell (CSCs), vaccines, CT-26, mouse colorectal model.

ICIA2018\Stem Cells Based Immunotherapy\Poster Discussion\8610
**8610**

# The Effects of Mesenchymal Stem cells on Clinical Scores of Multiple Sclerosis Patients

Fahimeh Lavi [1], Forouzan Yousefi [1], Mahmoud Mahmoudi [1]

*1. Immunology center, BuAli institute, Department of Immunology Mashhad University of Medical Science Mashhad, Iran*

**Background**: Multiple sclerosis (MS) is the most common disabilities among CNS diseases. The lack of treatments for multiple sclerosis patients in progressive stage demonstrates a major unmet clinical need. Mesenchymal stem cells have emerged to own beneficial effects in multiple sclerosis patients and substitutive animal model of MS.
**Methods :** we aimed to evaluate the safety and efficacy of MSCs derived from adipose tissue as an effective treatment for secondary progressive multiple sclerosis. Eligible participants were 10 female patients, non-pregnant, unresponsive to other approved treatments, lack of chronic and acute infection or tumor. Patients are predicted to continue to have progression of disease next year. We remove abdominal fat of patients and after harvesting we expand their adipose tissue mesenchymal stem cells (AT-MSC) to reach to a range of $5\times10^6$ MSC/Kg of body weight. Then we infuse their expanded AT-MSC in 2 dosage during 2 injection space 7 days apart. Next we evaluate the effect of AT-MSCs on patients by assessment of EDSS on baseline, 2 weeks, 1, 3, 6 and 9 months after first injection according to MacDonald criteria.
**Results:** in all of patients EDSS was decreased significantly from baseline to 3 months post first injection thereafter EDSS was increases in 2 patients by 1 point at endpoint of follow-up and 6 patients were progression free and in 2 patients EDSS was reduced significantly by 1 point from baseline to 9 months after first injection.
**Conclusion:** MSCs were safe and effective in secondary progressive MS patients and the most pronounced influence was from baseline to 3 months after first MSC administration.

**Keywords:** Adipose-derived mesenchymal stem cell, secondary progressive multiple sclerosis

ICIA2018\Stem Cells Based Immunotherapy\Poster Discussion\9780
**9780**

# Immunomodulatory Effects of Adipose Tissue-derived Mesenchymal Stem Cells in an Allograft Islets Composite Transplant for Experimental Autoimmune Type 1 Diabete

Bahare Niknam[1,2], Jamal Mohammadi Ayenehdeh[1,3] , Nader Tajik[1], Seyed Mahmoud Hashemi[4]

1. *Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran*
2. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
3. *Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
4. *Department of immunology, School of medicine, Shahid Beheshti University of Medical Sciences*

**Background:** Allogeneic islet transplantation could be an ideal alternative therapy for Type 1 Diabetes. Adipose Tissue-derived Mesenchymal Stem Cells (AT-MSCs) characterized by immunomodulatory and protective effects may have the potential to improve the outcome of this highly immunogenic transplant. Immunomodulatory properties have been shown to be exerted through both direct contact and production of soluble markers.
**Methods**: Syngeneic AT-MSCs along with allograft islets embedded in hydrogelic composite and transplanted intraperitoneally in Streptozotocin (STZ) induced diabetic C57BL/6 mice.
Five groups consist of Control, Hydrogel alone, Gel+MSC, Gel+Islet and Gel+MSC+Islet, delivered into the peritoneal cavity. 32 days after transplantation, allograft composites were Collected and Real-time PCR were performed with specific primers for β2M, IDO, iNOS, PDX1 genes.
**Results:** Co-transplantation of AT-MSCs significantly increased the transcript levels of IDO and iNOS in the Gel+MSC+ Islet group, in comparison with the Gel and Gel+Islet and Gel+ MSC groups. Similar results were also obtained for transcript levels of PDX1 which significantly increased in Gel+MSC+Islet group as compared to Gel and Gel+ Islet and Gel+ MSC groups.
**Conclusion:** According to results, AT-MSCs are capable to improving graft function with immunomodulatory effects through production of soluble markers.

**Keywords:** Mesenchymal stem cell, Type 1 Diabetes, Allograft islet transplantation

ICIA2018\Stem Cells Based Immunotherapy\Poster Discussion\9781
**9781**

# Assessment of Phenotype and Function of Tregs in an Allograft Islets and Fat Derived Mesenchymal Stem Cells Composite Transplant for Experimental Autoimmune Type 1 Diabetes

Bahare Niknam[1,2], Jamal Mohammadi Ayenehdeh[1,3] , Nader Tajik[1]

1. *Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran*
2. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
3. *Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Islet transplantation is one of the most promising therapeutic approaches in Type 1 Diabetes. In order to improve the viability and function of islet transplantation, it has been proposed to associate pancreatic islets with Adipose Tissue-derived Mesenchymal Stem Cells (AT-MSCs).
**Methods**: Allograft islets from BALB/c mice co-embedded with syngeneic AT-MSCs from abdominal fat tissue of C57BL/6 in hydrogelic composite and delivered in to the peritoneal cavity of Streptozotocin (STZ) induced diabetic C57BL/6 mice. Five groups consist of Control, Hydrogel alone, Gel+MSC, Gel+Islet and Gel+MSC+Islet, delivered into the peritoneal cavity. 32 days after transplantation, mononuclear cells from the Mesenteric lymph nodes (MLNs) and spleen were analysis For Treg and intracellular cytokine assay by flowcytometry.
**Results:** Analyses showed that AT-MSCs co-transplanted with allograft significantly increased Treg ($P<0.05$) in MLNs but there is no significant variation among the groups in spleen. According to the MFI for IL-10 and TGF-β1 in MLNs analysis, the IL-10 and TGF-β1 significantly increased in Gel+MSC and Gel+MSC+Islet groups, in comparison with the other corresponding groups ($P<0.05$); but in spleen, TGF-β1 significantly increased in Gel+MSC and Gel+MSC+Islet groups but variation in IL-10 was not significant.
**Conclusion:** These result show that AT-MSCs Can promote islet survival and function and can induced functional Treg cells. Moreover, local MSCs transplantation also exert immunomodulatory effects on infiltrating and resident immune cells.

**Keywords:** Type 1 Diabetes, Mesenchymal stem cells, Treg

# Tolerance and Autoimmunity

# Poster Discussion

ICIA2018\Tolerance and Autoimmunity\Poster Discussion\5230
**5230**

# Study of Therapeutic Plasmapheresis Effects on IL-6 and TGF-β and Their Receptors in Relapsing-Remitting MS Patients

Azam Jamshidian[1, 2]; Zahra Khademi[2]; Vahid Shaygannejad[3]

1. *Faculty of Paramedical Sciences, Shahrekord University of Medical Sciences, Shahrekord, Iran*
2. *Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran*
3. *Neurology dept. Medical school, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background**: Multiple sclerosis (MS) is a chronic demyelinating inflammatory disease of the central nervous system. Lack of sufficient information about the effects of plasma exchange (PE) therapy in multiple sclerosis (MS), has limited this treatment to individual patients with severe refractory relapses. Th17 cells play a promoting role in MS. The cytokines, interleukin 6 and TGF-β play a critical role in the development of Th17 cells. Evaluating the effect of PE on the expression levels of IL-6 and TGF-β and their receptors was the aim of this study.
**Methods**: Peripheral blood samples were obtained before and after a complete course of PE therapy, from 30 Relapsing Remitting-MS patients in relapse phase. IL-6 and TGF-β mRNA levels were assayed using real-time PCR. The IL-6 and TGF-β receptors were assessed using flowcytometry.
**Results**: Expanded disability scale score was significantly reduced after treatment (P< 0.01) and this relieve of symptoms was significantly higher in males compared with females (P= 0.039). A significant increase in TGF-β (p=0.009) and a significant decrease in IL-6 receptor were observed (P=0.028). Correlation analysis showed the changes in expression levels of two cytokines are directly correlated (P= 0.03). mRNA level of each cytokine after treatment was conversely related to the expression level of its receptor before therapy (TGF-β, P= 0.019 & IL-6, P= 0.028 ). The frequency of CD4+IL6R+ cells and CD4+IL6R+ TGF-βR+ cells before PE was conversely correlated to TGF-β mRNA expression levels after PE (p= 0.03 & p= 0.04, respectively). The frequency of CD4+IL6R+ cells was also correlated with disease severity (p= 0.001) and the disease severity was related with symptom relief (0.009).
**Conclusion:** This study showed increasing TGF-β mRNA and decrease of IL-6 receptor expression can be a way in which plasma exchange improves MS relapse symptoms, and this therapy impacts on the two cytokine and their receptor in an interrelated manner.

**Keywords:** Multiple sclerosis, Plasma exchange therapy, IL-6, TGF-β

ICIA2018\Tolerance and Autoimmunity\Poster Discussion\5312
**5312**

# Vitamin D Modulates the Expression of IL- 27 in the Central Nervous System in Experimental Autoimmune Encephalomyelitis (EAE)

Marziyeh mohammadi kordkheyli[1], Abdollah jafarzadeh[2]

1. *Tehran university of medical science, Iran, Tehran*
2. *University of medical science, Iran, Kerman*

**Background:** It has been reported that vitamin D has broad anti-inflammatory and immunomodulatory effects. To evaluate the effects of vitamin D on the expression of IL-in a model of experimental autoimmune encephalomyelitis (EAE).
**Methods**: EAE was induced in C57Bl/6 mice by immunization with myelin oligodendroglial glycoprotein mixed with complete Freund's adjuvant. The mice were administered with PBS or olive oil, intraperitoneally, in the control groups and vitamin D (200 ng every two days) in the treatment group, from day +3 to +30. At day 31, the mice were scarified and their spinal cords and brains were the expression of the IL-27 mRNA in the spinal cord was measured using real time-PCR.
**Results**: In PBS- or olive oil-treated EAE mice the expression of IL-27 P28 mRNA was significantly lower than that in the healthy control group ($p<0.002$). In both PBS- and olive oil-treated EAE groups, the expression of IL-27 EBI3 mRNA was also lower than that observed in the healthy group, but the differences were not significant. In vitamin D-treated EAE group, the expression of IL- 27 P28 and IL-27 EBI3 were significantly higher compared with the olive oil-treated EAE groups ($p<0.002$ and $p<0.04$, respectively). The PBS- or olive oil-treated EAE mice showed the clinical symptoms of EAE at days 9 and 10, respectively. The maximum mean pathological scores were also significantly lower in vitamin D-treated EAE group, in comparison with PBS- or olive oil treated EAE mice ($p<0.001$).
**Conclusion**: Vitamin D may modulate the expression of IL-27 in the spinal cord of EAE mice and also ameliorate the clinical symptoms of the disease.

**Keywords:** Vitamin D, EAE, IL-27

ICIA2018\Tolerance and Autoimmunity\Poster Discussion\6355
**6355**

# Down Regulation of Neuroimmune Semaphorin 3A in Peripheral Blood Mononuclear Cells (PBMCs) and Its Serum Level of Patients with Multiple Sclerosis

Mahsa Rezaeepoor[1], Shima Shapoori[1], Mazdak Ganjalikhani hakemi[1], Masoud Etemadifar[2], Fereshteh Alsahebfosoul[1], Nahid Eskandari[1], Marjan Mansourian[c]

*1. Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
*2. Multiple Sclerosis and Neuroimmunology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*
*3. Department of Biostatistics and Epidemiology, Faculty of Medicine, Isfahan University of Medical Science, Isfahan, Iran*

**Background:** Semaphorin 3A (Sema3A) as an immune modulator could be involve in the pathogenesis of autoimmune diseases and act as a suppressor of immune cell over-activation.
**Methods:** In the current study, we aimed to investigate expression of sema3A in peripheral blood mononuclear cells (PBMCs) and its serum level in relapsing-remitting multiple sclerosis (RRMS) patients. Fifteen newly determined and untreated RRMS patients were chosen and assessed in relapsing and remitting phases in compare with fifteen healthy individuals.
**Results:** In consistent with previous findings in other autoimmune diseases, our results revealed that serum level of Sema3A and its expression in PBMCs of RRMS patients were significantly lower than in normal subjects. We also evaluated this down regulation has predictive value with ROC analysis.
**Conclusion:** According to our data, we suggest that Sema3A could be participated in the pathogenesis of MS and might be a potential diagnostic biomarker for the disease.

**Keywords:** Semaphorin-3A, Multiple sclerosis, Autoimmune disease, Immune modulator

ICIA2018\Tolerance and Autoimmunity\Poster Discussion\7446
**7446**

# Evaluation of the *T-bet*, *RORγt* and *Foxp3* Gene Expression in Patients with Type 2 Diabetes Mellitus

Zahra Telikani [1,2], Vida Sheikh [3], Shiva Borzouei [3], Mortaza Ghasemi [3], Alireza Zamani [1], Iraj Salehi [4], Mahdi Alahgholi Hajibehzad [1,*]

1. *Department of Immunology, Hamadan University of Medical Sciences, Hamadan, Iran*
2. *Student Research Committee, Hamadan University of Medical Sciences, Hamadan, Iran*
3. *Department of Internal Medicine, Clinical Research Development Unit of Shahid Beheshti Hospital, School Of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
4. *Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran*

**Background:** T lymphocytes are related to inflammation and insulin resistance in type 2 diabetes mellitus (T2DM) and T2DM plus nephropathy (T2DMN). The imbalance between pro-inflammatory and inhibitory lymphocytes plays a major role in development of T2DM. The objective of this study is to investigate the gene expression of *T-bet*, *RORγt* and *Foxp3* related to T helper (Th) 1, Th17 and Regulatory T (Treg) cells subsets in T2DM.
**Methods**: Blood samples from 39 patients diagnosed with T2DM (mean: 55.7 ± 1.12; female=29, men=10), 25 patients diagnosed with T2DMN (mean: 55.8 ± 6.9; female=19, men=6) and 26 healthy control (HC; mean: 47.3 ± 9.2; female=14, men=12) were collected. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by Ficoll density gradient method. In order to determine mRNA expression of *T-bet*, *RORγt*, *Foxp3* and GAPDH (reference gene), total RNA was extracted from PBMCs and cDNA was generated. Expression of Th genes was tested/ performed with the SYBR green Real Time PCR method. The relative genes expression level for each sample were calculated by ΔΔCt method.
**Results:** The level of Foxp3 gene expression has shown significant decrease in T2DMN patients compared with HCs (p=0.04) and but there was no significant change in RORγt and T-bet gene expression. The level of Foxp3 gene expression has demonstrated significant decrease in T2DMN patients compared with T2DM patients (p=0.02), whereas there was no significant change in RORγt and T-bet gene expression. No significant differences were observed in gene expression of T-bet, RORγt and Foxp3 in T2DM patients compared with HC group.
**Conclusion:** This result show a decreased *Foxp3* gene expression in T2DMN, which could contribute to the pathogenesis of the disease and revealing a potential novel therapeutic target.

**Keywords:** T2DM, T-bet, RORγt, Foxp3, Nephropathy

ICIA2018\Tolerance and Autoimmunity\Poster Discussion\7460
**7460**

## Frequency and Functional Evaluation of Treg Cells in Patients with type 2 Diabetes Mellitus

Mahdi Alahgholi Hajibehzad [1], Elham Mahabadi Ashtiyani [1], Shiva Borzouei [2], Alireza Zamani [1], Vida Sheikh [2]

*1. Department of Immunology, Hamadan University of Medical Sciences, Hamadan, Iran*
*2. Department of Internal Medicine, Clinical Research Development Unit of Shahid Beheshti Hospital, School Of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*

**Background:** Regulatory $CD4^+CD25^+$ T cells (Tregs) can suppress harmful immunological reactions to self or foreign antigens. The aim of this study was to evaluate the number and suppressive capacity of Tregs in patients with type 2 diabetes mellitus (T2DM).
**Methods**: Tregs from 19 T2DM patients and 20 healthy controls (HC) were analyzed for the number and suppression capability using flow cytometry based on surface CD4 and CD25 markers. Treg and responder T cells (Tresp; $CD4^+CD25^-$) were sorted using magnetic activated cell sorting. CFSE-labeled Tresp were cultured and stimulated with anti-CD3 and anti-CD28 for 5 days. Tregs suppression assays were performed and analyzed by flow cytometry.
**Results:** The addition of Tregs to the culture of Tresp cells significantly decreased the proliferation of Tresp in both T2DM and HC. The % proliferation of Tresp in the presence of Treg cells was lower in HC than T2DM patients. The % suppression of proliferation was significantly lower in patients with T2DM compared with the HC group. No significant difference was observed between proportion of $CD4^+CD25^-$, $CD4^+CD25^+$, $CD4^+CD25^{low}$ and $CD4^+CD25^{hi}$ cells in T2DM and HC subjects.
**Conclusion:** These data demonstrate a normal number, but a decreased functional capacity of Tregs in T2DM, which could contribute to the pathogenesis of the disease and revealing a potential novel therapeutic target.

**Keywords:** Treg, Tresp, T2DM, Suppression

ICIA2018\Tolerance and Autoimmunity\Poster Discussion\7475
**7475**

# Nanocurcumin Improves Imbalance of Th17/Treg cells in Patients with Multiple Sclerosis

Sanam Dolati[1, 2], Mehdi Yousefi[1, 2]

*1. Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran*
*2. Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Multiple sclerosis is a chronic autoimmune disease that leads to brain inflammation. Th17 cells are considered to be important in MS pathogenesis. It was reported that a disturbance in the development and function of Tregs are associated with disability in MS patients. In this study, we aimed to identify nanocurcumin effects on Th17 and Treg cells frequency, cytokines secretion, and expression of transcription factors of these cells in patients with relapsing-remitting multiple sclerosis (RRMS).
**Methods:** 50 patients with RRMS were enrolled in this study in which 25 were treated for at least six months with nanocurcumin capsules while the other half received placebo capsules as the control group. At baseline and after a six-month treatment, the frequency of these lymphocytes, the expression of transcription factor and the serum levels of cytokines were assessed by flowcytometry, RT- PCR and ELISA, respectively.
**Results:** Nanocurcumin significantly increased the frequency of Treg cells in compared placebo group ($p=0.0027$). The levels of FoxP3, TGF-β and IL-10 mRNA increased following nanocurcumin therapy compared to the basal level ($p=0.0004$), ($p=0.0005$), and ($p<0.0002$), respectively. TGF- β and IL-10 levels were increased after treatment with nanocurcumin; from 193.7 ± 95.45 to 313.5 ± 203.6, and from 629.1 ± 355.1 to 1038± 496.8, respectively. Even though there is no significant difference in proportion of Th17 cells in placebo group compared with nanocurcumin treated group. RORγt and IL-17 mRNA levels were reduced in MS patients after nanocurcumin treated (0.0001) and ($p<0.04$) in compared with basal levels, IL-17A levels were decreased after nanocurcumin treated from 140.8 ± 57.24 to 88.59 ± 48.07 ($p=0.0011$) but there is no noticeable decrease in IL-23 mRNA levels and concentration in nanocurcumin group, control group.
**Conclusion:** The results of the current work indicate that nanocurcumin is able to restore the imbalance of Th17/Treg in MS patients.

**Keywords:** Multiple Sclerosis, Nanocurcumin, Th17, Tregs

ICIA2018\Tolerance and Autoimmunity\Poster Discussion\8105
**8105**

# MiR-326 was Over-expressed in T cell-derived Exosomes of Patients with Relapsing-Remitting Multiple Sclerosis

Maryam Azimi Mohamadabadi [1], Maryam Izad [1, 2]

*1. Department of Immunology, school of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
*2. MS Research Center, Neuroscience Institute, Tehran University of Medical Sciences, Tehran, Iran*

**Background**: Multiple sclerosis (MS) is an immune-mediated neurodegenerative disease of central nervous system (CNS). Invasion of autoreactive CD4+ T cells into CNS is believed to be an underlying pathogenic mechanism in MS. CD4+ T cells release exosomes which are highly enriched in microRNAs, reflective of physiological or pathological condition. Exosomal microRNAs were transferred between cells and affected the physiology of target cells. Thus exosomes could be potent agents to provide quantitative and qualitative information about immune cells involved in MS. We investigated the expression of pathogenic microRNAs in T cells-derived exosomes of MS patients or healthy controls.
**Methods:** Conventional T cells (Tconv) derived from relapsing-remitting (RR) MS patients or healthy controls were cultured for 3 days by soluble anti-CD3/CD28. Exosomes were purified from supernatants. After RNA extraction from exosomes pellet, the expression levels of miR-146a, miR-29a, miR-155, and miR-326 were quantified by real-time PCR.
**Results**: A statistically significant increased expression of miR-326 in Tconv-derived exosomes was observed in RRMS patients as compared with controls (7.5±1.88vs 2.51±0.9 P=0.03), On the contrary, no differences were found in the expression levels of miR-155, miR-146a, and miR-29a, in Tconv-derived exosomes of patients as compared with controls (P>0.05).
**Conclusion:** Our results point to an altered expression in exosome-derived microRNAs. MiR-326 was previously shown to play a role in the immunopathogenesis of MS by inducing TH17 differentiation and maturation. Therefore, miR-326 containing exosomes might also be a potential clinical target in course of MS. Moreover, the deregulation of this miRNA in exosomes may serve as a diagnostic and prognostic biomarker.

**Keyword**: Multiple sclerosis; Lymphocyte; Exosomes; microRNA

ICIA2018\Tolerance and Autoimmunity\Poster Discussion\8531
**8531**

# Methylation Status of the Human Telomerase Reverse Transcriptase Gene Promoter in Patients with Multiple Sclerosis

Seyed Alireza Mesbah Namin[1*], Sanaz Hesami[1], Mohammad Ali Sahraian[2], Rozita Doosti[2]

*1. Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
*2. Sina MS Research Center, Brain and Spinal Injury Research Center, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Multiple Sclerosis (MS) is a chronic inflammatory demyelinating disease of central nervous system. It is believed that both genetic and environmental factors play a role in causing the disease and shows the importance of epigenetic changes in this disease. There is also a relationship between telomere length and telomerase activity in autoimmune and inflammatory diseases which is associated with immaturity and inability of the immune system. The purpose of this study is to evaluate the promoter methylation of the human telomerase reverse transcriptase (hTERT) gene in MS patients.
**Methods:** In this study, the methylation status of the first CpG island of hTERT gene promoter is investigated by methylation specific PCR in two groups of normal subjects (females: 21 and males: 6) and MS patients (females: 38 and Males: 9). After DNA extraction of blood samples, the concentrated DNAs were treated and modified by sodium bisulfate. After performing of PCR using of specific primers, methylation status was analyzed by the presence of the methylated and unmethylated amplified products.
**Results:** The results of this study showed that hTERT gene promoter in control and patient's samples were both hypomethylated and there were no significantly differences between the hTERT promoter methylation in MS patients and normal controls, except for one case of patients.
**Conclusion:** It should be mentioned that study on the epigenetic modification of whole regions of the hTERT promoter is important and this field needs to be extended and further investigations would be considered in more samples.

**Keywords:** Multiple Sclerosis, Telomerase, Promoter, Methylation

ICIA2018\Tolerance and Autoimmunity\Poster Discussion\8665
**8665**

## Expression Analysis of PD-1 and Tim-3 Immune Checkpoint Receptors in Vitiligo Patients

Ali Rahimi[1], Hadi Hossein Nattaj[1], Zohreh Hajheidari[2], Abolghasem Ajami[1*], Hossein Asgarian Omran[1, 3*]

*1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
*2. Department of Dermatology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
*3. Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

**Background:** Vitiligo is a skin-related chronic autoimmune disease that degrades melanocytic cells, causing colorless and asymptomatic stains. In recent years, the contribution of immune checkpoint receptors has been addressed in the pathogenesis of multiple autoimmune diseases. In the present study, the expression profile of T-cell immunoglobulin and mucin-domain containing-3 (Tim-3) and programmed cell death-1 (PD-1) checkpoint molecules was investigated on $CD8^+$ T cells of patients with vitiligo as the main pathogenic effector cells in this autoimmune complication.
**Methods:** A total of 30 vitiligo patients and 30 sex- and age-matched healthy controls were included in this study. To determine the frequency of $Tim\text{-}3^+/PD\text{-}1^+/CD8^+$ T cells, PBMCs were stimulated with PHA for 72 h and a three-color flow cytometry method was applied. To measure the cytokines production, PBMCs were stimulated with PMA/ionomycin for 18 h and concentrations of IL-4, IFN-γ and TNF-α were measured in culture supernatants by ELISA. $CD8^+$ T cells were then positively isolated from all participants by magnetic beads separation method and the mRNA expression of PD-1 and Tim-3 molecules was determined by TaqMan based Real-Time PCR.
**Results:** Vitiligo patients have significantly showed more expression of Tim-3 and PD-1 on the surface of their $CD8^+$ T cells than that of normal controls. While, the production levels of TNF-α and IFN-γ were found higher by vitiligo patients than those of controls, IL-4 production was lower in patients with vitiligo. Expression analysis of Tim-3 and PD-1 mRNA confirmed the results obtained from flow cytometry and showed more expression of Tim-3 and PD-1 in $CD8^+$ T cells of vitiligo patients compared to normal individuals.
**Conclusion**: Our results indicate that Tim-3 and PD-1 are involved in immune dysregulation mechanisms of $CD8^+$ T cells in vitiligo. Further studies are needed for better understanding of Tim-3 and PD-1 roles in immunopathogenesis of vitiligo and may introduce useful biomarkers for disease progression and/or immunotherapy.

**Keywords:** Vitiligo, $CD8^+$ T cells, Tim-3, PD-1, Flow cytometry, Real-Time PCR

# Transplantation

# Poster Discussion

ICIA2018\Transplantation\Poster Discussion\7435
**7435**

# The Study of the Relationship between Serum Levels of Soluble VEGF Receptor-1 with Delayed Graft Function after Kidney Transplantation

Zahra Hooshyar[1], Saeede Shabanitabar[1], Javad Absari [2], Majid Faridgohar [3], Hassan Nikoueinejad [*4], Hossein Akbari [5], Behzad Einollahi [6]

*1. Medical Student; Student Research Committee, Kashan University of Medical Sciences, Kashan, Iran*
*2. Master Student; Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*
*3. Master Student; Department of Microbiology and Immunobiology, Kashan University of Medical Sciences, Kashan, Iran*
*4. Medical doctor & Immunologist; Nephrology and Urology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*
*5. Statistician; Trauma Research center, Department of Community medicine, Kashan University of medical sciences, Kashan, Iran*
*6. Nephrologist; Nephrology and Urology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*

**Background:** Delayed graft function (DGF) is a transplant complication which means needing dialysis throughout the first week after transplantation. This study aimed to ascertain the relationship between soluble VEGF receptor-1, as an immunomodulatory factor, and DGF after transplantation.
**Methods:** This case control study was done in a 6-month period among 2 groups of 58-member of transplanted patients with and without DGF. The control group included the patients who were operated in 2015 and didn't show DGF symptoms. Then, sFlt-1 level in all blood samples was measured by Elisa.
**Results:** Serum sFlt-1 levels were significantly higher in DGF group compared to those in control group ($P \leq 0.001$). SFlt-1 serum levels significantly affect DGF ($P<0.001$) in such a way that it increases the risk of DGF (OR=1.1).
**Conclusion:** This study showed a significant relationship between sFlt-1 and DGF. Therefore, plasma levels of sFlt-1 may be used as a diagnostic tool to determine the risk of DGF.

**Keywords:** Kidney Transplantation, soluble VEGF Receptor-1, Delayed Graft Function

ICIA2018\Transplantation\Poster Discussion\7681
**7681**

# The Absence of Immune marker HLA-DR Expression and Lack of Rejection in hEnSCs Derived Motor Neurons developed with Small Molecules (Purmorphamin) PMA

Azam Rahimi[1], Homa Mohseni Kouchesfahani[1], Somayeh Ebrahimi Barough[2], Jafar Ai[2]

*1. Department of Animal Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran*
*2. Department of Tissue Engineering, School of Advance Technology in Medicine, Tehran University of Medical Science, Tehran, Iran*

**Background**: Human endometrial stem cells (hEnSCs) are being applied in regenerative medicine. EnSCs derived motor neurons are one of the best targets for transplantation which do not stimulate immune system. Cell differentiation using small molecules provide a promising strategy for generating target cells for cell transplantation. We developed an effective and simple induction protocol for differentiating motor neurons from hEnSCs that is useful for transplantation to treat motor neuron disease. Treatments with differentiation media which contain PMA, promote the development of motor neurons.
**Methods**: During the first phase of differentiation cells were treated with DMEMF\12, FBS 20%, B27, FGF2, 2ME and IBMX for one day. Second environment contained DMEMF\12, N2, B27 and PMA. This phase lasted four days. Subsequent treatment with DMEMF\12, retinoic acid (RA) and PMA lasted 8 days. The last step involve adding environment contain DMEMF\12, N2, B27 and BDNF for 8 days. The whole period lasted 21 days. Then ICC test was done for the expression of Neurofilament (NF) and Acetylcholine transferase (Chat). Then Real-time PCR was performed in RNA stage for expression of neural markers such as NF, Chat, Nestin and GFAP (as glial marker). Then flow cytometry was done for the expression of the cell surface antigen HLA-DR in EnSCs and motor neurons derived from these cells.
**Results**: ICC results showed the expression of NF and Chat. Real-time PCR results demonstrated that the cell treated with PMA expressing neural markers NF and Chat. The results confirmed that hEnSCs and motor neurons derived from them are not expressing HLA-DR.
**Conclusion**: On the basis of our finding, in the present research it was stated that motor neurons developed by PMA, do not express HLA-DR. our finding indicated that EnSCs derived motor neurons developed by inducing small molecules do not stimulate immune system. Because of this unique future, they can be used in therapeutic cases such as transplantation and grafts.

**Keywords**: HLA-DR, hEnSCs, Motor neurons, Purmorphamin.

ICIA2018\Transplantation\Poster Discussion\7999
**7999**

# Comparison of BASILIXIMAB Monoclonal Antibody Transient Expression in two Mammalian Cell Lines

Reyhaneh Hoseinpoor[1], Masoumeh Rajabibazl[2], Azam Rahimpour[3]

*1. Department of Biotechnology, School of Advanced Technologies in Medicine, University of Shahid Beheshti Medical Sciences*
*2. Department of Medical Biochemistry, School of Medicine, University of Shahid Beheshti Medical Sciences*
*3. Department of Tissue Engineering and Applied cell sciences, Faculty of Advanced Technologies in Medicine, University of Shahid Beheshti Medical Sciences*

**Background:** Basiliximab (Simulect) is a chimeric monoclonal antibody against the alpha chain of interleukin-2 receptor (IL-2R) that is effective in reducing acute rejection in renal transplantation. The production of recombinant antibodies has been generally recognized as time-consuming and labor-intensive. For high level expression of recombinant antibodies, the most widely used mammalian expression systems are human embryo kidney (HEK-293T) and Chinese hamster ovary (CHO) cells. The aim of this study was to determine which mammalian expression cells can massively and cost effectively produce full-length antibodies.
**Methods:** The expression vector containing heavy and light chains of Basiliximab was constructed. HEK-293T and CHO-k1 cells were transfected for transient antibody expression using Lipofectamin LTX transfection reagent. To evaluate transfection efficiency, EGFP expression was analyzed 48 h post-transfection using the florescent microscope. The supernatant harvested from cells, was assayed to determine the level of production of antibody by ELISA. The microtiter plate wells were coated with the goat anti-human kappa light chain antibody and goat anti-human IgG (Fc specific)-peroxidase antibody was added for detection of IgG in cell culture supernatants. The amount of the antibody was assessed by comparison to a standard curve.
**Results:** EGFP fluorescence was observed in both cell lines. Antibody was detected in both culture supernatants. The estimate titer for CHO-K1 cells was up to 6 ng/ml while the titer for HEK293T cells was 3.5 ng/ml. Protein from transient CHO was more compared to protein produced from HEK293.
**Conclusion:** Production of IgG molecules with desired specificities has become an area of medical and basic research. The choice of host cell for high level expression of recombinant antibodies is critical. Our results here describe the superiority of CHO-K1 cells for transient expression compared with HEK293T cells.

**Keywords:** Basiliximab, transient Expression, CHO-K1, HEK293T

ICIA2018\Transplantation\Poster Discussion\8209
**8209**

# The Impact of Killer Cell Immunoglobulin-Like Receptors/Human Leukocyte Antigen Class One Combinations on the One-Year Prognosis of Allogeneic Hematopoietic Stem Cell Transplantation in Patients with Acute Leukemia

Marzieh Mansouri, [1] Jean Villard, [2] Mani Ramzi[3], Shirin Farjadian[1]

*1. Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran*
*2. Immunology and Transplant Unit and National Reference Laboratory for Histocompatibility (LNRH), Geneva University Hospital, Geneva, Switzerland*
*3. Department of Hematology-Oncology and Stem Cell* Transplantation*, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Hematopoietic stem cell transplantation (HSCT) is the best therapy for hematologic malignancies such as acute leukemia. Although, graft versus-leukemia (GVL) is an important approach for prevention of relapse, it could be associated with severe graft versus host disease (GVHD). Similar to T cells, NK cells may facilitate engraftment, combat infection, and control malignant cells but do not cause GVHD. KIRs are a family of inhibitory and activating receptors that are expressed mainly by NK cells which bind HLA as ligands. Because of high variability in KIRs, careful selection of donors based on HLA and KIR is essential to optimize HSCT outcomes. Since donor KIRs are potentially encountered with non-self recipient HLA class I epitopes, this study was designed to find out the impact of KIR/HLA combinations on the outcome of HSCT in patients with acute leukemia.
**Methods:** From March 2015 to March 2016, a total of 31 patients with acute leukemia who received HSCs were followed for one year by chimerism analysis (30, 60, 90, 180 and 365 days after HSCT). During this period, 17 of these patients were excluded from further analysis due to the death of GVHD or GVL. KIRs genotyping of the donors and their corresponding ligands in recipients was also investigated. All alive patients were received HLA-A/B/DRB1-matched HSCs from their siblings. Three patients received HSCs from opposite sex, four patients from ABO-incompatible and one patient from Rh-incompatible donor. Chimerism was not observed in none of the recipients at the studied time points after HSCT in 14 patients who were alive at least for the first year post-HSCT.
**Results and Conclusion:** All donors had KIR-B haplotype but just five of them carried KIR2DS1 while seven patients were C2/C2, three patients were C1/C2, four patients were C1/C1. Our results showed that HSCT from donors with KIR-B haplotype to C2-C2 or C1-C2 bearing recipients was associated with a better outcome during the first year after transplantation. Obscure points will be cleared by analysis of chimerism and KIR/HLA combinations in the samples of expired patients.

**Keywords:** Leukemia, Human leukocyte antigen , Killer cell immunoglobulin like receptor

ICIA2018\Transplantation\Poster Discussion\8571
**8571**

# Association of Epinephrine and Norepinephrine Plasma Levels and Expression Levels of β2-adrenergic Receptor Gene with Acute GVHD

Zahra Momeni[1], Aliakbar Movassaghpour[1], Mehdi Talebi[1], Mohammad Hossein Kazemi[2,3], Elham Roshandel[3], Abbas Hajifathali[3]

*1. Hematology & Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
*2. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*
*3. Hematopoeitic Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Graft versus host disease (GvHD) is the major complication of hematopoietic stem cell transplantation (AHSCT). Epinephrine (E) and norepinephrine (NE) are stress hormones recently found to have immunomodulatory properties and can decrease the probability of GvHD through interaction with adrenergic receptors. In this study, we have compared the levels of epinephrine and norepinephrine in plasma and also the expression of $\beta_2$-adrenergic receptor gene in peripheral blood mononuclear cells (PBMCs) of patients who develop acute GvHD (aGvHD) with recipients without aGvHD.
**Methods:** We have studied 15 candidates for AHSCT with median age of 38 years old (range: 25-45). Blood samples were taken from patients 7 days before and after of transplantation and plasma levels of E and NE were measured using Enzyme-linked Immunosorbent Assay (ELISA). After extracting RNA from buffy coats and cDNA synthesis, the expression of $\beta_2$-adrenergic receptor were measured using realtime-PCR. The occurrence of aGVHD were measured according patient's clinical manifestations. T-test was used to analyze data of the study.
**Results:** Our results showed that 5 patients out of 15 patients presented aGVHD during 100 days after AHSCT. The mean levels of E and NE in plasma and also the expression of $\beta_2$-adrenergic receptor in PBMCs of patients who presented aGvHD were significantly lower than recipients without aGVHD (P-value <0.05).
**Conclusion:** According to the lower levels of E and NE in plasma and lower expression of $\beta_2$-adrenergic receptor in PBMCs of patients with aGVHD in comparison with recipients without this complication, we concluded that these stress hormones and their receptor are associated with lower immunomodulation and consequently might have a role in developing aGVHD and could be a marker for prediction and management of aGVHD.

**Keywords:** Epinephrine, Norepinephrine, $\beta_2$-adrenergic receptor, aGVHD

ICIA2018\Transplantation\Poster Discussion\9741
**9741**

# Pre-transplant Serum Level of ferritin as Prognostic Markers of Bacterial Infection in Hematopoietic Cell Transplantation

Maryam Nikoonezhad[1,3], Mahshid Mehdizadeh[3], Elham Roshandel[2, 3] , Abbas Hajifathali[3*]

1. *Department of Immunology, faculty of Medical Siences, Tarbiat Modares University*
2. *Department of Hematology, faculty of Medical Science, Tarbiat Modares University*
3. *Taleghani Bone Marrow Transplantation Center, Shahid Beheshti University of Medical sciences, Tehran, Iran*

**Background**: Although the kind of antibiotics is used to prevent bacterial infection after hematopoietic cell transplantation (HCT), due to the growth of antibiotic-resistant microorganisms, bacterial infection is one of the barriers to the success of HCT, so it is important to identify patients who are at risk of infection. Ferritin, CRP and ESR are considered, as a potential biomarker for predicting bacterial infection. Also, recently some studies have shown high levels of pre-transplant ferritin associated with mortality and morbidity after autologous or allogeneic hematopoietic cell transplantation. In this study, we have evaluated the correlation between serum level of ferritin, ESR, CRP and, prevalence of bacterial infection in patients who underwent hematopoietic cell transplantation.
**Methods**: We studied 64 patients with hematological malignancies who received BM transplants without any antibacterial prophylaxis. We measured the serum level of ferritin, CRP and ESR of these individuals before and after HCT. Infection in these patients was confirmed by blood culture, then studied the association of these markers with post-transplant infections.
**Results**: The result showed 11 patients had mild to severe bacterial infection after HCT. Serum level of pre-transplant ferritin increased in 5 patients who have bacterial infection after HCT. Also, there was a significant correlation between high levels of ferritin before HCT and infection after HCT but there was no significant relationship between the serum level of CRP and ESR with HCT.
**Conclusion**: These results suggest that pre-transplant serum ferritin level may be a useful marker for predicting the risk of early bacterial complications after allogeneic HCT.

**Keywords**: hematopoietic cell transplantation (HCT), ferritin, C-Reactive Protein (CRP), *Erythrocyte Sedimentation Rate (ESR)*, Bacterial Infections.

ICIA2018\Transplantation\Poster Discussion\9797
**9797**

# Evaluation of the Serum Level of Interleukin-6 in Liver Ischemia-reperfusion Injury in Rat

Azad Bahrampour[1], Roonak Azizbeigi[2*]

*1. DVM Student, Faculty of Veterinary Medicine, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran*
*2. Assistant of Physiology, Department of Physiology, Faculty of Veterinary Medicine, Sanandaj Branch, Islamic Adzad University, Sanandaj, Iran*

**Background:** Liver ischemia-reperfusion injury occurs in a number of clinical settings, including liver surgery, transplantation and hemorrhagic shock with subsequent fluid resuscitation. It has been well demonstrated that there is a significant cause of morbidity and mortality which is characterized by oxidative stress accompanied with depletion of antioxidants. Interleukin-6 (IL-6) is an acute phase reactant cytokine with pleiotropic biological effects. This cytokine plays a critical role in hematopoiesis, host defense, and inflammation. The studies showed that IL-6 has a key role in anti-inflammatory properties. The aim of this study was to investigate the serum concentration of IL-6 in liver ischemia-reperfusion injury.
**Methods:** Adult male Wistar rats (n=10, weighing 220-250 gr) were used in this experimental. The animals were divided randomly into two groups 1) surgical control group, and 2) ischemia–reperfusion group. The control group underwent surgery but did not tolerate liver ischemia-reperfusion. The animals in the ischemia–reperfusion group were under surgical procedure and underwent ischemia for 45 minutes, then undergone liver reperfusion for 45 minutes. At the end of the study, serum concentration of IL-6 was evaluated by enzyme-linked immunosorbent assay kit (ELISA).
**Results:** The results showed that serum concentrations of IL-6 were 38.46±3.64 pg/ml and 385.79±12.96 pg/ml in the control group and ischemia–reperfusion group, respectively. There was a significant difference between the groups ($P<0.01$).
**Conclusion:** It can be concluded that liver ischemia-reperfusion injury can induce significant alterations in the serum concentrations of IL-6. It could form the basis for the performance of large scale prospective randomized clinical trials of liver ischemia-reperfusion injury.

**Keywords:** Liver ischemia-reperfusion, Rat, Interleukin-6

ICIA2018\Transplantation\Poster Discussion\9844.doc
**9844**

# Study of the Association between Genes Polymorphisms miRNA 146G/C and miRNA 196a2C/T and Outcome of Kidney Transplants in Iranian Patients

Anahita Abbasi,[1] Padideh Ebadi,[2,*] Mohammad Hossein Karimi,[1] Ramin Yaghobi[1]

1. *Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, IR Iran*
2. *Islamic Azad University of Shiraz, Shiraz, IR Iran*

**Background**: Acute rejection remains a serious clinical challenge and the most serious complication in the first year of transplantation. The interaction of microRNA (miRNA) in renal transplant rejection represents an important research area for developing clinical programs. The differential expression of miRNAs in the circulation and biopsy of the organism indicates a positive association with the allograft state. Therefore, in this study the pattern of miR-146a G>C and 196a-2 C>T gene polymorphisms was evaluated in kidney transplant patients.
**Methods**: Tissue samples were collected from 100 renal transplant patients between years: 1386-1392. The miR-146a G>C (rs2910164) and miR-196a-2 C>T (rs11614913) gene polymorphisms were evaluated in kidney transplant patients using in-house-PCR-RFLP methods.
**Results**: The CC genotype, C and G alleles of the miR-146a G>C (rs2910164) polymorphism is associated with increased risk of transplant rejection in kidney transplant patients (P= 0.003, P=0.01 and P=0.01), respectively. The CC genotype, T and C alleles of the miR-196a-2 C>T (rs11614913) were also significantly more frequent in transplanted patients versus Healthy sample ones (P= 0.02, P= 0.05and P= 0.05), respectively. But significant associations were not found between miR-196a-2 C>T (rs11614913) polymorphisms with outcomes in kidney transplant recipients.
**Conclusions:** The CC genotype, G and C allele of the miR-146a and also, the CC genotype, T and C alleles of the miR-196a may be genetic susceptible factors for transplant rejection especially in the patients. It is obvious that further studies are required to validate these findings in a larger population, as well as in patients with different ethnic origins.

**Keywords:** Kidney transplant, MicroRNA

ICIA2018\Transplantation\Poster Discussion\9938
**9938**

# The Effect of Exercise on Immune Factors Level and FOXP$_3$ Gene Expression of Renal Transplanted Patients

Jamshidian Tehrani Narges[1], Karimi MH[2*], Hemati N[3]

1. *Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
3. *Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Renal transplantation is considered to treatment for most patients with end-stage of renal failure. Kidney transplanted patients are exposed to different infectious diseases because of prolonged usage of immune suppressant drugs. On the other hand, exercise may induce beneficial effects on immune system. The present study was performed to examine the effects of 12-weeks selective exercise on interleukin 4 and FOXP3 in kidney transplanted patients.
**Methods:** Twenty three kidney transplanted patients voluntarily and objectively were selected and randomly divided to control (n=10) and training groups (n=13). Exercise groups participated in training program for 12 weeks, three days a week each day 60–90 minutes. During this time the control group did not participate in any regular exercise. The blood samples were taken before and after 12 weeks. Cytokines expression in blood samples were measured by ELISA and gene expressions quantified with real-time PCR. Data were analyzed using t dependent test.
**Results:** After 12 weeks, there was no difference of IL-4 level and FOXP3 gene expression, between training group and control group was seen however IL-6 was significantly increased and TNFα was significantly decreased in trained group.
**Conclusion:** It can be concluded that doing exercise may activated immune system in spite of usage of immune suppressant drugs.

**Keywords:** Renal transplant, Interleukin 4/6, Exercise training, FOXP3, TNFα

ICIA2018\Transplantation\Poster Discussion\9952
**9952**

## Evaluation of Lnc RNA Expreession in Rejection and non-Rejection Renal Transplantation

Zahra nekooee zadeh [1], Mohmmad Hosein Karimi[2], Padide Ebadi[3]

1. *Department of Biochemistry, Islamic Azad University of Shiraz*
2. *Department of Transplant Research Center, Shiraz University of Medical Sciences*
3. *Department of Biochemistry, Islamic Azad University of Shiraz*

**Background:** Transplantation is the process of moving cells, tissues or organs from one site to another site. In spite of technical progresses in graft science and immunosuppress drugs there are some barriers to achieve a successful transplantation. One of the most important issues in renal transplantation as an ordinary treatment method is rejection. Recognize of some biomarker and biology mechanism help to reduce the possibility of rejection. Non coding RNA is a functional RNA molecule that is transcribed from DNA but not translated in proteins. Those non coding RNAs that appear to be involved in epigenetic processes can be divided in to 2 main groups the short non coding RNAs(<30nts) and the long non coding RNAs. Lnc RNAs are defined as transcripts longer than 200 nucleotides. In general Lnc RNAs function to regulate gene expression at the transcriptional and post transcriptional level. According to Lnc RNAs in regulation of the expression of the gene, the purpose of this study was using Lnc RNAs as a biomarker in renal transplantation.
**Methods:** This study we used 30 patients with rejection and non-rejection and then extracted RNA by using of the Trizol and convert RNA to cDNA and examined amount of Lnc RNAs expression by NGS method. We chose more patients for confirmation of changes, first all RNA was extracted by using the Trizol. The second step is to convert isolated RNA to cDNA. By using real time PCR method we measured the amount of Lnc RNAs expression.
**Results:** This study indicate NR-110279 is up-regulation in rejected transplantation patients and down-regulated in patients with successful renal transplant. A down-regulation of NR-120448, NR-117089 in reject transplantation and up-regulation in patients with successful renal transplant was observed.
**Conclusion**: According to results maybe NR-110279 upregulation and NR-120448, NR-117089 is down-regulation in reject transplantation can be used as a biomarker. But this is not definitive, must be done by more patients and real time PCR methods, so we can definitely say that is right.

**Keywords:** Transplantation, Kidney, Lnc RNAs, Rejection.

# Vaccine

# Poster Discussion

ICIA2018\Vaccine\Poster Discussion\7350
**7350**

# Expression of OMP19 of *Brucella abortus* on the Surface of *Lactobacillus casei* and Evaluation of Its Immunogenicities in Laboratory Animals

Elham Mohammadi[1, 2], Mehdi Golchin[1]

*1. Department of Pathobiology, Faculty of Veterinary Medicine, ShahidBahonar University of Kerman, Kerman, Iran*
*2. Molecular Microbiology Research Group, Shahid Bahonar University of Kerman, Kerman, Iran*

**Background:** Brucellosis is a zoonotic disease threatening the public health and fails the trading of animals and their products; this has a negative impact on the economic development of a country. Vaccination is the most effective action to control brucellosis. There is no human vaccine against brucellosis, and attenuated live vaccines in animal are useful but have serious disadvantages. The recombinant vaccines are promising candidates for immunization in humans and animals with more efficacies which among them, vector vaccines have significant benefits. *Brucella* cell surface antigens are the first choice to produce a vaccine because they are the primary point of contact between the pathogen and host cells. In *Brucella* genus, there is superficial antigen named OMP19 which is present in all species and biovares of the genus. Theoretically, this antigen can provide protection against all 6 species of *Brucella*. Therefore, it can be considered to produce recombinant vaccine for consumption by majority of hosts. The aim of this study was to produce a vector vaccine using *Lactobacillus casei* strains carrying OMP19 gene and assessment of its immunogenicity in mouse model.
**Methods:** In this study, the gene encoding OMP19 antigen was amplified and cloned into an expression vector called pT1NX and transformed to *L. casei* cell via electroporation technique. The expression was confirmed using specific antibody against the recombinant protein via immunological screening tests such as western blot, dot blot and immunofluorescence. Finally, recombinant *L. casei* was fed to mice and results were recorded.
**Results:** Results showed that, the mice group which had received the OMP19 through *L. casei* based vaccine represented a very good general and mucosal immune responses, similar to commercial vaccine recipient group.
**Conclusion:** Therefore, the vaccine produced in this study could be a very good vaccine candidate against animal and human brucellosis.

**Keywords:** Brucellosis, vector vaccine, *Lactobacillus casei*, OMP19

ICIA2018\Vaccine\Poster Discussion\7415
**7415**

# Comparison of the LD50 Related to Herpes Simplex Virus type I Using Intraperitoneal and Intraocular Injection Routes in BALB/c Mice

Parisa Amir Kalvanagh[1], Hesam Karimi[2], Masoumeh Ebtekara[1*], Hoorieh Soleimanjahi[2], Parviz Kokhaei[3]

1. *Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
2. *Department of Virology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
3. *Department of Immunology, Semnan University of Medical Sciences, Semnan, Iran*

**Background:** In order to evaluate the efficacy of prophylactic and therapeutic candidate vaccines for dealing with herpes simplex virus-related diseases in vivo, the animal model should be challenged with the relevant pathogen. To meet the challenge, it is necessary to determine lethal dose 50 (LD50) of the pathogen in the animal model affected by it. One of the important parameters in this regard is the choice of the route of pathogen injection. Our aim in this study is to compare the LD50 of herpes simplex virus type I isolated from an Iranian subject using intraperitoneal (IP) and intraocular (IO) routes.
**Methods:** After measuring the infectious HSV-1 titer by the TCID50 Assay, 1, 10 and 100 X TCID50 of HSV-1 injected through IP and IO injection routes to the female 4-5weeks old BALB/c mice using insulin syringe (3mice/group). Signs of disease including paralysis, infection, redness, and swelling of the eyes, anorexia, and mortality were recorded during 15 days (twice daily).
**Results:** The LD50 in both injections routes for HSV-1 in BALB/c mice was 1.2X TCID50. IO route practically was more difficult than IP and required experienced people, and there also was the limitation for the volume of injections, and in most cases, the virus needed to be concentrated as well.
**Conclusion:** Applying the IP injection route for prophylactic vaccines and IO injection route for therapeutic vaccines seem more rational.

**Keywords:** Herpes Simplex Virus-1, LD 50, challenge, IP injection route, IO injection route.

ICIA2018\Vaccine\Poster Discussion\7663.doc
**7663**

# Immunogenicity of *Plasmodium falciparum* Merozoite Surface Protein-$1_{42}$ Antigen Formulated with poly (I: C) Adjuvant in BALB/c Mice

Niloufar Rezvani[1, 2], Akram Abouie Mehrizi[1]*, Sedigheh Zakeri[1], Laleh Babaeekhou[2]

*1. Malaria and Vector Research Group (MVRG), Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran, P.O.BOX 1316943551.*
*2. Islamshahr Branch, Islamic Azad University, Tehran, Iran*

**Background:** The C-terminal region of *Plasmodium falciparum* Merozoite Surface protein 1 (PfMSP-$1_{42}$) is a leading malaria vaccine candidate antigen. Nevertheless, the results of clinical trials of MSP-1 based vaccines have not shown complete efficacy. In this regard, the main obstacle is the lack of potent adjuvants suitable for using in human. In this study, to induce high titer of total antibodies and polarized Th1 immune responses against PfMSP-$1_{42}$, poly (I: C) as a potent and human compatible adjuvant was used.
**Methods:** The *pfmsp-$1_{42}$* gene was cloned and expressed in *E. coli* M15-pQE30 expression system. Five test groups of BALB/c mice were immunized with affinity purified recombinant PfMSP-$1_{42}$ antigen alone or in combination with poly (I:C), poly (I:C)/Alum, Alum or CFA/IFA adjuvants. The control groups received PBS1× or corresponding adjuvants without antigen. Immunization was carried out three times, 2 weeks intervals via subcutaneous rout. Anti-PfMSP-$1_{42}$ IgG and IgG subclasses were measured 10 days after last immunization.
**Results:** The results showed that antibody response was in comparable level in the mouse groups that received rPfMSP-$1_{42}$ in combination with poly (I:C) or CFA/IFA adjuvants. Interestingly, among the five test groups, the ratio of IgG2a/IgG1 antibodies was highest in the mouse group that received rPfMSP-$1_{42}$ in combination with poly (I:C) indicating a Th1 immune response.
**Conclusion:** In conclusion, the results showed that in compare with CFA/IFA adjuvant, the poly (I:C) adjuvant could improve the immune responses to rPfMSP-$1_{42}$ antigen, which could be applied in designing in a PfMSP-$1_{42}$-based vaccine.

**Keywords**: Malaria, *Plasmodium falciparum*, PfMSP1-42, Poly(I:C)

ICIA2018\Vaccine\Poster Discussion\7940
**7940**

## *In silico* Evaluation of RNA Based Vaccine Candidate Expressing LmSTI1 and PpSP15 Fusion Protein against Cutaneous Leishmaniasis

Nastaran Sadat Savar*[1], Arash Arashkia[2], Kenneth Lundstrom[3], Ali Khamesipour[4] , Hamid M. Niknam[1]

1. *Immunology Department, Pasteur Institute of Iran, Tehran, Iran*
2. *Department of Molecular Virology, Pasteur Institute of Iran, Tehran, Iran*
3. *Pantherapeutics, Switzerland*
4. *Center for Research and Training in Skin Diseases and Leprosy, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Leishmaniasis is among neglected diseases, 10 percent of the world population are at risk in 11 high-burden countries including Iran. However, there is still no vaccine available against any form of human disease. Self-amplifying mRNA-based vaccines are considered as the next generation vaccines. The main aims of the present study were to design a fusion form of the *Leishmania major* stress inducible protein 1 (LmSTI1) and *Phlebotomus papatasi* SP15 (PpSP15), and evaluate the stability and conformational changes of structures in RNA and protein levels compared to the native forms.
**Methods:** PpSP15/LmSTI1 and LmSTI1/PpSP15 fusion forms were designed by placing the PpSP15 gene sequence at the 5' or 3' ends of LmSTI1. RNA folding and minimum free energies were predicted using the RNAfold webServer. Physical and chemical parameters of proteins were computed by the ExPASY ProtParam tool and three dimensional structures of the proteins were modeled by the I-TASSER server. Modeled structures were validated and evaluated using the RAMPAGE and the ProSa web. Conformational studies on modeled fusion and native forms were performed using the Swiss-Pdb Viewer.
**Results:** According to the RNAfold results, PpSP15/LmSTI1 showed a higher minimum free energy compared to the LmSTI1/PpSP15 fusion form, and it was closer to the LmSTI1 native form. The ProtParam results showed that both fusion proteins were stable, although the half-life of PpSP15/LmSTI1 was estimated to be longer than that of LmSTI1/PpSP15 *in vivo*. Based on calculated RMSDs, the conformational changes of LmSTI1 and PpSP15 in PpSP15/LmsTI1 was shown to be smaller than that of LmSTI1/PpSP15 fusion form.
**Conclusion**: The RNA encoding PpSP15/LmSTI1 has optimal minimum free energy, at the protein level, smaller conformational changes and a longer half-life was seen. It seems reasonable to believe that this fusion construct can be used as a recombinant antigen in a self-amplifying mRNA vaccine platform against leishmaniasis.

**Keywords**: in silico study, RNA-based Vaccine, fusion, Leishmaniasis.

ICIA2018\Vaccine\Poster Discussion\8102.doc
**8102**

# Evaluation of the Adjuvant Effect of Agonists of Toll-like Receptor 4 and 7/8 in a Vaccine against *Leishmania major* in BALB/c Mice

Mosayeb Rostamian[1], Hamid M. Niknam[1]*

*1. Immunology Department, Pasteur Institute of Iran, Tehran 13164, Iran*

**Background:** There is no effective vaccine against human leishmaniasis. Achieving successful vaccines seems to need powerful adjuvants. Separate or combined use of toll like receptor (TLR) agonists as adjuvant is a promising approach in *Leishmania* vaccine research. In present study, we evaluated adjuvant effect of separate or combined use of a TLR7/8 agonist, R848 and a TLR4 agonist, monophosphoryl lipid A (MPL) beside soluble *Leishmania* antigen (SLA) in BALB/c mice.
**Methods:** Mice were vaccinated three times by SLA with separate or combined TLR7/8 and TLR4 agonists and were then challenged by *Leishmania major*. Delay type hypersensitivity, lesion development, parasite load, and cytokines (interferon gamma, and interleukin-10) responses were assessed.
**Results:** Results showed: 1) MPL can slightly assist SLA in parasite load reduction, but it is not able to increase SLA ability in evoking DTH and cytokine responses or decreasing lesion diameter. 2) R848 does not affect the DTH response and parasite load of mice vaccinated with SLA, but it decreases/inhibits cytokine responses induced by SLA, leading to increase lesion diameter. 3) MPL neutralized inhibitory effect of R848.
**Conclusion:** In overall, these data emphasize that MPL slightly assists SLA to make a more potent vaccine, but R848 is not a good adjuvant to induce T cell-dependent immune response in BALB/c mice, and therefore combination of these TLR agonists in the current formulation, is not recommended for making a more powerful adjuvant.

**Keywords**: *Leishmania major*, Monophosphoryl lipid A, R848, Soluble *Leishmania* antigen.

ICIA2018\Vaccine\Poster Discussion\8248
**8248**

# PPD Shifted Cellular Immunity into Th1 Pattern versus Commercial HBS Vaccine Formulated with Montanide ISA 720

Bahareh Golkaran[1], Setareh Haghighat [2], Mehdi Mahdavi [3,*]

1. *Department of Microbiology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, IR Iran*
2. *Department of Microbiology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, IR Iran*
3. *Department of Immunology, Pasteur Institute of Iran, Tehran, Iran*

**Background:** The best way for prevention of hepatitis B infection is vaccination. Commercial HBs vaccine is formulated in alum adjuvant and does not stimulate cellular immunity in an appropriate manner. PPD is the protein extract derivate from Mycobacterium promoting the cellular immunity strongly. Studies indicate that PPD could improve the immunity responses through affecting on dendritic cells. In this study, the novel formulation of HBs Ag vaccine in companion with PPD was used to stimulate cellular immune responses.
**Methods:** The commercial HBs Ag vaccine was formulated in 1 and 10 µg of PPD and the mice were injected subcutaneously three times with two week interval with different formulation of vaccines. Total antibody and IgG1, IgG2a isotypes were assessed in serum samples while cytokines level including IL-4, IFN-γ, TNF-α, IL-2 was evaluated in spleen cell culture via ELISA method.
**Results:** Our findings show that formulation of commercial HBs vaccine in PPD improved Th1 immune response via higher IFN-γ and IL-2 cytokine release
**Conclusion:** The results of this study showed the ability of PPD as immunopotentiator in vaccine formulation for stimulation of better Th1 cytokine response.

**Keywords:** HBS Ag1, Vaccine2, PPD3, adjuvant4, montanide ISA7205.

ICIA2018\Vaccine\Poster Discussion\8358
**8358**

# Naltrexone; as an Efficient Adjuvant in Induction of Th1 Immunity and Protection against *Fasciola hepatica* Infection

Hakim Azizi[1], Hadi Mirzaeei[2], Ali Akbar Nasiri[3], Hajar Yaghoobi[4]

*1. Department of Medical Parasitology, School of Medicine, Zabol University of Medical Sciences, Zabol, Iran*
*2. Department of Medical Genetics, School of Medicine, Zabol University of Medical Sciences, Zabol, Iran*
*3. Department of Anesthesiology, Zabol University of Medical Sciences, Zabol, Iran*
*4. Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran*

**Background:** Toxic effects of available therapeutics are major drawbacks for conventional management approaches in parasitic infections. Vaccines have provided a promising opportunity to obviate such unwanted complications. In present study, we examined immune augmenting and protective capacities of an emerging adjuvant, Naltrexone, against *Fasciola hepatica* infection in BALB/c mice.
**Methods:** Seventy BALB/c mice were divided into five experimental groups (14 mice per group) including 1- control (received PBS), 2- vaccine (immunized with *F. hepatica* E/S antigen), 3- Alum-vaccine (immunized with Alum adjuvant and E/S antigens), 4- NLT-vaccine (immunized with NLT adjuvant and E/S antigen), and 5- Alum-NLT-vaccine (immunized with Alum-NLT mixed adjuvant and E/S antigen). Lymphocyte stimulation index was assessed by MTT assay. Production of IFN-γ, IL-4, IgG2a and IgG1 were assessed by ELISA method.
**Results:** Results showed that NLT, either alone or in combination with alum, can induce immune response toward production of IFN-γ and IgG2a as representatives of Th1 immune response. Also, using this adjuvant in immunization experiment was associated with significantly high proliferative response of splenocytes/ lymphocytes. Utilization of Alum-NLT mixed adjuvant revealed the highest protection rate (73.8%) in challenge test of mice infected with *F. hepatica*.
**Conclusion:** These findings suggest potential role of NLT as an effective adjuvant in induction of protective cellular and Th1 immune response against fasciolosis.

**Keywords:** Naltrexone, vaccination, Fasciolosis, *Fasciola hepatica*

ICIA2018\Vaccine\Poster Discussion\8379
**8379**

# Immunogenecity Oral Value of *B.mellitensis* bp26 Gene Expressed in *Lactococcus lactis* as a Food Grate Vector in Mice Balb/c

Seyed Davood Hosseini [*1], Maryam.Azizpour.m (PhD) [2], Parvaneh Jafari [2], Mohadesehsadat Hosseini[3]

1. *Razi vaccine and serum Research institute, Arak Branch, Arak, Iran*
2. *Department of Microbiology, Islamic Azad University, Arak Branch, Arak, Iran*
3. *Faculty of Biotechnology.Alzahra University,Tehran, Iran*

**Background:** Brucellosis is still an important health problem in under developing countries. Several animal vaccines are produced but the complete protection is not achieved. A good candidate for brucellosis vaccine is *B.mellitensis* bp26 gen. Today, *Lactococcus lactis* with several positive characteristic are attractive for protein expression. These fast growing bacteria no need to aeration, easy to handling, have not exotoxin, endotoxin and protease, so the cost of culturing is inexpensive.
**Methods:** In this study, *B.mellitensis* bp26 gen was cloned in food grade PNZ 8149 vector and expressed in *L.lactis* NZ 3900. Fifteen BALB/c mice were divided into two groups (groups A–B). Group A mice were orally gavaged with 2-5 × $10^9$ CFU per mL of transformed *L.lactis* with PpNZ + bp26. Group B mice were orally gavaged with 2-5 × $10^9$ CFU per mL of *L.lactis* with pNZ. So, immunosorbent assay were conducted.
**Results:** Data shown the mean absorbance readings of the mice prior to and post immunization, were different significantly. In the three weeks following vaccination, the recombinant 8149 + bp26 treatment group showed an increase in antibody level whereas the non-recombinant *L. lactis* showed weak responses. The antibody levels were increased significantly in the 8149 + bp26 vaccinated group in relation to the non-recombinant *L. lactis* group ($P< 0.05$).
**Conclusion:** *L.lactis* can be used as a live delivery vector.

**Keywords:** *Lactococcus lactis*, *B.mellitensis,* bp26 gen, OMP28

ICIA2018\Vaccine\Poster Discussion\8455
**8455**

# A Lower mRNA Expression level of TLR2, 3, 4 in PBMCs Isolated from non-responders Children to HBV Vaccine

Kousar Smailneja ganji [1, 2], Mehdi Shahbazi [2], Mousa Mohammadnia-Afrouzi [2], Mojdeh Ghias Tabari[3], Motahareh Ehsani[2], Majgan Bagherzadeh[2]

*1. Student researchcommittee, Babol University of medical sciences, Babol,Iran*
*2. Department of Immunology, School of Medicine, Babol University of Medical Sciences, Babol, Iran*
*3. Department of Biochemistry, School of Medicine, Babol University of Medical Sciences, Babol, Iran*

**Background:** Toll like receptors (TLR) have an important role in the innate immune system and also able to recognize Hepatitis B virus that exert effect on immune response. Many host related factors such as the expression of TLRs, contribute to vaccine response. This study was designed to determine the expression levels of TLR2, 3, 4 genes as indicator of innate immunity response to the hepatitis B vaccine among 3-5 years old children.
**Methods:** The study was carried out among 60 children with age 3-5 years old who received HBV vaccine (43 responders, 17 non responders). Blood samples were collected, then the expression ofTLR2, 3, 4 genes in peripheral blood mononuclear cells (PBMCs) were analyzed by Real Time PCR method.
**Results:** The results of this study indicated that the average expression of TLR2, 3, 4 genes in the responder group was higher than the non-responder group. Additionally, the expression levels of TLR 3, 4 genes were significantly higher in responders compared to non-responders (P-Value≤ 0.01).
**Conclusion:** This study suggest that the expression of TLR2, 3, 4 could be considered as a marker of protection along with serological markers. Indeed, evaluation of the HBs antibody titer and the expression level of immune response genes together can also be used to determine efficiency of hepatitis B vaccine in responder and non-responder individuals.

**Keywords:** Hepatitis B vaccine, TLR2, 3, 4 genes and Real Time PCR

ICIA2018\Vaccine\Poster Discussion\9963
**9963**

# Oral Administration of Mice with Chimeric Protein Containing Urease, Omp31 and Omp19 Induces High Protection against *Brucella melitensis* and *Brucella abortus* Infections

Morteza Abkar [1], Saeed Alamian [2], Mahdi Fasihi Ramandi [3], Abbas Sahebghadam Lotfi [*4]

1. *Nanomedicine and Nanobiology research center, Shiraz University of Medical sciences, Shiraz, Iran.*
2. *Brucellosis Department, Razi Vaccine and Serum Research Institute, Karaj, Iran.*
3. *Molecular Biology Research Center, System biology and poisonings institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.*
4. *Department of Clinical Biochemistry, Faculty of Medicine, Tarbiat Modares University*

**Background:** Brucellosis is a group of closely related zoonotic bacterial diseases caused by members of the genus Brucella. It was demonstrated that Brucella (B) Urease, B. melitensis Omp31 and B. abortus Omp19 are promising candidates for subunit vaccines against brucellosis. It was hypothesized that application of three proteins can increase vaccine efficiency.
**Methods:** Three proteins were attached by hydrophobic linkers. Particle size and loading efficiency of the nanoparticles were determined. Immunogenicity of protein with Freund's adjuvant and N-trimethyl chitosan (TMC/antigen) nanoparticles as well as effect of its administration routes (i.p. and oral) on immunological responses and protection was investigated in mice. Antibody detection, cytokine measurement and protection assay were performed. Finally, immunized mice were challenged with the virulent *B. melitensis*16M and *B. abortus* 544.
**Results:** Bioinformatics analysis showed chimeric protein has proper stability and conformation, and it can potentially induce humoral and cellular immune responses. After protein expression and purification, chimeric protein was loaded onto TMC nanoparticles. The results indicated that i.p. immunization of chimeric protein with Freund's adjuvant and TMC nanoparticles induces Th1-Th2 immune responses, whereas oral administration of the chimeric protein elicited Th1-Th17 immune responses. Oral administration of chimeric protein encapsulated in TMC nanoparticles displayed the highest protection level (2.58 and 2.37, respectively) against virulent strains of *B. abortus* and *B. melitensis*.
**Conclusion:** The chimeric protein can be a proper oral vaccine candidate against brucellosis.

**Keywords:** Chimeric Protein, Trimethyl Chitosan, Oral Vaccine.

# Veterinary Immunology

# Poster Discussion

ICIA2018\Veterinary Immunology\Poster Discussion\7812
**7812**

# Production, Purification and Evaluation of Specific Egg Yolk Immunoglobulin (IgY) Against Vibrio Fluvialis of Hyper Immunized Hen Egg Yolks

Mojtaba Mirzaei[1], Mohsen Najimi[2], Mohammad Jahantigh[3], Firoz Ebrahimi[4], Abbas Jamshidian[2], Narjes Hasan Fakhrabadi[5]

1. *Student of Department of Pathobiology School of Veterinary Medicine University of Zabol. I.R. Iran*
2. *Department of Pathobiology School of Veterinary Medicine University of Zabol I.R. Iran*
3. *Department of Clinical Science School of Veterinary Medicine University of Zabol I.R. Iran*
4. *Department of Biotechnology School of Basic Science University of Imam Hossein I.R. Iran*
5. *Student of Department of Pathobiology School of Veterinary Medicine University of Zabol. I.R. Iran*

**Background:** New technologies, such as immunoglobulin egg yolk (IgY), has been in progress for therapeutic and diagnostic purposes and has been used to control many infections and results of various studies have shown that IgG immunoglobulin has inhibitory effect on the growth of many bacteria in liquid medium and animal body, the tendency to use egg yolk is increasing to produce polyclonal antibodies for application and economic purposes. *V. fluvialis* cause diseases in Human and fish. This bacterium is one of the most important factors in the development of cholera like diarrhea in human beings in underdeveloped countries.
**Methods:** The purpose of this study was to produce, purify and evaluate specific immunoglobulin (IgY) agenst *V. fluvialis* in hyper-immunized hen egg yolks. For this purpose, we immunized laying hens with formalin killed bacterial cells, IgG against *V. fluvialis* was detected in the serum of the laying hens, then IgY (IgG transferred to the yolk) using PEG 6000 from egg yolks were purified then Dialyzed. IgY evaluation was initially investigated by proteinometry to ensure produced product contains protein (IgY), then used SDS PAGE method to evaluate the purity and nature of protein. Therefore, the presence of this antibody in the purified product was confirmed. Then, to evaluate the effectiveness of this antibody, Indirect ELISA, accomplished.
**Results:** Concentration of 38 ng IgY/well and above, react with the antigen at Indirect ELISA. The microbial growth inhibitory assay was conducted and results showed that IgY specific anti-*V. fluvialis* with a concentration of 20 mg/ml would inhibit bacterial growth in liquid medium.
**Conclusion:** According to the results, it seems that the use of IgY biotechnology to produce specific antibody against *V. fluvialis* could control pathogenicity of bacterium easily and at low cost and can be a good alternative to antibiotics.

**Key words:** egg yolk immunoglobulin (IgY), *V. fluvialis,* Indirect ELISA, SDS-PAGE

ICIA2018\Veterinary Immunology\Poster Discussion\7978
**7978**

**Comparison of Three Precipitation Methods for Isolation of IgY from Chicken Egg Yolk**

Adibe Rahmani[1], Morad Rahimi[1], Shahrokh Navidpour[2], Ali Mostafaie[3c], Pantea Mohammadi[3], Shahram Parvane[3], Ahmad Bagheri[3]

1. *Department of clinical sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran*
2. *Razi Reference Lab. of Scorpion Research (RRLS), Razi Vaccine and Serum Research Institute, Karaj, Iran*
3. *Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran*

**Background***:* IgY, the major immunoglobulin class in egg yolk, first described in 1893 when Klemperer reported that immunized chickens produce antibodies detectable in their egg yolks as well as their blood. In this study we described three different precipitation methods to isolate IgY from egg yolk of chickens immunized against scorpion venom.
**Methods***:* Eggs from chickens immunized with scorpion venom in poultry farm (Veterinary Faculty, Kermanshah) were collected. For extraction, egg yolks were separated and diluted in distilled water adjusted to a pH 5. Then it was subjected to a freeze-thaw cycle and centrifuged to isolate the water soluble fraction. The supernatant was divided into three identical volume that precipitated by three methods: *Polyethylene glycol*, *ammonium sulfate and sodium chloride precipitation*. For each precipitants, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 12.5% separating and 5% stacking gels. Antibody activity of different extractions were identified by an indirect ELISA assay.
**Results***:* The total activity of IgY fractions precipitated by PEG, *ammonium sulfate* and NaCl were found to be similar and higher than untreated egg yolk. SDS-PAGE analysis indicated that purity of IgY isolated by PEG precipitation method has the highest purity of 94.7%.
**Conclusion***:* PEG precipitation is a simple and mild technique which separates proteins by virtue of their size and solubility. Based on the present results, PEG fractionation method appeared to be more efficient in the isolation of the IgY, while there is no need for any further purification techniques.

**Keywords**: IgY, Purification, PEG, precipitation, Salt

ICIA2018\Veterinary Immunology\Poster Discussion\8170
**8170**

## Evaluation of FMDV Vaccine on Immune Response

Abbas Azadmehr[1], Reza Norian[2], Niloufar Sadat Miri[1], Nowruz Delirezh[2]

1. *Department of Immunology, Babol University of Medical Sciences, Babol, Iran*
2. *Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Foot-and-mouth disease virus (FMDV) is a picornavirus which is highly infectious and economically devastating disease of animals. Inactivated foot-and-mouth disease (FMD) vaccines are currently used worldwide but disease still affects millions of animals around the globe.
**Methods:** This study measured the serum antibodies present in following vaccination with the tetravalent FMDV vaccine two times with a month interval. Serum antibody titers were measured in days 0, 7 and 14 after first vaccination and day 7 after booster, in order to determined high and low titer responder groups of antibodies. Two weeks after booster the PBMC of these groups were isolated, in order to evaluate lymphocyte proliferation at the present of mitogens and specific antigen, by MTT test. The stimulation index (SI) of proliferation in high responder group was higher than low and control groups ($P<0.05$).On the third day of culture, the concentrations of IL-2, IL-4, IL-5, IL-6, IL-10, IL-17, IFN-γ were assayed by ELISA method.
**Results:** The result showed that increased in IL-2, IL-4, IL-5 and IFN-γ level of high responder group means that the immune response to vaccine bias toward Th2 cells and humoral immune response, while in low responder group, poor response of Th2 cells was shown presumably became of Th17 cells.
**Conclusion:** In conclusion, the low immune response to FMDV vaccine may be because of higher stimulation and cytokine release of Th1/Th2 in comparison of Th2 cell types.

**Keywords**: FMDV, Immune response, Antibody, Cytokine, Vaccine, Mitogen

ICIA2018\Veterinary Immunology\Poster Discussion\8362
**8362**

# The Effect of Levamisole on the Immune System During Thiopental Sodium-induced Anesthesia in Male Mice

Mahdieh Raeeszadeh[1*], Javad Beheshtipour[2]

1. *Department of Basic Sciences, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran*
2. *DVM Student, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran*

**Background:** One of the most important concerns during surgery is anesthetic-induced immunosuppression. Immunomodulators are used to dominate this condition. Immunomodulators are compounds that are capable to regulate the immune system. It is frequently utilized to refer to substances that boost the immune response. Levamisole, a nonspecific immunomodulator, is able to amplify both humoral and cellular immune responses. It has been used to enhance immunity in various diseases. Hence, the aim of this study was to determine the effect of levamisole on the immune system during thiopental sodium-induced anesthesia in male mice.
**Methods:** This study was conducted on 30 male mice (30 ± 5 g). The mice were randomly divided into three groups (ten mice per group). The control group (N) received intraperitoneally (IP) normal saline. The first (T1) and second (T2) experimental groups received levamisole (20 mg/kg, IP) for 5 and 10 days, respectively. Then, all three groups were anesthetized by thiopental sodium (0.5%, 50 mg/kg, IP). In order to determination of the leukogram and neutrophil/lymphocyte ratio (NLR), blood samples were obtained from the orbital sinus before, during and after the study. After thiopental sodium administration, induction and recovery times of anesthesia were recorded. Data were analyzed using the ANOVA and Tukey's post hoc test.
**Results:** The leukocytosis was the remarkable leukogram outcome of the experimental groups. The NLR was significantly increased in the T2 group ($P<0.01$). The anesthesia induction time shows no significant difference between the control and experimental groups ($P>0.05$). The recovery time in the T2 group was significantly lower than the N group ($P<0.05$).
**Conclusion:** The results of this study showed that the levamisole can be used to enhance the immune system in anesthetic condition. Clinical trials are required to characterize the impact of levamisole on the immune system.

**Keywords:** Levamisole, Leukogram, Anesthesia, Mice

ICIA2018\Veterinary Immunology\Poster Discussion\8476
**8476**

## Bee Venom Melittin Effect on Human and Dog Neutrophil Respiratory Burst

*Fatemeh Noparast [1]; Sedigheh Nabian [1*]; Mohammad Taheri [2]; Mohammad Hasan Bemanian [3]; Abbas Gerami[1]; Toran Sepehrzad [3]*

*1. Department of Parasitology and Honey bee , Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran..*
*2. Rastegar Reference Laboratory, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
*3. Department of Allergy and Clinical Immunology, Hazrat Rasoul Hospital, Iran University of Medical Siences, ,Tehran,Iran*

**Background:** The therapeutic application of bee venom has been explained in traditional medicine for treatment of some diseases. Beneficial effects of bee venom and its components have been previously described. One of the most important components of bee venom is melittin. It has been demonstrated to be considered as the anti-inflammatory, anti-viral and anti-bacterial effects, in different cell types. This study was aimed to evaluate the effect of melittin on the respiratory burst of dog and human Neutrophils using chemiluminescence assay.
**Methods:** Neutrophils were collected from three heparinized blood samples of dogs and human using ammonium chloride lysis. The chemiluminescence assay was performed using the luminol to compare the effect of melittin on the respiratory burst function at different melittin doses.
**Results:** The findings showed decreased respiratory burst process in the majority of samples at different doses of melittin, especially at dose of 2.4 ng/ml, while melittin increased the respiratory burst in dogs and human at doses of 0.3 and 0.6ng/ml.
**Conclusion:** Our findings revealed that melittin might affect the respiratory burst at especial doses. Further comprehensive studies are needed to provide new possibilities for treatment of inflammation related diseases.

**Key words:** Melittin, Respiratory burst, Neutrophils, Dog, Human

ICIA2018\Veterinary Immunology\Poster Discussion\8490
**8490**

## Allelic Polymorphism of Ovar-DRB1 Second Exon in Iranian Lori-Bakhtiari Sheep Breed

Seyed Vahid Ganjiani[1], Elham Sadat Hosseini Nasab[2], Atefeh Esmailnejad[3]

1. *Department of Pathobiology School of Veterinary Medicine Shiraz University Shiraz Iran*
2. *Department of Pathobiology School of Veterinary Medicine Shiraz University Shiraz Iran*
3. *Department of Pathobiology School of Veterinary Medicine Shiraz University Shiraz Iran*

**Background:** The major histocompatibility complex (MHC) is the best-characterized genetic region associated with resistance and susceptibility to a wide range of diseases. Response to specific antigens is closely related to the MHC genes and their associations might therefore provide precious answers to main questions about the host–pathogen interactions. MHC of sheep is known as Ovar and is located on chromosome 20. Among Ovar MHC class II genes, DRB1 locus has been found to be highly polymorphic and associated with resistance and susceptibility to infectious diseases.
**Methods:** Ovar-DRB1 second exon allelic diversity was determined in 100 Iranian Lori-Bakhtiari sheep breed using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) technique using *Rsa*I restriction enzyme.
**Results:** PCR–RFLP identified seven distinct *RsaI* restriction patterns (*a, b, c, d, f, g,* and *h*) and 22 genotypes in Lori-Bakhtiari population. Pattern *g* had the highest (20.5 %), and Pattern *h* the lowest (1.0 %) frequency. High level of heterozygosity (82 %) and good genotype frequency fit to the Hardy–Weinberg equilibrium was observed in this population (P=0.26).
**Conclusion:** Ovar-DRB1 genotyping indicated a high level of polymorphism in the studied population. Direct sequencing is recommended for detecting allele sequences and subtypes.

**Keywords:** Major histocompatibility complex (MHC); Ovar-DRB1; Polymorphism; Lori-Bakhtiari

ICIA2018\Veterinary Immunology\Poster Discussion\8623
***8623***

***Selection of High-affinity ssDNA Aptamers for Diagnostic of Infectious Hematopoietic Necrosis Virus (IHNV)***

Rezvan MohammadiNezhad[1], Seyed Amir Hossein Jalali[2, 3], Hamid Farahmand[4]

1. *Department of Biotechnology, College of Agriculture and Natural Resources, University of Tehran, Iran*
2. *Institute of Biotechnology and Bioengineering, Isfahan University of Technology, Isfahan, Iran*
3. *Department of Natural Resources, Isfahan University of Technology, Isfahan, Iran*
4. *Department of Fisheries, Faculty of Natural Resources, University of Tehran, Iran*

***Background:*** Aptamers are single strand DNA or RNA molecules that can bind to a broad range of targets. Aptamers are selected by an iterative process known as Systematic Evolution of Ligands by Exponential Enrichment (SELEX). Due to various advantages such as high temperature-stability, animal free, cost effective production and high affinity and selectivity for the target molecule, aptamers are considered as attractive alternative to the monoclonal antibodies for diagnostic and therapeutic purposes. The objective of this study was to select DNA aptamers that can specifically bind to Infectious Hematopoietic Necrosis Virus (IHNV) nucleoprotein via SELEX method.
***Methods:*** The selection was started with a synthetic ssDNA library of $10^{18}$ random molecules with 80 bp length. The purified recombinant IHNV nucleoprotein immobilized on Ni-NTA resin was used as the target protein. The recovered aptamers of each round were amplified by asymmetric PCR and used as new pool for the next round. Starting from the sixth round, counter selection with a very similar protein to IHNV-nucleoprotein was also performed to improve specificity. After 12 rounds, selection of high affinity aptamers to target protein was assessed using Enzyme Linked Oligonucleotide assay (ELONA).
***Results:*** ELONA data from 12 rounds of selection showed that the percentage of specific aptamers to IHNV nucleoprotein was continuously increased during the first 9 rounds of selection and then reached a constant level. The binding affinity of aptamers form 9th round in ELONA assay was comparable to the binding affinity of anti-IHNV nucleoprotein monoclonal antibody in ELISA assay. No cross-reactivity was observed between selected aptamers and non-specific targets.
**Conclusion:** Finding of this study suggests that aptamers may be a good alternative detection probe to antibodies for rapid and specific detection of IHNV virus.

**Keywords:** Aptamer, SELEX, IHNV, Nucleoprotein

ICIA2018\Veterinary Immunology\Poster Discussion\9880
**9880**

# An Immunoblot Test Based on Whole Tachyzoites Lysate for Diagnosis of Humoral Response of Domestic Cats Infected with Toxoplasma gondii, Rh Strain

Morteza Hosseininejad*[1], Hosseini F[1]

*1. Faculty of Veterinary Medicine, Shahrekord University, Shahrekord-Iran*
*2. DVM Student of Veterinary Medicine, Shahrekord University, Shahrekord-Iran*

**Background**: Toxoplasma gondii, an obligate intracellular protozoan parasite infects human and all warm blooded animals. Three practical ways to diagnose T. gondii infection in living cats include: parasite isolation, fecal examination for oocysts and serologic tests. Isolation of the parasite is difficult, costly and time consuming and it is not easy to perform in routine diagnostic laboratories. The aim of this study was to detect anti-Toxoplasma gondii antibodies in cats infected with Rh strain of T. gondii by using an immunoblotting method.
**Methods:** eight cats were experimentally infected using tachyzoites harvested from Vero cell cultures. Tachyzoites were then lysed and transferred to polyacrylamide gels followed by blotting to PVDF membranes. An immunoblotting was performed using these membranes to detect IgG antibodies.
**Results**: In the present study, positive lanes were detected in regions related to the presence of T. gondii surface antigens, dense granular or rhoptry proteins. No positive lanes were detected in serum samples of kittens received only Vero cell lysates (controls). Positive or negative results were in concordant to the results of IFAT.
**Conclusion:** The results of this study revealed that whole tachyzoites antigen based immunoblotting is an appropriate diagnostic test for serological detection of anti-T. gondii antibodies in recently infected kittens with this Apicomplexan parasite.

**Keywords**: Immunoblotting, Cats, Toxoplasma gondii.

ICIA2018\Veterinary Immunology\Poster Discussion\9989
**9989**

**Beneficial Effects of *Lactobacillus paracasei* on Isoproterenol-Induced Heart Failure in Rat**

ZohrehHesari[1], Khatereh Kafshdouzan[2*], Parviz Kokhaei[3], Bahador Bagheri [4], Sahar Ghaffari[5]

1. *Faculty school of of Veterinary Medicine, Semnan University, Semnan, Iran*
2. *Department of pathobiology, School of Veterinary Medicine, Semnan University, Semnan, Iran*
3. *Cancer Research Center and Department of Immunology, Semnan University of Medical Sciences, Semnan, Iran*
4. *Cancer Research Center and Department of Pharmacology, Semnan University of Medical Sciences, Semnan, Iran*
5. *Veterinary Pathologist, , School of Veterinary Medicine, Tehran University, Tehran, Iran*

**Background**: Heart failure causes extensive complications on the heart by cardiac dysfunction and hypertrophy. The emerging evidences suggest that the administration of different species of probiotics has a number beneficial therapies on heart failure. This study was performed to determine whether oral probiotic administration can attenuate or prevent the heart failure induced by isoproterenol.
**Methods:** Adult male Wistar rats (n=40, 185±15g), were randomly assigned to five groups, including the control group, probiotic control (0.25 mg/kg/day, oral), isoproterenol control (5 mg/kg/day, subcutaneous), pretreatment group and treatment group were divided. The groups were studied for 30 days. Serum levels of ANP and chemerin were assessed by ELIZA and the standard histological examination was done.
**Results:** This study showed that ANP in the treatment group compared to control group and isoproterenol control, had a significant reduction ($p<0/05$) and chemerin were not observed a significant reduction between groups. Pretreatment with *L. paracasei* subpp. *paracasie* 8700:2 could not prevent the development of heart failure induced by isoproterenol.
**Conclusion**: According to this study, receiving *L. paracasei* subpp. *paracasie* 8700:2 attenuated the serum levels of ANP, a significant improvement in cardiac hypertrophy and the diminishment inflammation.

**Keywords**: heart failure, isoproterenol, *Lactobacillus paracasei* subpp. *paracasie* 8700:2, ANP and Chemerin.

ICIA2018\Veterinary Immunology\Poster Discussion\10010
**10010**

# Protein Solubilizing Strategies for Production viral Hemorrhagic Septicemia Virus (VHSV) Immunogenic Glycoprotein in *E. coli* Expression System

Marzieh Mohammadian Manesh[1], Seyed Amir Hossein Jalali[2, 3], mohammad zare mehrjerdi[4], Rezvan Mohammadi Nezhad[5]

1. *Department of Agricultural Biotechnology, University of Shirvan, Iran*
2. *Institute of Biotechnology and Bioengineering, Isfahan University of Technology, Isfahan, Iran*
3. *Department of Natural Resources, Isfahan University of Technology, Isfahan 84156–83111, Iran*
4. *Department of Agricultural Biotechnology, University of Shirvan, Iran*
5. *Department of Biotechnology, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran*

**Background:** Glycoprotein of VHSV, the causative agent for viral hemorrhagic septicemia disease in rainbow trout (*Oncorhynchus mykiss*), is a membrane protein which induce protective immunity against the virus. Hydrophobicity and toxicity of VHSV glycoprotein, prevents soluble expression of this protein in *E. coli* cytoplasm. The present study aimed at developing strategies for soluble expression of VHSV glycoprotein in *E. coli* to be used as subunit vaccine.
**Methods:** The ectodomain sequence of VHSV-G gene was amplified with specific primers. After digestion with *Hind* III and *Bam*H I restriction enzymes, the fragment was inserted into pET41a (containing GST solubilizing tag for cytoplasmic expression) and pMAL-p4X (containing MBP solubility tag for periplasmic expression) bacterial plasmids. Recombinant constructs were then transformed to three *E. coli* expression hosts including BL21 (DE3), Rosetta (DE3) and Shuffle T7 for protein expression.
**Results:** Based on SDS-PAGE and dot blotting assays, both GST and MBP fusion tags resulted in soluble expression of VHSV ectodomain. Protein expression level and solubility were greater in *E. coli* cytoplasm as compared to periplasm. No remarkable difference was observed between Rosetta and Shuffle T7 strains in terms of expression level and solubility, while BL21 strain produced low amount of recombinant protein. The expressed protein was purified using Ni-NTA affinity chromatography and its accuracy was validated with western blotting.
**Conclusion:** The results of this study demonstrated the positive effects of solubilizing tags and engineered host for enhancing soluble expression of highly hydrophobic membrane proteins in *E. coli*. The purified VHSV glycoprotein has the potential to be used as recombinant vaccine for immunization of rainbow trout fish against VHS disease as well as to produce anti-viral antibodies.

**Keywords:** Membrane Protein, Periplasmic Expression, Cytoplasmic Expression, Solubilizing Tags

# Allergy and Immunotherapy of Allergic Disease

# Poster Presentation

ICIA2018\Allergy and immunotherapy\Poster\6324
**6324**

**Epitope mapping of Hemiscorpiuslepturus scorpionvenom by means of phage display**

Nastaran Mohseni[1], Fatemeh Kazemi Lomedasht[1*]

*1. Venom &Biotherapeutics Molecules Laboratory of Biotechnology Research Center of Pasteur Institute of Iran, Tehran, Iran*
*FatemehKazemi-Lomedasht (Corresponding author) Ph.D of medical biotechnology (Assistant professor)*

**Background:** Hemiscorpiuslepturus envenomation is recognized as a serious health concern in tropical regions. Selection of peptides mimicking antigenic and immunogenic epitopes of toxins from random peptide libraries is a novel approach for the development of recombinant toxins and poly-epitopic vaccines.
**Methods:** To achieve this aim, a phage display peptide library and three rounds of bio panning were performed on immobilized antibodies (IgGs).
**Results:** Our results showed that the highest binding of the phage to immobilized horse antibodies occurred in the third round of bio panning. The sequencing results identified unique peptides mimicking the antigenic and immunogenic epitopes of Hemiscorpiuslepturus toxins.
**Conclusion:** The results of this study provide a basis for further studies and the development of a putative epitopic vaccine and a re-combinant toxin.

**Keywords:** Epitope mapping, Hemiscorpiuslepturus scorpion, venom, Phage display

ICIA2018\Allergy and immunotherapy\Poster\6325
**6325**

# Evaluation of food allergens in patients with Atopic dermatitis referring to Mahdieh Hospital in Tehran during the 2017

Sogand Nayeri[1], Mahdieh Amiri[1], Pouya Lahoori[1], FatemehTazari[*2]

*1. Department of biology, Faculty of Basic Sciences, Higher Education Institute of AndisheSazan, Neka, Iran*
*2. Department of biology, Instructor of Microbial Biotechnology, Higher Education Institute of AndisheSazan, Neka, Iran*

**Background:** Atopic dermatitis is a chronic inflammatory disease in the skin, which often is associated with severe itching. This disease is common in children, which usually is latent in half of the cases, in the first year of life; especially in 60% of cases, those ages are under 16. The purpose of this study was to determine the prevalence of common food allergens in people with atopic dermatitis using prick test in Iran.
**Methods:** In this study, all individuals referred to Mahdieh Hospital in Tehran during 2017 were examined and in case of allergy were selected. After diagnosis of atopic dermatitis in these patients, Prick skin test was performed by common food allergens.
**Results:** Among 1100 people with allergies, 306 (approximately 31.4%) were diagnosed with atopic dermatitis. 33% of people at least were sensitive (positive prick test) to one allergen. In people with atopic dermatitis, asthma, allergic rhinitis, and urticaria were 7.1%, 10.8%, and 4.8%, respectively. There was no relationship between gender and food allergy in people with atopic dermatitis. In this study the most common food allergens were yolk (41.2%), glair (39.1%), hazelnuts (35.7%) and peanuts (29.1%), respectively. Foods like rice (5.3%), barley (6.8%), and lamb meat (7.4%) rarely caused allergies.
**Conclusion:** The prevalence of atopic dermatitis in patients with allergies was more than expected. According to this research, several factors contribute to the prevalence of atopic dermatitis, including the early start of auxiliary feeding, especially with milk and eggs, smoking, woolen and nylon coatings, and keeping pets in the living environment, . Thus, this research have strong suggestion on avoiding these factors.

**Keywords**: Atopic dermatitis, Chronic disease, Allergic rhinitis, Prick skin test

ICIA2018\Allergy and immunotherapy\Poster\6359
**6359**

**Relationship between depression and job absenteeism in asthmatic patients**

Mostafa Aalipour[1]. Mohammad Ali Haghighati[2]. Pouria Karimi[3]. Abolfazl Yari[4]

*1. Department of Immunology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran*
*2. Department of Management and medical Information School of Management and medical Information Kerman University of Medical Sciences, Kerman, Iran*
*3. Department of Engineering environmental health, School of Health, Kerman University of Medical Sciences, Kerman, Iran*
*4. Department of Genetics, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran*

**Background:** Asthma is an immunological respiratory disease that has involved about 300 million people around the world in all races and age groups. The heavy economic burden caused by the disease, care and absence of work due to the illness make asthma a public issue. Previous studies have shown that asthmatic attacks are more in patients who develop both depression and asthma than who suffer from asthma only. Therefore, in this study, the relationship between depression and its impact on occupational life and absenteeism was studied in patients with asthma for the first time**.**
**Methods:** This descriptive cross-sectional study was performed on 124 patients with asthma in Kerman during the first half of 1396. The subjects were selected from among the patients referring to Besat Special Clinic and were approved before entering the study. To assess the level of depression and the amount of job absenteeism, the Persian version of the standard questionnaire PHQ-9 and WPAI-Asthma have been used, respectively. For data analysis, SPSS-24 software and Chi-square test have been used.
**Results:** The results showed that depression had a significant relationship with occupational absenteeism in patients with asthma ($P < 0.05$). Average weekly work in health was 48 hours in subjects under study. 50% of the subjects reported that the disease was effective in reducing their effectiveness in the workplace, and 22% had to rest at home for at least 28 hours a week. This was far higher for industrial workers and agricultural workers.
**Conclusion**: people who suffer from asthma and depression at the same time lose at least half of their useful time and, in addition, their effectiveness in the workplace is greatly reduced.

**Keywords:** Asthma, Depression, job absenteeism, PHQ-9,

ICIA2018\Allergy and immunotherapy\Poster\6360
**6360**

# Relationship between depression and quality of life in patients with asthma

Mostafa Aalipour[1]. Pouria Karimi[2], Mohammad Ali Haghighati[3], Abolfazl Yari[4]

*1. Department of Immunology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran*
*2. Department of Engineering environmental health, School of Health, Kerman University of Medical Sciences, Kerman, Iran*
*3. Department of Management and medical Information School of Management and medical Information Kerman University of Medical Sciences, Kerman, Iran*
*4. Department of Genetics, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran*

**Background**: Asthma and depression are among the most common diseases that threaten the human community today. Reports have shown that asthma and its deaths have increased dramatically over the past two decades. In recent years, attention has been paid to the correlation between depression and asthma. It has been shown that asthma is a disease associated with a mental condition and increase in people who suffer from depression, in addition to asthma and more asthmatic attacks occurred. This study examines the relationship between depression and quality of life in patients with asthma in Kerman, Iran.
**Methods:** This descriptive cross-sectional study was carried out on 200 patients with asthma (101 males and 99 females) confirmed their illness and referred to the specialized clinic of Besat in Kerman city in the first half of 1396. To assess the level of depression and quality of life, the Persian version of the PHQ-9 and ASQL questionnaires were used. Data were analyzed by SPSS software version 24 and Chi-square statistical test.
**Results:** The results showed that depression had a significant relationship with quality of life in patients with asthma ($P < 0.05$). 62% of asthmatic patients showed moderate to severe depressions. 95% of the patients had difficulty in their day-to-day activities, and 43% said that coexistence of depression and asthma create problems in their community activities.
**Conclusion:** The prevalence of depression in asthmatic patients shows a high percentage and according to the results, this factor has reduced the quality of life in patients. It seems that the study and monitoring of asthma patients with depression and their treatment can increase the quality of life in these patients and is effective in controlling the disease.

**Keywords:** Asthma, Depression, Quality of Life, PHQ-9, ASQL.

ICIA2018\Allergy and immunotherapy\Poster\7344
**7344**

**Evaluation of IL-10 gene expression following rush immunotherapy in patients with allergic rhinitis**

AmirabbasSalmani[1], FarahzadJabbariAzad[2]*, MojganMohammadi[2], RezaFaridHosseini[2], JalilTavakolAfshari[3], Maryamkhoshkhooi[2], AliFouladvand[1], SajadDehnavi[1]

1. *Department of Immunology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
2. *Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
3. *Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** Allergic rhinitis (AR) is the most common IgE mediated hypersensitivity disease in the world. Because of high prevalence of AR, it burdens high economic & public health-related cost. Rush Immunotherapy (RIT) is a rapid treatment for atopic diseases, but its immunological mechanisms are not fully understood. In present study, we aimed to evaluate the IL-10 gene expression, as one of cytokines produced by regulatory T-cells, following the rush immunotherapy in patients with AR.
**Methods:** Fifteen patients with AR who had a positive Prick test for common regional aeroallergens treated withNuevo RIT protocol (Three-day build up period) for three months. Before and after intervention, clinical symptoms was recorded by questionnaire and whole blood samples collected, RNA extraction from peripheral blood mononuclear was performed then cDNA synthesized to employ for SYBR Green real-time PCR technique. Finally gene expression of IL-10 was determined.
**Results:** The gene expression of IL-10 increased at three months following the RIT, but it was not significant. Significant improvement in the clinical symptoms was recorded following the RIT. ($P<0.05$).
**Conclusion:** As there is no significant difference between the IL-10 gene expression following the RIT, the expression of other cytokine candidate gene of T-regs, such as TGF-beta might be suggested for evaluation in further studies.

**Keywords:** IL10**,** Gene Expression, Allergic Rhinitis, Rush immunotherapy

ICIA2018\Allergy and immunotherapy\Poster\7365
**7365**

# Evaluation of Skin and RAST (radioallergosorbent test) test in patients with immediate hypersensitivity following betalactam(amoxicillin/penicillin) drug usage.

Zahra karami[12], Azam heidari[3]

1. *Shahidbeheshty university of medical sciences*
2. *Hamedan university of medical sciences*
3. *ShahidSadoughi University of Medical Sciences Yazd*

**Background**: Skin test is one of the most important methods for diagnosis of immediate hypersensitivity reaction in patients with following beta-lactam but also due to false positive results, in vitro tests should be done.
**Methods**: we decided to have skin test and radioallergosorbent test (RAST) in 50 patients who took betalactam(amoxicillin/penicillin) with other drugs and showed an immediate hypersensitivity reaction. Skin test was performed with intradermal drug solution and then we demonstrated antibetalactam (amoxicillin/penicillin) specific IgE antibodies by RAST.
**Results**: Out of 50 patients, 43 patients (86%) had positive skin test results (group A) and 7 patients (14%) had negative test results (group B) and of 43 patients group A , 33 patients (76.7%) have high level of allergen specific IgE (3.50-17.49 KU/L) and 10 patients (23.3%) have absent or undetectable allergen specific IgE (<0.35 KU/L) and of 7 patients(100%) group B, 7 patients have absent or undetectable allergen specific IgE (<0.35 KU/L).
**Conclusions**: The results show that in patients who took beta-lactam with other drugs and had immediate hypersensitivity reaction, the test results may be due to drugs other than beta-lactam.

**Keyword:** skin test, Radioallergosorbent test, immediate hypersensitivity reaction, betalactam

ICIA2018\Allergy and immunotherapy\Poster\7373
**7373**

# Relationship between quality of life and Job absenteeism in patients with asthma in Kerman province

Arash Samoudi[1], Abolfazl Yari[2], Mohammad Ali Haghighati[3], Pouria Karimi[4], Mostafa Aalipour[1]

*1. Department of Immunology, Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran*
*2. Department of Medical Genetics, Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran*
*3. Department of Health Management, Policy and Economics, Faculty of Health Management, Kerman University of Medical Sciences, Kerman, Iran*
*4. Department of Environmental Health Engineering, Faculty of Health Engineering, Kerman University of Medical Sciences, Kerman, Iran*

**Background:** Many health care centers are important indicators of life quality assessment studies and practice have understood. Asthma is one of the most common respiratory disorders that can impose serious social and economic consequences on individuals and society. So far few studies have been conducted on the consequences of occupational asthma and its impact on the quality and quantity of occupational life of individuals. The aim of this study is to determine how the impact of asthma on quality of life and ability to work of people employed in various sectors of society.
**Methods:** Cross-sectional and questionnaire study was carried out to determine the relationship between quality of life and job absenteeism in 124 patients with asthma referred to BESAT clinic (Kerman-Iran) in the summer of 2017. All patients were clinically confirmed by the expert and relevant tests. ASQL and WPAI-ASTHMA standard questionnaires were used to assess the quality of life and job absenteeism, which were used in similar studies, and their reliability and validity were confirmed. Finally, for analyzing the data, chi-square test was used in SPSS-16 software environment.
**Results:** Participants in the study included 124 patients, 83% of people aged 20-60 years.95% of people had daily activity limitations. Also, in 50% of patients, the effect of the disease on the effectiveness of the workplace, moderate to severe, was evaluated meanwhile, there was a significant relationship between job absenteeism with different dimensions of quality of life such as age ($p<0.015$), activity restriction ($p<0.005$), public health ($p<0.002$) and emotional states ($p<0.005$).
**Conclusion:** Based on the results of this study, it was found that there is a significant relationship between quality of life indicators and job absenteeism in asthmatic patients.

**Key words:** Asthma, Job absenteeism, Quality of life, ASQL, WPAI-ASTHMA

ICIA2018\Allergy and immunotherapy\Poster\7397
**7397**

# The anti-penicillin antibodies levels in sensitive and normal people to intradermal skin test

Mohammad Khosravi[1*], Fatemeh Boroun[2,] Mohammad Nemati[3]

*1. Department of Pathobiology, Faculty of VeterinaryMedicine, Shahid Chamran University, Ahvaz, Iran*
*2. Student of Veterinary Medicine, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran*
*3. Razi Vaccine & Serum Research Institute ,Agricultural Research, Education & Extension organization (AREEO)*

**Background:** The hypersensitivity reaction to penicillin is a public health problem. Immunological responses to penicillin and other beta-lactam antibiotics can be classified in immediate and non-immediate. The immediate hypersensitivity is mediated by IgE; however the non-immediate sensitivity facilitated by other isotypes of antibody or T lymphocytes. This research detected the non IgE antibody value against penicillin in allergic and normal people.
**Methods:** Thirty-eight samples from patients with positive or negative intradermal skin testing results of penicillin allergy were included in this study. The IgG and IgM levels against penicillin G were defined by in-house ELISA test.
**Results:** The results showed a significant ($P < 0.05$) elevation in total immunoglobulin and IgG of the sensitive groups; whoever the anti-penicillin IgM was significantly greater in non-sensitive people.
**Conclusion:** However the sensitized people to penicillin cannot be certainly detected with the total antibody, specific IgG and IgM value against penicillin; these values are a good indicator for prediction of immediate and late response of the immune system to penicillin.

**Keywords:** intradermal skin test, sensitive and normal people, anti-penicillin

ICIA2018\Allergy and immunotherapy\Poster\7455
**7455**

# Fungal waterborne contamination as a threat for allergic infections in hot springs

Ali Ghajari*[1], Ensieh Lotfali[1]

*1. Department of Medical Parasitology and Mycology, School of Medicine, ShahidBeheshti University of Medical Sciences, Tehran, Iran*

**Background:** Fungi cause allergies and many other adverse health effects. The prevalence of respiratory allergies by fungi such as *Aspergillus spp, Penicilliumspp, Cladosporiumspp, Alternaria spp*. has dramatically increased over the past decades. Hot springs are places for therapeutic purposes. Water of these pools could be carriers for transmission of various fungal diseases. The objective of this study was to survey of allergic fungal in these pools in Iran.
**Methods:** In this cross-sectional study, 150 samples were collected from 50 hot springs. The collected samples were filtered and cultured on SC (Sabouraud's dextrose agar with Chloramphenicol) and SCC (Sabouraud's dextrose agar with Chloramphenicol and Cyclohexamide). The plates were incubated at 25°C for 1-4 weeks. Then according to colony morphology and microscopic characteristic of fungi, the presence of allergic fungi were studied. The grown fungi were identified by macroscopic and microscopic methods.
**Results:** Out of 150 samples, 43 (28.6%) were positive. A total of 356 colonies observed, *Penicillium.spp* 161 colonies (45.2%), *Aspergillus.spp* 134 colonies (37.6%),*Cladospporium.spp* 44 colonies (12.3%), *Alternaria. spp* 17 colonies (4.7%). 134 colonies of *Aspergillus.spp* included *Aspergillusniger* 102 colonies (76%), *Aspergillus flavus* 17 colonies (12%), *Aspergillus fumigatus* 15 colonies (12%). The most common allergic fungi were *Penicillium.spp, Aspergillus.spp*, *Cladosporium.*spp and *Alternaria.spp* respectively.
**Conclusion:** The results of this study showed the presence of allergen fungal in hot springs. Sensitivity to *Aspergillus.spp* has been associated with severe persistent asthma. Allergic bronchopulmonary aspergillosis (ABPA) is caused by *Afumigatus*, *Alternaria.spp* and *Cladosporium.spp*, with the development, persistence, and severity of asthma. Therefore, experimental studies are essential to reduce the pollution of allergic fungal diseases in hot springs in our country.

**Keywords:** Fungal contamination, Allergic bronchopulmonary aspergillosis

ICIA2018\Allergy and immunotherapy\Poster\7496
**7496**

# Seroepidemiology of Toxocariasis in Iranian asthmatic children, A study in Karaj district, Iran

Homa Sadri[1], Mohammad JavadGharavi[2]

1. *Non-Communicable diseases Research Center, Alborz University of Medical Sciences, Karaj, Iran*
2. *Department of Parasitology, Alborz University of Medical sciences, Karaj, Iran Department* of Parasitology ,Iran University of Medical sciences, Tehran, Iran

**Background:** The prevalence of asthma varies widely between countries and further studies are needed to evaluate risk factors for asthma in each region. A relation between asthma and toxocariasis has been reported. We aimed to investigate the possible association between Toxocara infection and childhood asthma in our region.
**Methods:** This was a case-control study involving 192 children aged 2 to 15 years, 92 of whom had asthma and 100 of whom did not have asthma. Blood samples were tested for the presence of Toxocaraantibodies, using ELISA method.
**Results:** Only one patient (1.09%) had positive levels of anti-Toxocara antibodies in case group and no one in the control group. more than 90% of children in both case and control groups were residents of urban area.
**Conclusion:** Toxocara infection is not a common risk factor for childhood asthma in our urban area

**Keywords:** Asthma, Children, Toxocara

ICIA2018\Allergy and immunotherapy\Poster\7546
**7546**

# IgG variables related to age, sex and season in patients with allergies referred to allergy clinic of Ali ibn Abitalib Hospital in Zahedan

FatemehRanjbar*[1], Mahla Jafari[2]

*1,2 Bachelor's degree in laboratory science and member of Young and Elite Researchers Club, Department of Laboratory Sciences, Islamic Azad University, Zahedan Unit, Iran*

**Background:** Allergic diseases are among the chronic diseases. When an extreme immune system is exposed to an allergen, the body has begun to produce a specific antibody called IgG to fight the substance and specifically secreted against antigens.
**Methods:** This descriptive- analytical research was done on a cross- sectional study on 88 individuals who were referred to the allergy clinic of Ali ibn Abi Talib Hospital in Zahedan. Patients reviewed included the ISAAC (International study of asthma and allergies in childhood) Standard and Global Questionnaire, Performing a Skin Prick Test and IgG was measured by ELISA method. Data was analyzed using SPSS version 21 and compiled by using Pearson correlation test.
**Results:** The number of patients was 88, of which 53 were females and 35 were males of these, 11.4% were less than one year, 14.8% were between one to five years, 11.4% between six to fifteen years and 62.5% of the adult population. According to the Pearson correlation test, the correlation between IgG variables with age, gender and season was performed. Results showed that this variable was not related to age and sex (P <0.05) and had only a significant relationship with the season (P> 0 / 05).
**Conclusion:** There is a positive correlation between the IgE variable and the incidence of allergy. Therefore, IgE may predict a higher incidence of allergic diseases.

**Keywords:** Relationship, Variable, Allergy, Zahedan, Season

ICIA2018\Allergy and immunotherapy\Poster\7547
**7547**

# Measuring Immunoglobulin Titer (IgE, IgM, IgG and IgA) in Patients with Allergy Referring to the Allergy Clinic of Imam Ali Hospital

Fatemeh Ranjbar[2] ,MahlaJafari[*1]

*1,2 Bachelor's degree in laboratory science and member of Young and Elite Researchers Club, Department of Laboratory Sciences, Islamic Azad University, Zahedan Unit, Iran*

**Background:** Allergy is a reaction to the immune system's excesses of various factors, when an extreme immune system is exposed to allergens, the body produces immunoglobulin to fight. Immunoglobulins are biological molecules that are active in the immune system and are specifically secreted against antigens.
**Methods:** In this study, changes in immunoglobulins in people with allergies were investigated. For this purpose, 88 patients with allergies referring to the Allergy Clinic of Imam Ali Hospital (Zahedan) in 1396, blood samples were taken and the immunoglobulin IgM, IgG, IgE and IgA were measured by ELISA method. Then, the data were analyzed by SPSS V21 software. Then, Pearson correlation test was used to determine the relationship between immunoglobulins and age, sex, and seasonal variables was investigated.
**Results:** 53 cases were male and 35 were male, 11.4% less than one year old, 14.8% between one and five years old, 11.4% between the ages of six and 15 years, and 62.5% of adults. The most visited was the summer season. Results showed that IgE, IgM, IgA variables had no significant relationship with age, sex and season ($P < 0.05$) and IgG had only significant relationship with seasonal variable ($P > 0.05$). The most changes were seen in IgE header, so that 34 People had abnormal IgE levels.
**Conclusion:** Considering the amount of IgE titer changes in the region and the severity of clinical signs in the inhabitants of this climate, further studies are needed to identify the antigenic action of allergens in different climates to reduce allergic symptoms.

**Keywords:** Allergy, Immunoglobulin, Imam Ali Hospital, Zahedan

ICIA2018\Allergy and immunotherapy\Poster\7553
**7553**

# High Level of Herbal Food Additive Induces Inflammatory Responses in the Allergic Asthma Mice

Seyyede Masoume Athari[1], Bahram Yavari[2], Seyyed Shamsadin Athari[3*]

1. *Department of Biology, Faculty of Basic Sciences, Maragheh University, Maragheh, Iran*
2. *Department of Genetic and Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
3. *Department of Immunology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran*

**Background:** Herbal food additives are used routinely for rich of foods which may exacerbate asthma. The prevalence of allergic asthma has been increased over the past decades, so that prevention is an important strategy in the management of asthma. The aim of this study was to investigate the role of herbal food additive as a stimulus in asthma.
**Methods:** BALB/c mice were divided into four groups as followings; two OVA asthmatic and normal groups, fed with a diet containing herbal food additive and two healthy groups diet with normal food. Inflammation of airway and cytokines level in broncho-alveolar lavage fluid were measured.
**Results:** The group treated with both OVA and herbal food additive showed higher inflammation, interleukin-5 and 13 levels than the group sensitized only with OVA.
**Conclusion:** OVA and herbal food additive group showed more severe allergic asthma symptoms in comparison to the group only sensitized with OVA. Therefore, the herbal food additive can be asthmatic agents.

**Key Words:** Food, allergic Asthma, Immunology

ICIA2018\Allergy and immunotherapy\Poster\7554
**7554**

# Pickles in Iranian food maybe allergic Asthma trigger in mouse model

Seyyed Shamsadin Athari[1], Seyyede Masoume Athari[2*]

1. *Department of Immunology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran*
2. *Department of Biology*, Faculty of Basic Sciences, Maragheh University, Maragheh, Iran

**Background:** Pickles is used in large amount of Iranian family that can be irritant compound for the respiratory system. The prevalence of allergic asthma is increased. The aim of this study was to investigate the role of Pickles as an asthmatic agents.
**Methods:** Fifteen mice were divided into three groups as followings; ovalbumin sensitized asthmatic groups, group that fed with a diet containing Pickles and normal group. Lung histopathology were examined.
**Results:** The group treated with Pickles showed higher lung eosinophilic inflammation and mucus hyper secretion similar to OVA (asthmatic) mice.
**Conclusion:** The Pickles consumption can be important trigger of allergic asthma and it should be used carefully in atopic people.

**Key Words:** Pickles, Allergy, Inflammation

ICIA2018\Allergy and immunotherapy\Poster\7713
**7713**

# The Evaluation of Amount of TGF-β Produced in Supernatant of Co-culturing of DC Pulsed by Somatic Antigenic Extract of *Marshallagiamarshalli* and Peripheral T Lymphocyte in People Suffering from Asthma

Fatemeh Hemati[1], HadiMoheb Alian[2], Hasan Borji[3], Milad Hemati[4], Majid Mirsadrai[5]

1. *Department of Veterinary School of Ferdowsi University of Mashhad, Iran*
2. *Department of Veterinary School of Ferdowsi University of Mashhad, Iran*
3. *Department of Veterinary Faculty of Ferdowsi University of Mashhad, Iran*
4. *Atherosclerosis prevention research center, Medical Sciences University of Mashhad, Iran*
5. *Department of Medical science of Islamic Azad University of Mashhad, Iran*

**Background:** Asthma is a chronic inflammatory disease with high prevalence and socio-economic impacts that causes respiratory dysfunction.According to the hygiene hypothesis due to decreasing of bacterial and parasite infection the prevalence of allergic asthma especially in developed countries is going up.Also dendritic cells have special potential to elect immune responses and ploraizing naive T cells in to different subtypes of helper Tcells. Nowadays DC based immunotherapy is a good alternative approach to treat many disease including cancer. In this study we utilized monocyte derived DC to skew naive T cells in to regulatory T cells by co culturing of DCs with somatic extract of *Marshallagiamarshalli* in order to treat allergic asthma.
**Methods:** In this study 10 people were chosen, 5 healthy people and 5sick people that affected by asthma. Their blood sample were collected and their PBMCs were isolated and then monocytes separated from lymphocytes by adding of IL-4 and GM-CSF, monocytes differentiate to DC and 4th day, antigen extract of *Marshallagiamarshalli* added to culture medium .Then at 7th day of culture, these DCs cultured by autologous T cells and finally at 12th day of culture, the supernatants were collected and content of TGF-β were measured by sandwich ELISA.
**Results**: The average amount of TGF-β contents of supernatants in asthma group significantly increased (225±6.1 pq/ml) in comparsion with healthy groups (210±8.5 pq/ml).($P<0.05$) **Conclusion:** With regarde to hygiene hypothesis in which parasitic worms contain regulatory molecule of immune system, it could be possible producing of tolergenic DCs by antigenic extract of *Marshallagiamarshalli*. *Marshallagiamarshalli* and this associated with polarizing of T cells toward regulated T cell in the blood of individuals affected by asthma; by evaluating amount of TGF-β content of supernatant of cell culture, it is clear that DCs could polarize immune responses.

**Keywords:** Dendritic cells, Allergic asthma, *Marshallagiamarshalli*, TGF-β

ICIA2018\Allergy and immunotherapy\Poster\7831
**7831**

# The Specific IgE Assay to Common Respiratory Allergens in Subjects with Allergic Symptoms in Ahvaz

Maryam Moradi[1], Abbas Fayezi[2], Mana Momeni[1], Asyeh Javanian[1], Suzan Amini[1], Sara Yaghotian[1], Mohammad Shahrooei[1, 3]

*1. Specialized Immunology Laboratory of Dr. Shahrooei, Ahvaz, Iran*
*2. Department of Allergy and Clinical Immunology, School of Medicine, Ahvaz University of Medical Sciences, Ahvaz, Iran*
*3. Department of Microbiology and Immunology, Experimental Laboratory Immunology, KU Leuven, Leuven, Belgium*

**Background:** The results of the specific IgE assay to respiratory allergens differs extensively between different geographical regions. This study was designed to assess the specific IgE to common respiratory allergens using ImmunoCAP system among allergic cases in Ahvaz province.
**Methods:** Four hundred and eight patients with allergic symptoms entered this cross-sectional study in Ahvaz from 2014-2017. After fulfilling a specific questionnaire and medical assessment, 5 ml sample was taken from the cases. The total IgE and specific IgE for 9 respiratory allergens was measured using the ImmunoCAP system (Thermofisher-Phadia, Uppsala, Sweden) according to manufacturer's instructions.
**Results:** A total of 57% of the patients were male. The mean age of the subjects was 23 y (3 m to 68y). The majority of subjects were older than 18 years (n=195, 48%). As the results depict, the most common outdoor aeroallergens were Russian thistle (52%), Willow (47%), and Mesquite (41%), respectively, while among indoor allergens, German cockroach (38%) and Dermatophagoides farinae (11%) were the most prevalent. Sensitization to one allergen and more was found in 66.4% of the patients. Mono-sensitization and poly-sensitization were considered in 11.76% and 54.6% of patients, respectively.
**Conclusion:** The prevalence of sensitization to respiratory allergens has increased during the past decade in this city. The pattern of allergen-specific IgE showed Russian thistle as the most common aeroallergens in patients. Regarding the air pollution in some cities such as Ahvaz, the relationship between air pollution and longer pollination season and as their results increasing the allergic diseases, attention to the determination of the common respiratory allergens especially pollens seems necessary.

**Keywords:** Specific IgE, Respiratory allergens, Ahvaz.

ICIA2018\Allergy and immunotherapy\Poster\7837
**7837**

# Pattern of Skin Sensitivity to Aeroallergens among Allergic Rhinitis Patients in Birjand City, Iran

Mohammad Fereidouni[1], Neda Mahavar[1], Mohammad Sadegh Naghizadeh[1], Mohammad Mofateh[2]

*1. Asthma, Allergy & Immunology Research Center, School of Medicine, Birjand University of Medical Sciences, Birjand, Iran (Times New Roman, font size11 italic)*
*2. Department of Otolaryngology, School of Medicine, Birjand University of Medical Sciences, Birjand, Iran*

**Background:** Allergic disorders are among the most common health problems around the world and their prevalence has been increased during the last decays. Allergic rhinitis and conjunctivitis are the most prevalent forms of allergy and have negative impacts of quality of life as well as productivity of patients. Pollens and indoors allergens are the main triggers of allergic symptoms but the pattern of sensitization is varied in different parts of the world. The aim of this study was to identify the most common aeroallergens in allergic rhinitis patients at Birjand city of Iran.
**Methods:** patients who were referred to Birjand Allergy clinic during 2013 to 2017 because of any allergies, clinically evaluated by specialist and skin prick test with a battery of at least 20 common outdoor and indoor allergenic extracts performed based on standard method.
**Results:** 875 patients (mean age: 26 ±11.9 years, range 2-64 years, M/F ratio: 0.89) who suffered from allergic rhinitis or allergic rhinoconjunctivitis enrolled in this study. Highest rate of skin sensitivity was for weeds/ grasses pollen including Salsola Kali, AmaranthusRetroflexus, Chenopodium Album and Composite family (74.3%, 62,5%, 50.9% and 39.3% respectively). Among tree's pollen; Ash (49%), Walnut (46.9%) and Mesquite (29.3%) were the most common. Less than 20% of patients showed skin reactivity to indoor allergens and Storage mites, mix of Cockroaches and house dust were the most common (17.6%, 17.3% and 9.8% respectively).
**Conclusion:** The results of current study confirmed the importance of weed/grass and trees pollen as the major source of allergic sensitization in our area. Interestingly the rate of sensitization to indoor allergens was low which can be explained by geo-climatic situation.

**Keywords:** Allergy, Rhinitis, Conjunctivitis, Skin prick test

ICIA2018\Allergy and immunotherapy\Poster\7865
**7865**

# Evaluation of the Relationship between Exposure to Hepatitis A Virus and Allergic Disease in Birjand City in 2014

Zahra Asghari[1], Mohammad Fereidouni[2]

1. *medical doctor, Faculty of Medicine, Birjand University of medical Sciences*
2. *Associated Professor, Faculty of Medicine, Birjand University of medical Sciences*

**Background:** Childhood infections affect the immune system resulting in decrease in allergic diseases. This study aimed to evaluate the relationship between a History of Hepatitis A and Allergic Disease in Patients in Birjand Allergic Clinic of Imam Reza (AS) in 201۴
**Methods:** This is a case control study on 352 patients had been referred to allergy and asthma clinic of Imam Reza (AS) in 2013. Sampling method was non-random in both groups (allergic 113 people and non-allergic 239 people). Both group were matched based on gender, education, age, job, income and living place. Allergic diagnosis was based on skin prick test and serum IgE beside the clinical examination. Then a questioner was completed and blood sample obtained for HAV-ab (total) anti evaluation. Data were analyzed with the software SPSS21 ($p \leq 0/05$)
**Results:** Overall 352 patients were enrolled in this study. Prevalence of hepatitis A and allergy was 66.5% and 32.1% respectively. Prevalence of hepatitis A were higher in allergic patients in compare to none allergic (n=90 79.6% Vs n=118 60.3% p=0.0001).
**Conclusion:** The results of this study, unlike most studies in this area show a higher incidence of allergic patients had a history of hepatitis. Given the high prevalence of hepatitis-A in developing countries due to the low level of health on one hand and their transition period(increasing urbanization, changing life styles and increasing the level of dealing with other environmental allergens) that independently causes atopy, it seems that a greater role these factors play in the development of atopy.

**Key Words:** Allergy, HAV, Hygiene hypothesis

ICIA2018\Allergy and immunotherapy\Poster\8035
**8035**

# Increased Serum Levels of Resolvin E1 but No Difference in Serum Levels of Resolvin D1 in Allergic Rhinitis Patients Compared to Healthy Subjects

Ramin Lotfi[1], SeyedHamidreza Mortazavi[2], Ali Gorgin Karaji[3], Farhad Salari[3*]

*1. Student research committee, School of Medicine, Kermanshah University of medical sciences, Kermanshah, Iran*
*2. Department of Pediatrics, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*3. Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*

**Background:** Allergic rhinitis (AR) is a common inflammatory disorder of nasal mucosa, which is characterized by pruritus, sneezing, rhinorrhoea, and nasal congestion. *Resolvins* (Rvs) are anti-inflammatory and pro-resolving lipid mediators *derived* from *omega-3*polyunsaturated *fatty acids* (PUFAs). Resolvin E1 (RvE1) and resolvin D1 (RvD1) are produced from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), respectively. The aim of this study was to evaluate the serum levels of RvE1 and RvD1 in AR patients and healthy subjects.
**Methods:** Serum samples were obtained from 37 patients with AR and 30 age- and sex-matched healthy subjects. RvE1 and RvD1 concentrations were measured by ELISA technique according to the manufacturer´s instructions.
**Results:** RvE1 concentrations were significantly increased in patients compared to healthy subjects, but no significant difference in RvD1 concentrations between patients and healthy subjects was observed.
**Conclusion:** This study showed that concentrations of RvE1 were significantly increased in patients than healthy subjects, suggesting that this increment is an effort to help to timely resolution of nasal mucosa inflammation and prevention of acute inflammation progression to chronic inflammation. Our findings suggest that RvE1 or its analogues may help rational development of therapeutic approach for AR.

**Keywords:** Allergic rhinitis, Inflammation, Pro-resolving, Resolvin E1

ICIA2018\Allergy and immunotherapy\Poster\8061
**8061**

# The Purification Process of *Toxocaracanis* Antigen Fragments in Order to Immunomodulate Airways Inflammation and Allergies

M.Badri[1], FGhaffarifar[1], ZS.Hasan[2], A.Dalimi[1]

1. *Department of parasitology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
2. *Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

**Background:** Parasitic infections are very widespread in human populations. According to WHO, about 2 billion people suffer from parasitic infections. For example, people with asthma (approximately 819 million) are more affected than asthma (approximately 300 million). The common feature of parasitic infections and allergies is often the activation of Th2 and the increase of IL4, IL5 and IL13 cytokines, which increases the eosinophils and mast cells and increases the production of IgE antibodies. In populations where parasitic infections have already been endemic and have been significantly reduced due to increased community health levels, this trend of balance adjustment of immunological responses has been impaired and consequently an increase in the incidence of diseases such as asthma, in the community. For example, the prevalence of Ascaris in 1976 in endemic regions of Iran was 79.71%, and a significant decline to 0.5%, while a significant increase in MS cases per 100,000 people since 2006 (26.6%) To 2011 (44.53).
**Methods:** The Toxocaracanis nematode was collected from 5 stray dogs and sonicated for isolating of antigens. The soluble antigen at first the lipid was removed by ether then we used Sephadex G200 column for purification of antigens. For evaluating the characterization of purified antigen we used SDS-PAGE.
**Results:** After that crossing the chromatography column the three-peak antigens were isolated and after the SDS-Page, the molecular weight (10, 27 and 50 kD) was used as a candidate. The Bradford Spectrometry method was used to determine the protein content of each fraction.
**Conclusion:** In these evaluation the antigenic fractions will be used for exposing to human lung cells under in vitro conditions as well as injection into laboratory mice after exposure to Ovalbumin.

**Keywords:** Toxocaracanis, Antigen fragments, Immunomodulation, Airways inflammation

ICIA2018\Allergy and immunotherapy\Poster\8179
**8179**

# Budesonide and Fexofenadine Treatment Reduce Blood Eosinophils and Serum IgE in Allergic Rhinitis

Elham Bagherinia[1], *Seyed Hamidreza Mortazavi*[2], *FarhadSalari*[3], *Ali Gorgin Karaji*[3*]

*1. Student research committee, School of Medicine, Kermanshah University of medical sciences, Kermanshah, Iran*
*2. Department of Pediatrics, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*3. Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*

**Background:** Allergic rhinitis (AR) is an IgE-mediated inflammation of the upper airway. The aim of this study was to evaluate the effect of treatment with Fexofenadine and Budesonide on the level of serum IgE and percent of peripheral blood eosinophil.
**Methods:** As part of a clinical trial, 27 AR patients (17 men and 10 women, age 18-60) were treated for one month with Fexofenadine and Budesonide. Before treatment and one month after treatment, blood was taken from AR patients. Serum IgE level was measured using IgE ELISA kit and the percentage of peripheral blood eosinophil was counted using peripheral blood smear.
**Results:** The results of this study showed that after one month treatment, both the percentage of peripheral blood eosinophils and serum IgE level were significantly decreased in AR patients.
**Conclusion:** Taken together our results showed that treatment with Fexofenadine and Budesonide can reduce serum IgE levels and blood eosinophil count.

**Keywords:** Allergic rhinitis, Blood eosinophil percent, Fexofenadine, Budesonide

ICIA2018\Allergy and immunotherapy\Poster\8180
**8180**

# Treatment of Allergic Rhinitis with Fexofenadine and Budesonidereduces Clinical Symptoms and Improves the Quality of Life

Elham Bagherinia[1], *SeyedHamidreza Mortazavi[2], FarhadSalari[3], Ali Gorgin Karaji[3*]*

*1. Student research committee, School of Medicine, Kermanshah University of medical sciences, Kermanshah, Iran*
*2. Department of Pediatrics, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*3. Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*

**Background:** Allergic rhinitis (AR), an inflammation of upper airway, is the most common allergic disease. AR signs and symptoms including: a runny nose, sneezing, redness, itchy, and watery eyes, and nasal congestion and obstruction, despite having a safe nature, can seriously affect patient's quality of life. Treatment of AR is a way to reduce RA symptoms and improving the quality of life. The aim of this study was to evaluate the effect of treatment with Fexofenadine and Budesonide on the quality of life and on the clinical symptoms of allergic rhinitis patients.
**Methods:** In this clinical trial, 30 AR patients (18 men and 12 women, age 18-60) were treated for one month with Fexofenadine and Budesonide. For each patient, before treatment and after one month treatment, two questionnaires were used, one for patient symptoms and the other for patient quality of life.
**Results:** Our results showed that, in AR patients, one month treatment with Fexofenadine and Budesonide significantly reduce the severity of patient's symptoms and improve the quality of patient's life.
**Conclusion:** Generally, our results showed that dual treatment with Fexofenadine and Budesonide could be an effective way to treat allergic rhinitis.

**Keywords:** Allergic rhinitis, Fexofenadine, Budesonide, Quality of life.

ICIA2018\Allergy and immunotherapy\Poster\8229 allergy
**8229**

# The Effectiveness of Using AllergyCut® in the Allergic Rhinitis Treatment

Farid Semsarha[1*], Saleh Ramazani Khorshiddoost[2], Zahra Moeinibakhsh[2], Nika Khoshnevis[2], Maral Nazarifar[2], Niloofar Basrehizad[3], Reza Ramazani Khoorshiddoost[4]

1. *MIM Daroo Pharmaceutical Co. Tehran, Iran.*
2. *Aramesh multi professional pain clinic, Tehran, Iran.*
3. *Islamic Azad university of Science and Research Branch, Tehran, Iran*
4. *Faculty of Systems Management, Amirkabir University of Technology, Tehran, Iran*

**Background:** Allergic rhinitis is one of the most common chronic diseases affecting adults. AllergyCut® is a registered herbal drug with nasal spray dosage form that its efficacy has been approved during the present study.
**Methods:** In this study, that has been done at Aramesh clinic under supervision of shahidbeheshti university of medical sciences and health services, patients with allergic rhinitis were selected and some who were in need of further tests for diagnosis of immunological sensitivity were referred to the physician for confirming their allergy. After completing the treatment sessions, the physician re-examined the patients and their data. Then a follow-up program has been set up and the patients have used the AllergyCut® spray between mostly 60 days to 160 days (for about less than 10% of patients). The patients provided the information about their allergic attacks in a daily form, which has been designed for this study.
**Results:** From110 patients who have been treated with the AllergyCut® spray, only the data of 70 patients could be cited and studied because of the accuracy in filling out the forms and the correct usage of the spray. The comparison of the data about allergy symptoms has been done according to indicators, guidelines and forms of the world allergy organizations (WAO). In first month after treatment, the condition and clinical symptoms of patients have improved significantly to 33% in average value of allergy indices, and after second month these decreased by 70% compared to the first month. The follow of patients has been done for about 2 years and this improvement maintain in this period.
**Conclusion:** The herbal nasal spray AllergyCut® have been used in a certain time by 70 patients who have suffered from allergic rhinitis and about 74% of the patients reported the removal of allergic rhinitis symptoms during usage and also after two years follow.

**Keywords**: Allergic rhinitis, AllergyCut®, Herbal medicine

ICIA2018\Allergy and immunotherapy\Poster\8277
**8277**

# Does Air Pollution Change Common Children's Inhaled Allergens in Isfahan?

Fatemeh Rezayat[1], Sadegh Feyzollahzadeh[2], Abbas Rezaei[1]

*1. Department of Immunology, School of Medicine, Isfahan University of medical Science, Isfahan, Iran*
*2. Department of Immunology, School of Medicine, Urmia University of medical Science, Urmia, Iran*

**Background**: The prevalence of respiratory allergy in children depends on their habitat. Beyond genetic factors, many environmental factors specially air pollution in large cities have been implicated in increased risk of allergy. It has believed that common inhaled allergens are different in each area. Moreover, air pollution may change the prevalence of allergens or generation of new allergens in the population. In the present study we compared prevalence of allergens in children younger than 5 years old during last year with in Isfahan province.
**Methods**: 75 children younger than 5 years old who were suffering from skin and respiratory symptoms, referred to Dr. Rezaei Immunology Laboratory were chosen. The total serum IgE levels were measured using ELISA. For determination of allergens, CAP RAST (radio allegro sorbent test) and membrane strip containing 50 different allergens test (EUROLINE) were performed for each patient.
**Results**: The Mean of total serum IgE concentration was more than normal values. Considering the results, Rough pigweed, Rose and cultivated oats were detected as the most common inhaled allergens for children in Isfahan province. IgE concentration was significantly ($P<0.05$) higher in hypersensitive children to Rough pigweed compared with Rose and cultivated oats. It was however the most number of children had allergic reaction to Rose. All of these results have been compared with existed data in earlier studies on common allergens in Isfahan.
**Conclusion**: In this study, three allergens including Rough pigweed, Rose and cultivated oat was detected as the most common inhaled allergens in Isfahan. Since last decade Rough pigweed was known as the most common inhaled allergen in children. Until today air pollution in this province did not have any effects on common inhaled allergens in children and its effects may involve in adult respiratory allergy.

**Keywords**: Respiratory allergy, Inhaled allergen, Isfahan, Children.

ICIA2018\Allergy and immunotherapy\Poster\8445
**8445**

# Effect of treatment with Fluticasone propionate and Fexofenadine on blood Eosinophil percentage and Serum IgE level in Allergic Rhinitis

Atefe Mehrmanesh[1, 3], Elham Bagherinia[1, 3], Seyed Hamidreza Mortazavi[2], Farhad Salari[3], Ali Gorgin Karaji[3]

*1. Student research committee, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*2. Department of Pediatrics, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*3. Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*

**Background:** Allergic rhinitis (AR) is an IgE-mediated inflammation of the Upper respiratory tract and is the most common allergic disease. The goal of this study was to investigate the effects of treatment with Fexofenadine and Fluticasone propionate on the level of serum IgE and the percentage of blood eosinophil in AR patients.
**Methods:** In this study, 26 AR patients (12 men and 14 women, aged 18-60) were treated for one month with Fexofenadine and Fluticasone propionate. Before treatment and one month after treatment, blood was taken from AR patients. Serum IgE level was measured using IgE ELISA kit and the percentage of peripheral blood eosinophil was determined using peripheral blood smear.
**Results:** The results showed that after one month treatment, the percentage of blood eosinophils was significantly decreased. On the other hand, after one month of treatment, AR patient's serum IgE level was significantly increased.
**Conclusion:** Overall, our results showed that one-month treatment of AR patients with fexofenadine and fluticasone propionate reduces the percentage of blood eosinophil, but increases serum IgE level.

**Keywords:** Allergic rhinitis, Serum IgE titer, Blood eosinophil percentage, Fexofenadine, Fluticasone propionate

ICIA2018\Allergy and immunotherapy\Poster\8449
**8449**

## Correlation of Serum *Resolvins* and the Quality of life in Allergic Rhinitis

Razieh Rezaeianpour[1], Ramin Lotfi[1], Firoozeh Khamoushi[2], Seyed Hamidreza Mortazavi[3], Ali Gorgin Karaji[4], Farhad Salari[4*]

*1. Student research committee, School of Medicine, Kermanshah University of medical sciences, Kermanshah, Iran*
*2. Student research committee, School of Nutritional Science & Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*3. Department of Pediatrics, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*4. Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*

**Background:** Allergic rhinitis (AR) is an airway inflammatory disorder with high and increasing prevalence. Although it does not have a dangerous nature, it can seriously affect the patient's quality of life (QOL). Resolvins such as resolvin E1 and D1 (RvE1 and RvD1) are omega-3 fatty acids-derived lipid mediators that have anti-inflammatory and pro-resolving effects. The major aim of this study was to investigate the relationship between quality of life and serum levels of RvE1 and RvD1 in AR patients.
**Methods:** This cross-sectional study included 36 AR patients with age 15–57 years. The serum concentrations of RvE1 and RvD1 were measured by ELISA technique according to the manufacturer´s instructions. The Rhinitis Quality of Life Questionnaire (RQLQ) was used to assess the symptoms severity of patients. Data were analyzed by SPSS V21.
**Results:** The mean age of 36 patients was 34.7±10.4. 69.4% of patients were women. The mean of RvD1 and RvE1 serum concentrations in patients were 5.6 ng/ml and 242.4 pg/ml, respectively. The significant positive correlation was found between the practical problems and RvE1 serum concentrations (correlation coefficient: 0.360; P= 0.031; $P < 0.05$), but there was no association between the serum concentrations of RvD1 and any subscale of RQLQ.
**Conclusion:** Our results showed that serum levels of RvE1might be effective in improving the quality of life of AR patients.

**Keywords:** Allergic rhinitis, Quality of life, Omega-3 fatty acid, Resolvin

ICIA2018\Allergy and immunotherapy\Poster\8495
**8495**

# Determination of the Most Common Respiratory and Food Specific IgE Patients with Allergic Symptoms Using Immunoblotting method

Sepideh Moradkhani[1,2], Majid Khoshmirsafa[2,3], Maryam Mahjoub[2], Alireza Rezaei Manesh[2,4], Seyed Mehdi Ebrahimi[2,5], Farhad Seif[3], Parisa Nourbakhsh[6], Mehdi Shekarabi[2,3*]

1. *Department of Immunology, Faculty of Medicine, Kerman University of Medical Science, Kerman, Iran*
2. *Massoud Clinical Laboratory, Tehran, Iran*
3. *Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*
4. *Department of Immunology, Faculty of Medicine, Kermanshah University of Medical Science, Kermanshah, Iran*
5. *Department of Clinical Biochemistry, Faculty of Medicine, TarbiatModares University of Medical Science, Tehran, Iran*
6. *Departments of Immunology, Faculty of Medicine, Arak University of Medical Science, Arak, Iran*

**Background:** The prevalence of allergic diseases, as one of the global health problems, is rising particularly in developing countries in recent years. Different allergens can cause allergic symptoms. The aim of this study was to determine the frequency of the respiratory and food IgE in patients with suspicion of allergy.
**Methods:** In this cross-sectional study, 162 (52% female, 48% male) patients with suspicion of allergy were investigated in Massoud Clinical Laboratory, Tehran, Iran, between May 2016 and January 2018. Specific IgE for 30 selected respiratory and food allergens was measured by immunoblotting method. Data were analyzed by SPSS-23 software. A *p* value less than 0.05 were considered as significant.
**Results:** The median (IQR) age of recurrence were 4 (1.5-9). Mixed grasses (GX) was the most frequent specific IgE among 30 selected respiratory allergens (42.8%) and Pistachio nut (F203) was the most frequent specific IgE among 30 selected food allergens (18.3%). In addition, the frequency (positive reaction) of respiratory and food allergens was higher in children in comparison to adults (Pv<0.05).
**Conclusion:** The results of this study indicate that evaluation of specific IgE by Immunoblotting method can be useful for precise diagnosis and follow up of desensitization therapy.

**Keywords:** Allergy, Food Allergens, Respiratory allergens, Specific IgE

ICIA2018\Allergy and immunotherapy\Poster\8514 allergy
**8514**

**Association between Education and Management of Asthma Exacerbation**

Babak Khodadadi*[1], Arash Kalantari [2], MortezaSagharjoghi Farahani[3]

*1. Young researchers and Elite Club, Khorramabad Branch, Islamic Azad University, Khorramabad, Iran*
*2. Assistant professor of Tehran University of medical sciences. Imam Khomeini hospital complex vali-e-asr hospital Department of Pediatrics, Tehran, Iran.*
*3. Young researchers and Elite Club, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran*

**Background:** Asthma is the most common chronic disorders in the world that affect children. The prevalence of childhood asthma is increasing over the last two decades, many children are able to achieve good levels of control. So this study focus on the relationship between education and asthma exacerbation control.
**Methods:** In this quasi-experimental study, 104 children (6-14 years old) with asthma referred to asthma and allergy clinic of Children's Medical Center in Tehran at 2011-2015, which selected by convenience sampling method and divided into two equal groups of intervention and control. Data were analyzed by SPSS version 18.0 and T-test.
**Results:** The results of the study indicated that reducing asthma symptoms, improving PFT(Pulmonary function test), *decreasing the frequency* of *exacerbations* and salbutamol spray using in the intervention group; while changes in the control group were not significantly different ($p>0.05$).
**Conclusion:** *The results* suggest that good education have an excellent effect *on asthma exacerbation control* in children.

**Keywords:** Asthma, Education, exacerbation,

ICIA2018\Allergy and immunotherapy\Poster\8520
**8520**

# An Attempt to Harness the Deathful Peanut Allergy by Naturally Existing CAM Named Flavonol Quercetin

Lotfollah Behroo[1]

*1. Vice-chancellor for Student, Farhangian University, Nutrition, Tehran, Iran*

**Background:** Epidemiologically, ever-growing Allergies-rates are actually/drastically baleful. Accordingly, "Peanut Allergy" is especially, of critical significance considering the pertinent fatal or life-threatening anaphylactic events. "Avoidance"- focused upon as a mainstay of remedy-, seems to be indeed a difficult labor to perform principally if the suspected food is found everywhere. As a consequence, the majority of people with food allergy seek for natural/plant-origin Complementary and Alternative Medicines (CAMs), said to be more effectual and concurrently, quite safe. A "flavonol" subgroup-member of flavonoids named Quercetin is of prime interest to investigators. To inspect the benefits of a naturally-occurring nutritional substance-Quercetin- on peanut-induced sequelae in a murine model.
**Methods:** Male Wistar-rats were sensitized with peanut-extract in the presence of cholera-toxin and {Al (OH) 3}. The sensitized-animals were subsequently divided into 3-group; "Positive-control", "Quercetin-treated" and "Sham-sensitized". One-week post-sensitization, the wistars in treatment-group were gavaged daily, with Quercetin dissolved in 5% dimethyl-sulfoxide (DMSO), at a dose of 50 mg/kg body-weight (BW)/mL/rat, for 4-week. Duly, all the study animals were challenged intragastrically, with peanut-allergens. Naive rats (n=7) served as negative-controls. Then, Plasma Histamine and Total serum IgE levels were measured.
**Results:** Following daily-repeated orally-administering for four weeks, Quercetin significantly lowered Plasma Histamine and Total serum IgE Levels in the Quercetin-treated wistars {($p<0.000$) and ($p<0.000$)}, respectively.
**Conclusion:** It is revealed that the flavonoid "Quercetin" has suppressed thoroughly the respected IgE responses as to peanut-allergens and is efficient enough to abrogate the peanut allergy's complications. Hence, it can be assumed/endorsed as a substitute compound against IgE mediated food allergies in human beings.

**Keywords:** CAMs, Flavonoid, Flavonol, Murine Model, Peanut Allergy Treatment, Quercetin

ICIA2018\Allergy and immunotherapy\Poster\8522
**8522**

## Frequency of Common Aeroallergen in Allergic Rhinitis Patients of Gorgan in 2016

Morteza Sagharjoghi Farahani[1], Babak Khodadadi*[2], Arash Kalantari[3], Seyed Mohammad Hadi Bagheri Rostami[4,5]
Hosna salmani

1. *Young Researchers and Elite Club, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran*
2. *Young Researchers and Elite Club, Khorramabad Branch, Islamic Azad University, Khorramabad, Iran*
3. Assistant professor of Tehran University of medical sciences. Imam Khomeini hospital complex vali-e-asr hospital Department of Pediatrics, Tehran, Iran
4. *General physician, Gorgan University of medical science, Gorgan, Iran*
5. *Department of School of health managment and information sciences, Iran University of medical sciences, Tehran, Iran*

**Background**: In recent years, allergic diseases have increased in the region and identifying the factors that affect these diseases is very important. The aim of this study is to determine the prevalence of common inhalers allergens based on pericardial tests in allergic rhinitis patients in Gorgan.
**Methods**: In this descriptive study, Prick test was performed on 270 patients who had allergic rhinitis to asthma and allergy center of Gorgan during 2016. After completing the demographic questionnaire including age, gender history of asthma, allergic rhinitis, eczema, and conjunctiva, information was recorded. Finally, the data was analyzed by SPSS16. Chi-Square test is used to evaluate the difference between the results of the test for each of the variables (P <0/05)
**Results:** According to this study, 54 patients were seasonally and 47 persons had mixed allergic rhinitis. And disease associated with allergic rhinitis were conjugate (24%), asthma (12%) and eczema (3%) respectively. 40% of patients had allergies to Mite allergies and 40% of patients had allergic to weeds, 30% of patients were allergic to grass and allergic rhinitis, and the lowest sensitivity was related to dogs and cat's allergens. In Patients with Permanent Allergic Rhinitis (PAR), 50% of patients were sensitive to two types of mites, dermatophagouspterocnemia (DP) and dermatophagousfina (DF), and in Seasonal versus Perennial (SAR) patients, 60% of the patients were allergic to weeds and grasses.
**Conclusion**: Based on the findings, more than half of the patients with permanent allergic rhinitis had the highest sensitivity to the mites and then, showed the highest sensitivity to weeds and cockroaches, respectively. In patients with seasonal allergic rhinitis, the most susceptibility was allergenic to weeds and then grasses.

**Keywords:** Allergic rhinitis, Allergen inhalation, Prick test

ICIA2018\Allergy and immunotherapy\Poster\8529
**8529**

# Investigating the Effect of Using Latex Gloves on Causing Skin Allergies in the ICU Ward of Kowsar Medical Center, Sanandaj

Jalil Adabi[1], Elaheh Amani[1], Sho'aub Dehghani[1], Pejman Allahvaisy, Golnaz Babamiri

*1. Kurdistan University of Medical Sciences, Sanandaj, Iran*

**Background:** Latex is recognized as a flexible substance which has been used in producing plastic products. It has been estimated that wearing gloves made of latex can cause allergies among 1% of the individuals and 3- 17 % of personnel working in medical centers. It has not been calculated that to what extent using products made of latex such as gloves can cause allergies. A stockpile of research argue that individuals might be different in terms of indicating allergies by using latex products. This study aims to investigate the effect of using latex gloves on indicating allergies among personnel of ICU ward in Kowsar Hospital of Sanandaj.
**Methods:** Since personnel working in ICU wards are required to use latex gloves, the subjects of this study were chosen among the personnel of ICU in Kowsar hospital of Sanandaj. Forty of them completed the checklist given to them. The collected data was analyzed through SPSS 18.
**Results:** The analyzed data obtained from the checklists revealed that 20% of the subjects suffer from skin redness and itching when they use latex gloves. 80% of them reported that they prefer using prep gloves.
**Conclusion:** Concerning the fact that latex gloves are being used increasingly specially in ICU wards, it seems significant to consider potentials of causing allergies.

**Key words:** Allergy, ICU Personnel, Latex Gloves

ICIA2018\Allergy and immunotherapy\Poster\8540
**8540**

# Pathology of Patients with Allergies Referring to Ali ibn Abi Taleb Zahedan Hospital In 1396

Fateme ranjbar[1]*, Mahla jafari[2],Hanie jafardokht bonjar[3], Hamide danesh[4], Fateme akbari mehni[5]

*1,2,4,5. Bachelor's degree in laboratory science and member of Young and Elite Researchers Club, Department of Laboratory Sciences, Islamic Azad University, Zahedan Unit, Iran*
*3. Bachelor of Science in Nursing and Member of Young Researchers and Researchers Club, Islamic Azad University, Unit of Sciences, Iran*

**Background:** Allergy is an immediate deformed reaction to the re-entry of allergenic substances that have previously been triggered by the person's immune system. Allergic diseases, the prevalence of which has increased significantly in recent years, particularly in industrialized areas, are among chronic diseases. The aim of this study was to determine the pathology of allergy.
**Methods:** The present study was descriptive-analytic. The statistical population consisted of allergic patients referred to allergy clinic of Ali ibn Abi Talib Hospital in Zahedan. This study included 404 patients referring to Ali ibn Abi Taleb Zahedan Hospital in 1396, based on laboratory criteria. Patients included 214 females and 190 males. Data extraction was done from the statistical sources of the hospital and then the data were analyzed by SPSS v21 software.
**Results:** Of the patients studied, 53% of the women and 47% of the men suffered from the disease. These patients were examined for sex, marital status, occupation, educational level, place of residence, and season of bone disease. Of these, 246 were single and married. In terms of occupational parameter, including cultural, military, administrative, etc. Cultural people earned a higher percentage. Those who were under the diploma were those who lived in Zahedan and also had more of this complication in the winter.
**Conclusion:** There is a significant difference between the rates of the disease and the parameters mentioned, and it is better to prevent the spread of the disease by raising awareness of the people and taking appropriate measures. How to cope with predisposing factors in the list of health education programs and it is suggested to identify the predisposing populations of the futuristic research.

**Key words**: Pathology, Allergy, Immune system

ICIA2018\Allergy and immunotherapy\Poster\8574
**8574**

## Laboratory Findings in Patients with Urticaria

Maryam Moossavi[1], Mohammad Fereidouni[2,*], Afshin Derakhshani[1]

*1. Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran*
*2. Asthma, Allergy & Immunology Research Center, School of Medicine, Birjand University of Medical Sciences, Birjand, Iran (Times New Roman, font size11 italic)*

**Background:** Urticaria known as hives is a common disorder with various etiologies. Studies manifested that urticaria is associated with hepatitis B and C, gastritis, thyroid malfunction and autoimmune diseases like rheumatoid arthritis. Therefore, the only screening tests that can be measured are immunologic factors. The aim of the present study was to evaluate and measure C-reactive protein (CRP), HbsAg, liver function tests, TG, thyroperoxidase (TPO), H pylori IgG, antinuclear antibody (ANA) titers, rheumatoid factor (RF), IgM and IgA in a sample of patients with urticaria in Birjand, South Khorasan province.
**Methods:** 337 Individuals who were referred to Birjand Allergy clinic during 2013 to 2017 because of urticaria were recruited to this study. C-reactive protein (CRP), HbsAg, liver function tests, thyroperoxidase (TPO), H pylori IgG, antinuclear antibody (ANA) titers, rheumatoid factor (RF), IgM and IgA were measured for all individuals.
**Results:** In our study, 69% of the individuals were women and 31% were men. Most of the urticaria individuals showed normal range of H.pylori (76.3%), IgM (92.1%), IgA (86%), TPO (90.5%), CRP (89.2%), LFT (92.4%) and TG (93.3%) followed by negative HbsAg (98%), RF (96.8%) and ANA (94.9%) test. However, some of them showed high and severs value that was seen in H.pylori (1.3%), IgM (0.4%), IgA (0.8%), TPO (0.4%), CRP (0.4%) and LFT (0.4%) tests and positive ANA (0.4%) as well.
**Conclusion:** In our study, it seems that high and sever value of H.pylori was the most important factor in urticaria patients.

**Keywords:** Urticaria, Laboratory findings, Screening

ICIA2018\Allergy and immunotherapy\Poster\8636
**8636**

# Distribution of Different Allergic Disorders among Patients Referred to Birjand's Allergy Clinic, a Five Years Study

Afshin Derakhshani[1, 2], Mohammad Sadegh Naghizadeh[1, 2], Maryam Moossavi[1,2] Mohammad Fereidouni[3]

*1. Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran*
*2. Asthma, Allergy & Immunology Research Center, School of Medicine, Birjand University of Medical Sciences, Birjand, Iran*
*3. Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran*

**Background:** The prevalence of allergic disorders has been increased during the recent decays. The World Allergy Organization (WAO) reports that approximately one of five people suffer from some form of allergic diseases such as allergic rhinitis (AR), asthma, conjunctivitis, eczema, food allergies and so on. High frequency of allergic disorders causes huge economic burden and negatively impact patients' quality of life and productivity. However, distribution of allergens and risk factors may be varies in different geographic areas. The aim of this study was to evaluate the prevalence of different allergic disorders among patients referred to allergy clinic during a 5 years period in Birjand, Iran.
**Methods**: patients who were referred to Birjand's Allergy clinic during 2012 to 2017 because of any allergy related symptoms evaluated by the specialist and relevant tests including serum total IgE, skin prick test and spirometry were performed for proper diagnosis.
**Results:** 2493 patients (mean age: 27 ±15.4 years, range 1-83 years, M/F ratio: 0.77) who suffered from asthma, allergic rhinitis (AR), allergic rhino conjunctivitis (ARC), and other allergic disorders enrolled in this study. 47.4% (n=1182) of patients had allergic rhinitis or rhino conjunctivits. 21.7 % and 16% had urticarial and atopic dermatitis respectively. Prevalence of asthma and history of drug allergy was 12% and 10% respectively.
**Conclusion:** The results of this study showed that allergic rhino conjunctivitis and urticaria are the most common causes of visiting specialist while asthma and drug allergy are the least.

**Keywords:** Allergic rhinitis, Asthma, Prevalence

ICIA2018\Allergy and immunotherapy\Poster\8639
**8639**

# Estimating the Prevalence of Aero-allergens and Food Allergens in Patients with aAtopic Eczema/ Dermatitis

Mohammad Sadegh Naghizadeh[1, 2], Afshin Derakhshani[1, 2], Mahsa Najafzadeh[1, 2], Mohammad Fereidouni[3]

*1. Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran*
*2. Asthma, Allergy & Immunology Research Center, School of Medicine, Birjand University of Medical Sciences, Birjand, Iran)*
*3. Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran*

**Background:** Prevalence of allergic diseases such as atopic dermatitis is reported to be increasing in recent decade. Identifying the many allergens is very important for diagnosis and treatment of allergic diseases. The purpose of this study was to investigate the prevalence of common allergens in patients with eczema at Birjand city of Iran.
**Methods:** Prevalence of 26 common aero and food allergens evaluated in atopic dermatitis patients who were referred to Birjand Allergy clinic during 2013 to 2017 by standard skin prick test (SPT).
**Results:** 123 patients (mean age: 28.63 ± 15.00 years, range 2-71 years, M/F ratio: 0/62) were enrolled in this study. Highest rate of skin sensitivity in food allergens were for coffee (15%), cereal mix (13%), peanut (10.4%), melon (10.3%) and black pepper (7%), and among aeroallergens, Russian thistle (53%), weed mix (48%), mugwort (38%), tree mix (36%) and chenopodium (29%) were the most common.
**Conclusion:** Recognition and avoidance of allergens in combination to medical therapy has an important role in management of atopic dermatitis. The rate of sensitization to indoor allergens was interestingly low which is probably because of geo-climatic situation. We conclude that the prevalence of allergens in each region is different depending on environmental conditions, food habits, ethical diversities and life style.

**Keywords:** Allergy**,** Skin prick test**,** Atopic dermatitis, Food allergens, Aeroallergens

ICIA2018\Allergy and immunotherapy\Poster\8646
**8646**

# Prevalence of Allergies and Pattern of Skin Sensitization among Patients Suffering from Otitis Externa

Kosar Salehitorabi[1, 2], Afshin Derakhshani[1], Mahsa Najafzadeh[1], Mohammad Reza Mofateh[3], Mohammad Fereidouni[2, 4]

*1. Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran*
*2. Asthma, Allergy & Immunology Research Center, School of Medicine, Birjand University of Medical Sciences, Birjand, Iran*
*3. ENT department, Medical school. Birjand University of Medical Sciences, Birjand, Iran*
*4 . Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran*

**Background:** Otitis externa is an inflammatory condition of external auditory canal. It is a common problem that can be seen at any ages and can be disabling for patients. The most common cause of otitis externa is bacterial infection, but fungal overgrowth, trauma and dermatologic conditions may be involved. There are some evidences that support the role of allergy in development of otitis externa. The aim of this study was to evaluate the frequency of allergies and pattern of skin sensitivity to different allergens among patients suffering from otitis externa.
**Methods:** Patients suffering from otitis externa evaluated for presence of any allergic disorders and skin prick test with a battery of at least 20 different allergens as well as positive and negative control performed by an allergist. 5 milliliters of venous blood was taken and after serum separation, the level of serum total IgE was measure by ELISA method.
**Results:** 78 patients (mean age: 31.95, in range of 4-75, M/F ratio: 0.33) were enrolled in this study. Prevalence of allergic rhino conjunctivitis, eczema, and asthma and food allergy was 24.7%, 2.6%, 1.3% and 1.3% respectively. 35.9% and 23.3% of patients had positive skin reactivity to mix of weeds and mix of trees extracts respectively.
**Conclusion:** The results of this study showed that except the allergic rhino conjunctivits, other allergies are not common among OE patients and weeds' pollen are the most common allergens among OE patients.

**Keywords:** Allergy, Otitis extern, Skin Prick Test.

ICIA2018\Allergy and immunotherapy\Poster\8647
**8647**

# Comparison of the Pattern and Severity of Skin Prick Test Sensitivity to Different Allergens between Male and Females at Birjand

Mahsa Najafzadeh[1] , Mohammad Fereidouni[2*]

*1. Student Research Committee, Birjand University of Medical Sciences, Birjand, IR Iran*
*2. Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, IR Iran (corresponding author)*

**Background**: Studies have shown that allergens are very important sensitizing agents in respiratory disorders such as asthma and allergic rhinitis that are common in Iran. The aim of this study was to determine the pattern and severity of skin prick test sensitivity to different allergens between male and females at Birjand, south Khorasan, Iran.
**Methods**: Skin Prick Test (SPT) was performed on 1484 patients for 33 common allergens. The blood sample was taken for measuring IgE concentration and eosinophil count.
**Results:** In the present study, the most skin reactions were observed for Russian thistle, mugwort, weed mix, lambsqauarter and cat allergen which were 88.61, 64.728, 59.49, 46.326 and 39.71 $mm^2$ , respectively. In contrast, the least skin reactions were seen for tree mix, peanut and betulaceae which were 12.268, 12.838 and 13.121 $mm^2$, respectively.
There were no significant association between the mean IgE levels in females (237.98 ± 12.11) and males (246.45 ± 13.64). However, the difference in the rate of eosinophilia in females (4.13% ± 0.11) and males (4.88 ± 0.15%) was statistically significant ($p<0.001$).
In addition, a direct correlation was identified between total IgE and sum of the wheals ($p= 0.002$) and between IgE level and mean sum of the wheals ($p= 0.01$); however, there was no correlation between total IgE levels and mean of positive wheal.
**Conclusion:** The present study revealed the hypersensitivity to different allergens in Birjand city.

**Keywords:** Skin test, Allergens, Total IgE, Eosinophil

ICIA2018\Allergy and immunotherapy\Poster\8652
**8652**

# Pattern of Allergies and Skin Prick Sensitization among Patients Suffering from Adenotonsillar hypertrophy

Neda Mahavar[1, 2], AfshinDerakhshani[1,2], Mohammad RezaMofatteh[3,], Mohammad Fereidouni[1,4]

*1. Asthma, Allergy & Immunology Research Center, School of Medicine, Birjand University of Medical Sciences, Birjand, Iran)*
*2. Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran*
*3. ENT department, Medical school. Birjand University of Medical Sciences, Birjand, Iran*
*4. Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran*

**Background:** Adenotonsillar tissue is a part of Waldeyer ring, which are responsible for antigen sampling from mouth cavity and antibody formation against antigens entering the body including allergens. Pathological growth of the adenoid and tonsil are mainly caused by antigenic stimulation as a part of inflammatory process. Adenotonsillar inflammation is one of the oldest and common problems and adenotosillectomy is the most common operation in small children although is seen in adult as well. In spite of the high prevalence of adenotonsillar hypertrophy, the cause of this problem is unclear but some researches have shown that allergy may be a risk factor and Sensitization to a variety of allergens is believed to be involved in the development of adenotonsillar hypertrophy. The aim of this study was to evaluate the prevalence of allergic disorders in patients with adenotonsillar hypertrophy referring to Birjand's allergy clinic of Imam reza hospital.
**Methods:** 238 patients with adenotonsillar hypertrophy (mean age 14.12 years, range 3 to 51 years, M/F ratio: 1) were enrolled in our study. For all patients an allergy questionnaire were filled and skin prick test with a battery of 20 common allergen including tree and grass pollen, fruits, mites, molds and cockroach was done for all patients.
**Results:** 48.3% of patients had a positive skin reaction to at least one allergenic extract. The most common allergens were weeds including Russian thistle, Mugwort (42%), Rough pigweed (35.5%), weed mix (33.2%), Chenopodiacea (22.31%) followed by Ragweed (14.8%) kiwi (14.3%), Melon (12.5%), coffee (12.3%) and cockroach (11.7%).
**Conclusion:** The results of this study show that a fairly large proportion of patients with adenotonsillar hypertrophy are sensitized to allergens particularly aeroallergens and therefore it is possible that allergy have a role in development of this problem.

**Keywords:** Adenotonsillar hypertrophy, Allergy,Skin prick test

ICIA2018\Allergy and immunotherapy\Poster\8661
**8661**

# Comparison of the Skin Prick Test Wheal Size, Eosinophilia and Total Serum IgE between Allergic Male and Females at Birjand City, Iran

Mahsa najafzadeh[1, 2], Neda Mahavar[1, 2], Mohammadsadegh Naghizadeh[1,2], Mohammad Fereidouni*[1,3]

*1. Asthma, Allergy & Immunology Research Center, School of Medicine, Birjand University of Medical Sciences, Birjand, Iran)*
*2. Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran*
*3. Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran*

**Background**: Studies have shown that allergens are very important sensitizing agents in different types of allergies especially allergic rhinitis and asthma but the pattern of sensitization and the allergenicity of different allergens are vary in different. The aim of this study was to determine the pattern of skin prick test sensitivity to different at Birjand city, Iran.
**Methods**: patients who suffered from any kind of allergies evaluated by an allergist and skin Prick Test (SPT) was performed.
**Results**: 1456 allergic patients (mean age: 26.03 ± 14.2, range: 2-72 years, M/F ratio: 0.81) enrolled in this study. The average number of positive skin test in male and female was 1.8 and 1.7 respectively. The mean of skin wheal area because of allergenic extracts was larger in men than women but the difference was not significant (15.3 mm2 vs 13.6 mm2, P>0.05).There were no significant difference between the mean IgE levels in females and males (237.98 ± 12.11 vs 246.45 ± 13.64, P> 0.05). However, the percentage of blood eosinophilia was significantly higher is males than females (4.88 ± 0.15% vs 4.13 ± 0.11%, p<0.001).
**Conclusion**: The present study revealed that except for blood eosinophilia which is higher in males, there is no sex disparities in case of skin prick test reactivity to different allergens or total serum IgE.

**Keywords:** Skin test, Allergens, IgE levels

ICIA2018\Allergy and immunotherapy\Poster\9795
**9795**

# The Relationship between Vitamin A and Serum IgE Level with Quality of Life in Patients with Allergic Rhinitis

Akram Davoodi[1], Firoozeh Khamoushi[2], Mohsen Molaei[1], Seyed Hamidreza Mortazavi[3], Ali Gorgin Karaji[4], Farhad Salari[4*]

*1. Student research committee, School of Medicine, Kermanshah University of medical sciences, Kermanshah, Iran*
*2. Student research committee, School of Nutritional Science & Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*3. Department of Pediatrics, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*4. Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*

**Background:** Allergic rhinitis (AR) is a common inflammatory disease of nasal mucosa that considerably affects the quality of life (QOL) of patients. Vitamin A (Vit A) and its metabolites such as retinoic acid (RA) play critical roles in development and regulation of the immune responses. Immunoglobulin E (IgE), a B cells-produced antibody, is involved in the pathogenesis of allergic diseases such as AR. The aim of this study was to investigate the relationship between dietary vit A intake and serum levels of IgE with QOL in AR patients.
**Methods:** This cross-sectional study included 36 AR patients with age 15–57 years. The serum level of IgE was measured by ELISA technique according to the manufacturer´s instruction. The dietary vitamin A intake was measured by Food Frequency Questionnaire (FFQ) and analyzed by Nutrition 4 software. The Rhinitis Quality of Life Questionnaire (RQLQ) was used to assess the symptoms severity in AR patients. Data were analyzed by SPSS V21.
**Results:** The mean age of 36 patients were 34.7±10.4. 69.4% of patients were women. The mean of IgE serum concentrations in patients was 84.2 IU/mL. The daily intake of vit A was 781.8±500.1 gr/day and so it was less than the recommended amount by recommended dietary allowances (RDA). Significant positive correlation was found between low intake of vit A with emotional symptoms (correlation coefficient: 0.367; P= 0.028; P<0.05) and activity limitations (correlation coefficient: 0.330; P= 0.05; P<0.05). Significant negative correlation was observed between IgE serum levels and practical problems (correlation coefficient: -0.376; P= 0.02; P < 0.05).
**Conclusion:** This study showed that IgE serum levels and dietary vitA intake are factors that can affect the life quality of AR patients.

**Keywords:** Allergic rhinitis, Immunoglobulin E, Quality of life, Vitamin A

ICIA2018\Allergy and immunotherapy\Poster\9904
**9904**

# The Main Complaint of Patients with Allergies Referring to the Hospital of Ali ibn Abi Talib in Zahedan in 1396

Mahlajafari[1] , fateme ranjbar[2]

*1,2 - Bachelor of Science in Laboratory Sciences and Member of Young and Elite Research Club, Department of Laboratory Sciences, Islamic Azad University, Zahedan Unit, Iran*

**Background:** Allergies are the reaction of the immune system to extremes over various factors. People who are hypersensitive have an ultrasignificant immune system against apparently harmless substances, and show an excessive response. Although the immune system protects the host against infections and alien factors, immune responses can cause injury and even disease. The aim of this study was to investigate the main complaint of patients with allergies referred to Imam Ali Hospital in Zahedan
**Methods:** The present study is a descriptive-analytical type. This study was performed on 404 patients referred to the Allergy Clinic of Zahedan Imam Ali Hospital in 1396. Information was collected using questionnaires completed by the patients and then analyzed by SPSSV21 software. And statistical analysis
**Results:** Of the patients, 53% of the patients were female and 47% were male, of whom 247 were single and the rest were married and the most frequent visits were in winter. 44.1% of patients had seasonal allergic reaction, 2.33% of cases of colds, 8.6% of cases of asthma and 9.5% of cases of febrile seizure, and 56.5% of them had no history of allergies
**Conclusion**: Allergic diseases are chronic diseases, affecting the quality of life of humans and are considered as the main causes of referral to health centers. It is better for authorities to prevent more social and economic harm than others by raising awareness of the people and adopting appropriate approaches

**Keywords**. Allergy, Complaint, Ali ibn Abi Talib, Zahedan

# Cancer Immunology

# Poster Presentation

ICIA2018\Cancer Immunology\Poster\5201
**5201**

# Differential effects of inhibitory and stimulatory anti-HER2 monoclonal antibodies on intracellular signaling pathways

Tahereh Soltantoyeh[1], Tannaz Bahadori[2], Reza Hosseini Ghatar[1], Jalal Khoshnoodi[1], Azam Roohi[1], Maryam Mobini[1], Forough Golsaz Shirazi[1], Mahmood Jeddi Tehrani[2], Mohammad Mehdi Amiri[1], Fazel Shokri[1,2]

1. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
2. *Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran*

**Background:** Homo- and hetero- dimerization of the receptor tyrosine kinase HER2 hyperactivate several downstream signaling pathways, leading to uncontrolled growth and proliferation of tumor cells. Anti-HER2 monoclonal antibodies (mAbs) may induce different effects on HER2 dimerization and signaling.
**Methods:** The effect of two inhibitory (2A8, 1T0) and one stimulatory (1H9) anti-HER2 mAbs either alone or in combination with Trastuzumab was investigated on AKT and ERK signaling pathways and HER2 degradation by Western blotting.
**Results:** While 1H9 mAb had no significant effect on AKT and ERK signaling pathways, 1T0 and 2A8 mAbs inhibited phosphorylation of both pathways. Combination of 1T0 mAb with Trastuzumab resulted in significant synergistic inhibition of both pathways and HER2 degradation, much more potently than the combination of Trastuzumab and Pertuzumab.
**Conclusion:** Our data indicate that anti-HER2 mAbs may induce different signaling pathways depending on their effect on tumor cell growth and proliferation. The significant inhibition of ERK and AKT phosphorylation by 1T0 alone or particularly in combination with Trastuzumab suggests its potential therapeutic application for targeted immunotherapy of HER2 overexpressing malignancies.

**Keywords:** Breast cancer, Signaling Pathways, Protein Kinase B, Extracellular Signal-Regulated Kinase, Monoclonal Antibody, Human Epidermal Growth Factor Receptor 2.

ICIA2018\Cancer Immunology\Poster\5232
**5232**

# Effect of extremely low frequency of electromagnetic fields (ELF-EMF) on the argyrophilic nucleolar organizer regions (AgNORs) in bone marrow cells

Mohammad Rafiee[1,2], Mohammad Abbasi[3], Mohammad Yousef Alikhani[4], Alireza Zamani[5]*

*1. Department of Medical Laboratory Sciences, School of Para medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
*2. Department of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Department of Internal Medicine, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
*4. Department of Microbiology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
*5. Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*

**Background**: we are continuously exposed to increasing amount of the extremely low-frequency electromagnetic fields (ELF-EMF) in our daily life. This caused to generate concerns regarding the biological effects of such exposure. Therefore, this study was conducted to evaluate the influence of ELF-EMF on Argyrophilic nucleolar organizer regions (AgNORs) of bone marrow (BM) cells.
**Methods**: Eighty male rats were separated into four exposed groups (1,100,500 and 2000 µT intensities) and a control group. The exposed groups were received ELF-EMF in solenoids which fed with the constant 50Hz sinusoidal power two hours a day for two months. The bone marrows were aspirated from bones of sacrificed rats and smears were prepared on slides. To analysis of BM cells, AgNOR staining was done on slides and Total AgNORs area (TAA), total AgNORs number (TAN) and total AgNORs length (TAL) were calculated by Scion Image Software and presented as pixels.
**Results**: The different intensities of ELF-EMF had no effect on the weights of rats. Significant reduction was seen in TAA, TAN and TAL in group of 1µT compared with groups of 2000 µT and control. In addition, there was significant positive correlation between intensities increase of ELF-EMF and increase of TAA, TAN and TAL amounts.
**Conclusion**: It can be concluded that very low intensity (1µT) of ELF-EMF decreases AgNORs indices in bone marrow cells. But high intensity of 2000 µT caused to return of AgNORs indices to control levels.

**Keywords:** Bone marrow, Argyrophilic nucleolar organizer regions, Extremely low-frequency electromagnetic fields, Proliferation, weight.

ICIA2018\Cancer Immunology\Poster\7422
**7422**

**Cytotoxicity and Apoptosis Evaluation of *Artemisiaabsinthum*plant on AGS Gastric Cancer Cell Line**

Elahe Aleebrahim Dehkordy[1, 2], RaziyehKarami [2] , Amin Soltani[2], SadraAnsaripour [3] NajmeShahinfard[2], Mahmoud Rafieian kopaei[2]

1. *Young Researchers and Elite Club, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran*
2. *Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran*
3. *Student Research Committee, Shahrekord University of Medical Sciences, Shahrekord, Iran*

**Background:** Gastric cancer remains the third leading cause of cancer related mortality worldwide. Natural products and derivatives of medicinal plants can play an important role to cure cancer. So Products derived from natural plants that induce apoptosis are used for cancer treatment. Artemisia is a genus belonging to the Asteraceae family with commonly fragrant species.This plant is widely used in Iranian traditional medicine. The current study was designed to evaluate the anticancer effect of this herb on AGS cancer cell lines.
**Methods:** Cytotoxic effect of hydro alcoholic extract on AGS cells was studied by MTT colorometric assay and viability was monitored using cell counting and trypan blue. In order to investigate apoptosis, flow cytometry was performed by using Annexin V and PI apoptosis detection kit.
**Results:** The findings of the present study showed that the extract of *Artemisia absinthu* had inhibitory and apoptotic inducing effects on AGS cell lines. The results of MTT assay showed that the anticancer effect of *Artemisia absinthum* dependent to time and dose. The percentage of dead cell increased as dose arose. The 50% growth inhibitory concentration ($IC_{50}$) of extract in 48 and 72 h was 501± 21.15 and 413±16.12, respectively.
**Conclusion:** Our data well established the anti-proliferative effect of *Artemisia absinthum* extract, and clearly showed that the extract can induce apoptosis AGS cells. Therefore *Artemisia absinthum* could have growth inhibitory effect on AGS cell line dose and time dependent manner. It seems to come with further research, and utilizes its compound in cancer treatment.

**Keywords:** Cytotoxicity, Apoptosis, *Artemisia absinthum*, Gastric Cancer

ICIA2018\Cancer Immunology\Poster\7428
**7428**

# FoxP3 Gene Variants and Breast Cancer: rs3761548 as Susceptibility not Prognosis Marker

Nadereh Naderi[1], Fahimeh Arabpour[1], Mahsa Rahimzadeh[2], Amin Shafizad[3], Marzieh Norouzian[1]

*1. Molecular Medicine Research center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran*
*2. Department of Biochemistry, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran*
*3. Department of Radiation oncology, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran*

**Background:** Recent studies have characterized FoxP3 as a marker of regulatory T cells (Tregs) and an X-linked tumor suppressor gene which is involved in the pathogenesis of breast cancer. Therefore, we investigated the potential influence of a single-nucleotide polymorphism (SNPs) of FoxP3 gene on the development of breast cancer in Iranian women.
**Methods:** The association between FoxP٣ rs3761548 polymorphism and breast cancer risk was assessed in 100 breast cancer patients and 108 healthy controls using sequence-specific primers (PCR-SSP).
**Results:** We identified significant difference of rs3761548 in both allele and genotype frequencies between cases and control groups. Our results showed that individuals carrying a FoxP3 rs3761548 AA genotype had about 4.3 fold increased risk of breast cancer compared with CC carriers.
**Conclusion:** This study has provided the first genetic data on the FoxP3 gene in south of Iran and proposes the rs3761548 polymorphism of FoxP3gene as a risk factor in the development of breast cancer in Iranian population.

**Keywords:** Breast Cancer, FoxP3, Polymorphism, Treg cells

ICIA2018\Cancer Immunology\Poster\7430
**7430**

# TGF-β Strengthens Cytotoxic effect and Senescence Induced by PI3K/ AKT/ MTOR Inhibitor in Human Colorectal Cancer Cells

Raziyeh Karami[1], Hedayatollah shirzad[1], Amin Soltani[1], Elahe Aleebrahim Dehkordy[2,3], Mahdi Ghatrehsamani[1]*

1. *Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran*
2. *Young Researchers and Elite Club, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran*
3. *Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran*

**Background:** Although NVP-BEZ235 and XAV-939 have been shown a promising result in the treatment of colorectal cancer (CRC), our data is little with regard to the effect of environmental cytokine on these drugs. In order to explore the reaction of CRC to this novel therapy, the current study proposes to investigate the inhibitory effect of NVP-BEZ235 and/or XAV-939 in CRC cells in combination with TGF-β as a tumor environmental cytokine to analyze their effect on cancer cell proliferation, cell death and expression of cancer promoting genes.
**Methods:** The cells were treated with single or combination of NVP-BEZ235, XAV-939 and TGF-β. Cell viability was evaluated by MTT assay. Flow cytometry method was used to determine cellular death. Real-time PCR was utilized to identify the effect of treatments on expression of snail, slug, c-myc and notch-1 genes.
**Results:** The results revealed that NVP-BEZ235 inhibited CRC cells proliferation and induced senescence cells more than XAV-939. Although, XAV-939 increased cellular death and inhibited senescence induction of CRC cells better than NVP-BEZ235. TGF-β pretreatment sensitizes CRC cells to the cytotoxic effect of NVP-BEZ235 and XAV-939. Real-time PCR analysis showed that resistance cells to NVP-BEZ235 increased expression of slug. Expression of notch enhanced remarkably when cells treated with both NVP-BEZ235 and XAV-939.
**Conclusion:** The results of the present study demonstrated that presence of anti-inflammatory cytokine like TGF-β augments the inhibitory effect of XAV-939 or especially NVP-BEZ235.

**Keywords:** TGF-β, Colorectal Cancer, NVP-BEZ235, XAV939

ICIA2018\Cancer Immunology\Poster\7457

**7457**

# Invitro increasing cytotoxicity of CMV-specific CD8+ Tcell in CMV-seropositive colorectal cancer patients

Shaker Ardakani M[1], Fazeli MS[2], Kokhaei P[1] ,Shakiba Y[3], Tabatabaei Yazdi SM[4], Abbasian A[4], Emami AH[5], Pak F[1], Nourizadeh M[6]

1. *Department of Immunology, Semnan University of Medical Sciences, Semnan, Iran.*
2. *Department of Surgery, Imam Khomeini Complex Hospital, Tehran University of Medical Sciences, Tehran, Iran*
3. *Regenerative medicine Research center, Kermanshah University of Medical Sciences, Kermanshah, Iran*
4. *Tehran Blood Transfusion Center, Tehran, Iran.*
5. *Department of Hematology/Oncology, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran*
6. *Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Colorectal cancer (CRC) is the third most common cancers with a high incidence of mortality and morbidity. A potential oncogenic role of HCMV in different types of cancers including CRC has been recently shown by detecting the CMV peptides in tumor tissues compared to the adjacent normal tissues. Targeting the CMV peptide-expressing tumor cells has been considered as an effective immunotherapy approach. Therefore, we tried to induce CMV-specific CD8+ cytotoxic T cells (CTLs) in vitro in CMV-seropositive CRC patients and compare it to healthy controls.

**Methods:** Sixteen CMV+ colorectal cancer patients and eight CMV+ healthy controls were enrolled the study. Patients 'blood samples were obtained during tumor surgery (before chemotherapy and radiotherapy treatments). Cytotoxicity was measured by detecting the CD107a on the surface of CMV-specific CD8+ T cells using flow cytometry before and after 14-day culture of PBMCs in the presence of CMV peptide epitopes and IL-2 cytokine. Data were analyzed using FLOWJO software.

**Results:** CD107a expression on CD8+T cells in both patient and control groups increased significantly after 14-day culture in the presence of CMV peptide epitopes and IL-2 wich was an indicator of the potential cytotoxicity function of CTLs.

**Conclusion:** The results of this study specifies that in vitro stimulation of PBMC in the presence of CMV peptide epitopes and IL-2 can be an applicable method to generate potent CD8+ CTLs for future T cell therapy in CRC patients.

**Keywords:** Colorectal Cancer, CMV, CD107a

ICIA2018\Cancer Immunology\Poster\7468
**7468**

# High Molecular weight Fractions derived from *Lactobacillus Reuteri* Cell-Free Supernatant Decrease Colon Cancer Stem- like Cell Invasion in Vitro

Faezeh Maghsood[1], Mahdi Rohani[2], Mehdi Kadivar[1]*

1. *Department of Biochemistry, Pasteur Institute of Iran, Tehran, Iran*
2. *Department of Microbiology, Pasteur Institute of Iran, Tehran, Iran*

**Backgroud:** Cancer stem cells (CSCs) have essential role in tumor metastasis and recurrence. Several factors are dysregulated in CSCs such as decreased expression of E-adherin, and tissue inhibitor of metalloproteinases, especially TIMP-1, and increased expression and activity of matrix metalloproteinases (MMPs), especially MMP-9 which are involved in tumor invasion and metastasis. Many studies have reported anti-cancer effects of *lactobacillus reuteri* as a probiotic bacterium. In this study, we investigated the effect of different molecular weight fractions derived from *L.reuteri* supernatant on invasion factors in a previously established human colon cancer stem-like cells derived from HT-29 cell line (HT29-CSC like).
**Methods:** Cell free supernatant (CFS) of *L.reuteri*was fractionated into defined molecular weight ranges (<3kD, 3-10 kD, 10-50 kD, 50-100 kD, >100 kD) using ultra-filtration tubes. HT29-CSCs were treated with different molecular weight fractions of CFS, and uninoculated bacterial growth media (MRS) as a control. Subsequently, cell invasion assessed using matrigel-coated insert plates. The expression of MMP-9, and TIMP-1 mRNAs were determined using real-time PCR, and the gelatinolytic activity of MMP-9 was evaluated by zymography.
**Results:** Cell invasion was significantly decreased following treatment with different molecular weight fractions of CFS in comparison with MRS-treated group ($p<0.05$). Also, 10-50 kD, 50-100 kD, and >100 kD fractions, significantly decreased activity and expression level of MMP-9 ($p<0.05$). Furthermore, expression level of TIMP-1 was significantly up-regulated by crude CFS, and >100 kD fraction ($p<0.05$).
**Conclusion:** Our results indicate that CFS of *L. reuteri* possesses anti-metastatic properties. Since fractions containing high molecular weight secretory factors decreased cell invasion, it suggests that the inhibitory compound(s) may fall into a macromolecule range such as a protein, nucleic acid, or a polysaccharide.

**Keywords:** Invasion, Lactobacillus reuteri, Matrix metalloproteinase, Probiotic

ICIA2018\Cancer Immunology\Poster\7498
**7498**

# NK cell purification and expansion in the presence of IL-2, IL15 and IL18 with or without PBMCs feeder cells

Alireza Andalib, Anahita Najafalizadeh, Nafiseh Esmaeil, Fariborz Mokarian, Abass Rezaei

*Immunology Department, Isfahan Medical School, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background**: NK cells are progressively considered as medical tools for cancer immunotherapy. The development of applicable methods to generate large numbers of functional NK cells is a crucial step to maximize the potential of this approach. So finding the appropriate condition for augment the NK cell number is part of the main aim of the study.
**Methods:** NK cells were isolated from PBMCs using Magni-Sort Human NK cell Enrichment methods. Purified NK cells were activated *in vitro* by IL-2 and IL-15 for two weeks. The HL-60 cell line was used as a target to assess NK *cytotoxic activity*. The cells were stained with FITC-conjugated anti-CD107a mAb, with PE-conjugated anti-CD3 mAb and PE/CY5-conjugated anti-CD56 mAb then NK cells were determined with flow cytometry method.
**Results:** The purity of NK cells was up to 92% by using the MACS method. Expanded NK cells were highly cytotoxic (90%) and more than 80% were viable. NK cell culture without using PBMCs as feeder cells even using IL-2+IL-15+IL18 induced no expansion of CD56+CD3- cells, but the culture including IL-15 + IL-2 has 1.3-fold expansion without using PBMCs as feeder cells.
**Conclusion:** NK cells did not expand significantly without feeder cells even using different cocktails of the cytokines. In contrast to T cells, NK cells cannot proliferate without feeder cells. The results using combination of IL-2, IL-15 and IL-18 were not significantly different from using IL-2 and IL-15 without using feeder cells.

**Keywords:** NK cells, IL-2, IL-15, IL-18, CD107a, feeder cells

ICIA2018\Cancer Immunology\Poster\7538
**7538**

# Evaluation of TGF-β Expression in Mcf-7 Cell Line after Treatment with Plant Extract

HesamBabaei Khameneh[1], Narges Amirjamshidi[1], Azin Aghamajidi[1], Abbas Azadmehr[1], Ebrahim Zabihi[2], Sadegh Fattahi[3],AmrollahMostafazadeh[*2]

1. *Department of Immunology, Babol University of Medical Sciences, Babol, Iran*
2. *Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran*
3. *Cellular and Molecular Biology Research Center, Babol University of Medical Sciences, Babol, Iran*

**Background:** Breast cancer is one of the most common neoplasm and the second leading cause of cancer related deaths in women worldwide. Studies have been shown that the expression of TGF-β increased up in several subtype of breast cancer. Because of these findings, it has been suggested that, reduction of TGF-β expression can be a suitable target for prevention of tumor growth.
**Methods:** MCF-7 cells were cultured under cell culture conditions. MTT assay in dose and time dependent manner was conducted to find GI50 (growth inhibition 50) for each extraction. Then, the cells were exposed to different doses of plant extracts in triplicate. The level of TGF-$\beta_1$ gene expression was obtained by real-time RT-PCR.
**Results:** The results suggested that high concentration of *L.usitatissimum* extract significantly reduced TGF-$\beta_1$ mRNA levels and low concentration of *S.striata* extract dramatically increased the TGF-β1 mRNA levels in MCF-7 cells.
**Conclusion:** According to the results, *L.usitatissimum* probably has some compounds that have negative effect on the TGF-β expression in MCF-7 cells.

**Keywords:** MCF-7, TGF-β, *linumusitatissimum*, *scrophulariastriata*

ICIA2018\Cancer Immunology\Poster\7608
**7608**

# NK cell purification and expansion in the presence of IL-2, IL15 and IL18 with or without PBMCs feeder cells

Alireza Andalib, Anahita Najafalizadeh, Nafiseh Esmaeil, Fariborz Mokarian, Abass Rezaei

*Immunology Department, Isfahan Medical School, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background**: NK cells are progressively considered as medical tools for cancer immunotherapy. The development of applicable methods to generate large numbers of functional NK cells is a crucial step to maximize the potential of this approach. So finding the appropriate condition for augment the NK cell number is part of the main aim of the study.
**Methods:** NK cells were isolated from PBMCs using Magni-Sort Human NK cell Enrichment methods. Purified NK cells were activated *in vitro* by IL-2 and IL-15 for two weeks. The HL-60 cell line was used as a target to assess NK *cytotoxic activity*. The cells were stained with FITC-conjugated anti-CD107a mAb, with PE-conjugated anti-CD3 mAb and PE/CY5-conjugated anti-CD56 mAb then NK cells were determined with flow cytometry method.
**Results:** The purity of NK cells was up to 92% by using the MACS method. Expanded NK cells were highly cytotoxic (90%) and more than 80% were viable. NK cell culture without using PBMCs as feeder cells even using IL-2+IL-15+IL18 induced no expansion of CD56+CD3- cells, but the culture including IL-15 + IL-2 has 1.3-fold expansion without using PBMCs as feeder cells.
**Conclusion:** NK cells did not expand significantly without feeder cells even using different cocktails of the cytokines. In contrast to T cells, NK cells cannot proliferate without feeder cells. The results using combination of IL-2, IL-15 and IL-18 were not significantly different from using IL-2 and IL-15 without using feeder cells.

**Keywords:** NK cells; IL-2; IL-15; IL-18, CD107a, feeder cells

ICIA2018\Cancer Immunology\Poster\7615
**7615**

**Thymolhas selective cytotoxic effects**

Pourya Tahmasebi, Seyyed Meysam Abtahi

*Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Thymolis a phenolic compound that possess pharmacological activities, like anti-inflammatory, antioxidant, anticancer, and antimicrobial effects. Here, we investigated the selective cytotoxic effects of Thymolon peripheral blood mononuclear cells (PBMCs) and two cancer cell lines.
**Methods:** $۱\times۱۰^{6}$ live cells of PBMCs, LS174T (a colon cancer cell line) or T47-D (an invasive milk ductal carcinoma) were incubated with serial logarithmic dilutions of Thymol (0-400µM) for 24 h. Finally, by the time, the survivability of cells was determined by MTT method.
**Results:** Gained data indicated that Thymol had a profound cytotoxic effect on the LS174T and T47-D cell lines in a dose dependent manner. Interestingly, the IC50 value of this compound in two cancer cell lines was lower than the IC50 value in PBMCs**.**
**Conclusion:** As a result, Thymol provides favorable cytotoxicity in LS174T and T47-D cell lines without any additive cytotoxicity in PBMCs.

**Keywords:** Thymol, Peripheral Blood Mononuclear Cells, LS174T, T47-D, Anticancer.

ICIA2018\Cancer Immunology\Poster\7675
**7675**

# The study on Hemiscorpiuslepturus (scorpionida: Hemiscorpiidae) venom cytotoxicity effects on K562 cells: in a in vitro study

Nazanin Fathi[1],Golnaz Rashidi[2], Ali Khodadadi[*]

*1, 2, *.Department of Immunology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*

**Background:** Cancer is one of the most important health problems in the world. In Iran, cancer is the third cause of death after cardiovascular disease and trauma. Evaluation of the therapeutic course of cancer is a very considered area, especially the using of natural products. Recently, using of animal venoms is to be interested by scientists to inhibit the cancerous cells. Scorpion toxins are a promising approach to confront cancer, since they have shown both *in vitro* and *in vivo* fights effects on cancer cells. The purpose of this study was to evaluate the susceptibility of *Hemiscorpiuslepturus* venom on K562 cell lines, had been derived from human chronic myeloid leukemia (CML).
**Methods:** Initially, calculating the concentration of protein venom by Bradford method, then K562 cells were treated with *H.lepturus* venom using an increasing rate of concentrations during a 24 hour incubation period and inhibition of CML growth was assessed by MTT assay.
**Results:** The amount of protein concentration in *H.Lepturus*crude venom was calculated 3.35 mg/ml. The MTT assay result showed that the IC50 value was 14µg/ml after 24h incubation of K562 cells with scorpion venom.
**Conclusion:** The results of the current study suggest that H.lepturus venom increase the inhibition of growth activity of K562. Therefore, H.lepturusis hoped to be a step to *drug discovery* from natural toxins to treat the cancers.

**Keywords:** Hemiscorpiuslepturus, Venom, MTT, IC50, K562 cells

ICIA2018\Cancer Immunology\Poster\7688
**7688**

# Genistein promotes VEGF expression and Secretion in lymphoblastic leukemia cell lines

Mohsen Shahmoradi[1], Batoul Pourgheysari[2], HedayatollahShirzad[1], Fatemeh Shafiee[3]

*1. Department of Microbiology and Immunology, Faculty of Medicine, Shahrekord University of Medical Science, Shahrekord, Iran*
*2. Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran*
*3. Natural Language Processing (NLP) Research Lab, Faculty of Computer Science and Engineering, Shahid Beheshti University, Iran*

**Background:** Genistein, a soy isoflavone, has the potential to arrest various types of cancers, partially through its anti-angiogenesis property. Vascular endothelial growth factor (VEGF), one of the angiogenic mediators, can be secreted by leukemic cells and plays an important role in tumor invasion and metastasis. In the present study, we examined the in vitro expression of VEGF mRNA and protein after treatment with genistein in two human hematopoietic tumor cell lines.
**Methods:** Two human lymphoblastic leukemic cell lines (Molt-4 and Jurkat ) were used in this study. The cells were cultured in complete RPMI medium, and then incubated with $ic_{50}$ concentration of genistein for 24, 48 and 72 hours. The mRNA expression of VEGF gene was determined by real-time polymerase chain reaction (RT-PCR). Furthermore, the level of VEGF secreted in the cell culture supernatants was measured using enzyme-linked immunosorbent assay kits (R and D systems).
**Results:** In both cell lines, VEGF protein secretion and mRNA expression were increased after treatment with Genistein in all three time periods. Likewise, genistein could act as an antineoplastic activity against jurkat and molt-4 cell lines.
**Conclusion:** Although the importance of angiogenic factors, like VEGF, was clearly established in solid tumors, this effect has not been fully elucidated in human hematopoietic neoplasms. In solid tumor cell lines, genistein usually decreased mRNA expression and secretion of VEGF while in our study, this pattern was different. This Difference could be useful to recognition of VEGF modulators and perhaps new treatment strategies for leukemia and other VEGF related disorders.

**Keywords:** Lymphoblasticcell line, Genistein, VEGF

ICIA2018\Cancer Immunology\Poster\7758
**7758**

# Knowledge and Attitudes of Midwifery Students about Human Papilloma Virus Infection, Cervical Cancer and related Factors in Ahvaz Jundishapur University of Medical Sciences, Iran

Abdolhussein Shakurnia[1], Ata Ghadiri[1], Maede Hamidi[2], Samaneh Delavari[1]

1. *Department of Immunology, School of Medicine, Ahvaz University of Medical Sciences, Ahvaz, Iran*
2. *Student of Midwifery, School of Nursing and Medwifery Ahvaz University of Medical Sciences, Ahvaz, Iran*

**Background:** Yearly, around 500,000 cases of invasive cervical cancer are diagnosed worldwide. Human papilloma virus (HPV) is a leading cause of cervical cancer and sexually transmitted infections are common health problems among young females. The knowledge and attitude of Midwifery have a considerable effect on HPV infection in women. The aim of this study was to determine the level of knowledge and attitude of Midwifery students regarding HPV infection, cervical cancer and related factors in Ahvaz Jundishapur University of Medical Sciences.
**Methods:** This descriptive cross-sectional study was conducted on 112 midwifery students. Data were collected using a questionnaire containing 20 items on demographic, items related to knowledge and attitudes the HPV infection and cervical cancer. Reliability and validity of the questionnaire was evaluated and confirmed. Scores higher than the average were defined as good knowledge and attitudes. The data was analyzed in SPSS 18 software by Exact Fisher and Kruskal-Wallis tests.
**Results:** In this study, mean age of medical students was 21.59±2.41 years and all were female. The mean score of the knowledge was 26.61±22.64 and 89.2% of students had poor knowledge about HPV. The mean score of attitude was 86.52±10.52 and 97.3% of students had good attitudes. Only 2.7% of the students have been vaccinated against HPV. The relationship between the students' current educational year and their knowledge on HPV was not significant (p=0.186).
**Conclusion:** This study showed that the level of Knowledge of midwifery students about HPV infection was low. Therefore educational programs are needed to increase students' knowledge about HPV during their educational course. It seems that higher knowledge level of Midwifery can have significant effect on justification and informing target group for vaccination. Better knowledge and understanding of Midwifery regarding role of vaccination and decreasing mortality of cervical cancer can take a step to promote the condition of societal health.

**Keywords:** Human Papilloma Virus, Cervix Cancer, Knowledge, Attitude, Midwifery Students.

ICIA2018\Cancer Immunology\Poster\7780
**7780**

# Evaluation of immune checkpoint molecules, PD-L2 and 4-1BB ligand (CD137L) expression on tumor tissue of colorectal cancer patients

Parastoo Shakerin[1], Kaveh Baghaei[1], Bijan Sedighi Moghaddam[2], Maryam Nourizadeh[3]

*1. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research institute for Gastroenterology and Liver Diseases, ShahidBeheshti University of Medical Sciences, Tehran, Iran*
*2. Semnan Medical University, Semnan, Iran.*
*3. Immunology, Asthma, and Allergy Research Institute (IAARI) Tehran University of Medical Sciences,Tehran, Iran*

**Background:** Immune checkpoint (ICP) molecules modulate the immune responses by either inducing or preventing T cell activation. Expressing some immune checkpoints on malignant cells has been shown to externally regulate anti-tumor immune responses.
In this study we aimed to investigate the expression levels of two immune checkpoint molecules, PD-L2 and 4-1BBL in tumor tissues of colorectal cancer (CRC) patients compared to the adjacent normal tissues.
**Methods:** RNA extraction and cDNA synthesis were done from tumor and adjacent normal tissues of 30 CRC patients. Expression of PD-L2 and 4-1BBL molecules was evaluated by real-time PCR. Protein levels of these molecules were assessed using immunohistochemistry (IHC) staining of the tissues.
**Results:** According to our IHC data, the expression of 4-1BBL ($P$ = 0.0006) significantly decreased in tumor tissues of CRC patients compared to the controls, while considerable changes were not detected for PDL2 molecule. These results were in accordance to our real time PCR data.
**Conclusion:** Low expression of 4-1BBL,as an immune system activator, on cancer cells of CRC patients can be proposed as a target molecule to be synergized by applying agonistic anti-4-1BBL anti-bodies as an alternative immunotherapy approach for CRC patients.

**Keywords**: Immune Checkpoint Molecules, PD-L2, 4-1BBL

ICIA2018\Cancer Immunology\Poster\7857
**7857**

# 4-1BBL expressing B cells in breast cancer draining lymph nodes

Fereshteh Mehdipour[1], Mohsen Arabpour[2], Abdol Rasoul Talei[3], Abbas Ghaderi[1, 2]

*1. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
*2. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
*3. Breast Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** B cells can positively regulate immunity against tumor by increasing granzyme B expression in cytotoxic T cells via 4-1BBL/4-1BB axis. The aim of this study was to determine the frequency of 4-1BBL$^+$ B cells in the tumor draining lymph nodes (TDLNs) of breast cancer patients and its change during disease progression.
**Methods**: Fresh axillary lymph nodes' specimens were taken from 46 patients and mononuclear cells were isolated using Ficoll-Hypaque gradient centrifugation. Cells were stimulated for 4 hours with PMA/Ionomycin. Stimulated and unstimulated cells were stained for CD19 and 4-1BBL and subjected to flow cytometry.
**Results:** 2.2±1.7 of unstimulated B cells were able to express 4-1BBL while 25.7±12.3% of B cells expressed this co-stimulatory molecule after stimulation. The level of 4-1BBL expression on B cells was not significantly different in the metastatic and non-metastatic lymph nodes. However, the frequency of 4-1BBL$^+$ B cells in stimulated samples showed a tendency toward decrease in patients with grade III as compared with grade II+I (P=0.069). Moreover, the percentage of 4-1BBL$^+$ B cells was significantly higher in TDLNs of ER+ or PR+ compared to ER- or PR- patients (P=0.031 and P=0.013, respectively).
**Conclusion**: The frequency of 4-1BBL$^+$ B cells in TDLNs showed positive associations with good prognostic indicators such as ER+/PR+ and lower breast tumor grade which might be an indicative of their positive role in immunity against tumors.

**Keywords:** 4-1BBL, B cells, Tumor draining lymph nodes, Breast cancer

ICIA2018\Cancer Immunology\Poster
**7889**

# Identification of antibody reactive proteins in pancreatic cancer using two-dimensional immunoblot coupled to mass spectrometry

Marzieh Rezaei[1, 2], Saman Nikeghbalian[3], Zahra Mojtahedi[1], Abbas Ghaderi[1*]

1. *Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
3. *Department of Surgery, Nemazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy characterized by early invasiveness and resistance to treatment. Surgery in early stages is the only effective treatment, and the finding of new biomarkers for early detection of PDAC is still a major challenge.The present study aimed to compare immunoproteome between PDAC patients and healthy controls using serological proteome analysis (SERPA) method.
**Methods:** Cell lysates from two different pancreatic cancer cell lines were first separated by two dimensional (2D) gels, and then transferred onto the membranes probed with sera from 20 PDAC patients and 10 healthy controls. Proteins differentially reacted with autoantibodies in PDAC patients and the control groups were identified by mass spectrometry.
**Results:** This process led to the finding of the 18 pancreatic immunoreactive antigens can be classified into extra-cellular matrix and cytoskeletal proteins, enzymes, chaperones, signal transduction proteins and transcriptional regulators and containing of Laminin subunit alpha-5, Transcriptional regulator, Superoxide dismutase [Mn], ATP synthase subunit beta, mitochondrial ATP synthase beta subunit, Heterogeneous nuclear ribonucleoproteins C1/C2, Serine-threonine kinase receptor-associated protein, Protein SEC13 homolog, Eukaryotic translation initiation factor 3 subunit I, Chloride intracellular channel protein 1, Rho GDP-dissociation inhibitor 2, Elongation factor I-gamma, Mitochondrial Ef-Tu, chain A, Septin 2, Glyceraldehyde 3-phosphate-dehydrogenase, Phosphoglycerate mutase B isozyme, Prohibitin isoform 1, Tubulin beta 8 channel.
**Conclusion:** In the present study, we identified 18 immuno-reactive proteins in PDAC. While the identified proteins were critically involved in PDAC pathogenesis, further investigations in large scale population will determine the applicability of these potential biomarkers for early diagnosis or treatment of the disease.

**Keywords:** pancreatic ductal adenocarcinoma, biomarker, SERPA, autoantibody

ICIA2018\Cancer Immunology\Poster\7934
**7934**

# Decrease of rankl and pthrp expression, genes involved in bone metastasis, in response to arbutin as an antioxidant in lncap cell line

Hajar Safari[1], Ebrahim Zabihi[2], Mahdi Pouramir[3] , Hamid Reza Nouri [2]

*1. Student Research Committee, School of Medicine, Babol University of Medical Sciences, Babol, I.R. Iran.*
*2. Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, I.R. Iran.*
*3. Cancer Research Center, Health Research Institute, Babol University of Medical Sciences, BabolI.R.Iran*

**Background:** Prostate cancer is the sixth highest cause of death in men worldwide. Prostate cancer lines such as LNCap express activated NF-κB. The high levels of reactive oxygen species (ROS) lead to activation of NF-κB, which affect expression of Receptor Activator of NF- kB Ligand (RANKL) and parathyroid hormone-related protein (PTHrP) that are involved in osteoclast differentiation and skeletal localization of tumors. Therefore, in this study, effects of Arbutin as an antioxidant were evaluated on expression of these genes.

**Methods:** The human prostate carcinoma cell line, LNCaP, cultured in RPMI 1640 medium containing 5% fetal calf serum (FBS). Cells were stimulated with tert-Butyl hydroperoxide (tBHP) as ROS inducer and then treated by Arbutin in different doses. Intercellular ROS was measured by flow cytometry via a fluorogenic dye (H2DCFDA). After 12 h stimulation with Arbutin, cells were harvestedand total RNAs were extracted using Trizol. The RNAs were reverse transcribed using random primers. The specific primers used to amplify RANKL and PTHrP by SYBER Green Real-time PCR. Gene expression was normalized to GAPDH by the $2^{-\Delta\Delta ct}$ method.
**Results:** Based on the flow cytometry obtained data, Arbutin effectively decreased intercellular ROS in dose dependent manner. It has been able to significantly reduce ROS in 1/10 and 1/100 dose of IC50. Analysis of RANKL gene expression significantly showed low level of expression in Arbutine treated group compared to tBHP as positive group. In addition, expression of PTHrP showed the same response to the Arbutin.
**Conclusion:** Taken together, the results of our study indicate that Arbutin in LNCap cells can decrease expression of osteoclast differentiation associated genes, thereby may be affects tumor-induced bone metastasis.

**Keywords:** Arbutin, ROS,Prostate cancer; RANKL; PTHrP.

ICIA2018\Cancer Immunology\Poster\7955
**7955**

# Evaluation of prostate specific antigen (PSA) level in Suspicious Patients Who Referred to a Medical Diagnostic Laboratory in Ahvaz, Iran (2012-2016)

Abdolhussein Shakurnia[1], Asadollah Baesi[2], Masomeh Tehrani[2] Maryam Karami[3]

1. *Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
2. *Department of health education, culture and research (ACECR), Branch of Khuzestan, Ahvaz, Iran*
3. *Verterinary Doctor, Shahid Chamran University, Ahvaz, Iran*

**Background:** Prostate cancer is one of the most prevalent malignant cancer in men that is increasing mortality. Due to the easiest and most sensitive test for prostate cancer is serologic test, measurement of serum level of prostate specific antigen (PSA) is abundantly used for screening and diagnosis of prostate cancer. Considering the epidemiologic trend of prostate cancer, the aim of this study was to investigate the level of PSA in the males in a 5 period years from 2012 to 2016.
**Methods:** This study was retrospective cross sectional-descriptive. The required information of all patients who were suspected to Prostate cancer by physicians was extracted by laboratory software. The serum PSA level of 15178 patients was measured by ELISA method. According the procedure the subjects divided base on PSA values to 2 groups; lower level normal and higher level normal as health and patients men, respectively. Data was analyzed in SPSS 18 software by Chi-square tests.
**Results:** Mean age of the subjects was 57.56±12.46 years, and the mean PSA in patients was 21.87±24.35 ng/ml. The mean of PSA in patients over 50 years was 24.44±25.43 ng/ml, which was significantly higher than the values of the patients under 50 years of age with 8.72±10.80 (p=0.001). The prevalence rate of positive cases of PSA based on serological test was 2.09% (317/15178). Frequencies of positive cases of PSA has gradually increasing trend, so that the percentage of cases recorded between 2012 to 2016 was 15.4%, 17.3%, 19.5%, 22%, and 25.8%, respectively. However, this difference was not statistically significant.
**Conclusion:** Trends of increasing in prostate cancer in recent years are consistent with studies in other countries. Due to the aging of the Iranian population, developing one screening program for men is the way for prevention of prostate cancer progression and reducing mortality of this cancer recommended.

**Keywords:** Prostate cancer, prevalence, Prostate specific antigen (PSA), Ahvaz, Iran

ICIA2018\Cancer Immunology\Poster\7998
**7998**

# Investigation of cytotoxic effect of *Tanacetumparthenium* and *Cuscutaepithymum* extracts on MCF7 human breast cancer cells

Homa Davoodi*[1], Mahdieh Naghavi Alhosseini[2], Masoumeh Mazandarani[3], SeyedehNaemeh javid[4]

1. *Cancer Research Center, Golestan University of Medical Sciences, Gorgan, Iran*
2. *Department of Immunology, School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran*
3. *Department of botany, gorgan branch, Islamicazad university, gorgan, Iran*
4. *Department of Microbiology, School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran*

**Background:** Breast cancer is one of the most common carcinomas in women. Therapy on breast cancer including surgical operation, chemotherapy, radiotherapy, etc. were not completely efficient because of the serious side effect and resistance to anticancer drugs so that breast cancer still remains one of the leading causes of cancer-related death. Recently Complementary and alternative medicine for breast cancer considered to reduce or eliminate the disadvantages of conventional treatments. For centuries, traditional medicines especially natural products with herbal origin have been used for medicinal purpose. The present study was designed to explore the cytotoxic effects of *Tanacetumparthenium* and *Cuscutaepithymum* extracts on breast cancer cell line (MCF-7).
**Methods:** In order to evaluate the cytotoxicity properties of *T. parthenium* and *C. epithymum*, hydroalcoholic extract of these plants were prepared and then stored for further investigation. Cancer cells (MCF-7) were seeded in 96 well plates. Cultivated breast cancer cells were incubated with different concentrations (0.312-40 mg/ml) of the extracts for 48 hours and cell viability was determined using MTT assay.
**Results**: The results showed that in vitro exposures of the MCF-7 cells with different concentration of total extracts of these two plants significantly inhibited their growth and viability ($P<0.05$). In addition, the highest percentages of cell growth inhibition were found in concentration of 0.312 mg/ml with the values of 73.7% and 91.9% for *Tanacetumparthenium* and *Cuscutaepithymum,* respectively.
**Conclusion:** Hydroalcoholic extracts of *T. parthenium* and *C. epithymum* had cytotoxic effect on MCF-7 cell line and lead to cancer cell growth inhibition consequently. These findings are worth studying further and could have implications for future treatments and management of human breast cancer.

**Keywords:** Breast cancer, *Tanacetumparthenium*, *Cuscutaepithymum*, Cytotoxicity

ICIA2018\Cancer Immunology\Poster\8004
**8004**

**Down-regulation of HSP40 gene family followed OCT4B1suppression in human tumor cell lines**

Shima Khajouee Ravari[1], Malek Hossein Asadi[2], Seyed Javad Mowla[3], Mohammad Reza Mirzaei[1*]

*1. Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
*2. Departments of Biotechnology, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran*
*3. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran*

**Background:** OCT4B1, as one of OCT4 variants, were expressed in cancer cell lines and tissues more than other variants. We showed that this variant has an important role in regulation of cellular pathways as well as apoptosis and stress (heat shock protein). The aim of this study was to determine the effects of OCT4B1 silencing on expressional profile of HSP40 gene family in three different human tumor cell lines.
**Methods:** OCT4B1 expression were suppressed followed specific siRNA transfection in AGS (gastric adenocarcinoma), 5637 (bladder tumor) and U-87MG (brain tumor) cell lines with Lipofectamine reagent. The real-time PCR array technique was employed and the fold changes were calculated using RT$^2$ Profiler PCR array data analysis software version 3.5.
**Results:** Our results indicated that the expression profile of 36 studied genes from HSP40 family in studied tumor cell lines after OCT4B1 suppression showed approximately similar pattern of expression. Fifteen genes were down-regulated and tow genes (DNAJC11 and *DNAJC5B*) were up-regulated in all three studied tumor cell line (more than 2 folds) and other studied genes (19 genes) showed different expressional pattern (up or down-expression) based tumor cell lines.
**Conclusion:** we suggest there is a direct correlation between OCT4B1 expression in tumor cell lines (and tissues) and HSP40 family gene expressions to escape from apoptosis and cancer expansion.

**Key words:** OCT4B1, HSP40 gene family, siRNA, tumor cell lines

ICIA2018\Cancer Immunology\Poster\8052
**8052**

## Sirt1 Natural antisense transcript Down-regulates in Human Cancers

Neda Mokhberian[1], Seyed Mahmoud Hashemi[2], Vahid Jajarmi[1], Hossein Ghanbarian[1]

*1. Department of Biotechnology, School of Advanced Technologies in Medicine, ShahidBeheshti University of Medical Sciences, Tehran, Iran*
*2. Department of Immunology, School of Medicine, ShahidBeheshti University of Medical Sciences, Tehran, Iran*

**Background:** Sirtuin1 (Sirt1) is a NAD-dependent deacetylase which is involved in the development and progression of human cancers. Recent studies have shown that a natural antisense transcript (NAT) is present in Sirt1 locus. Natural antisense transcripts (NATs) have recently been associated with the development of human cancers. Here, we identified the expression of Sirt1 sense and Antisense transcripts in several human cancers.
**Methods:** Following RNA extraction and cDNA synthesis, the expression level of Sirt1 mRNA and Sirt1-NAT was detected by quantitative real time-PCR assay in cancer cell lines and cancer clinical specimens.
**Results:** While Sirt1 mRNA level was upregulated in cancer cell line and cancer clinical specimens, the expression of Sirt1 antisense decreased in cancers compared to controls.
**Conclusion:** A converse relation was observed in the expression of Sirt1 sense and antisense transcript in normal and cancer tissues.

**Keywords:** Sirt1, Natural Antisense Transcript, Cancer, Cell line

ICIA2018\Cancer Immunology\Poster\8121
**8121**

# HLA-G prognostic value in malignant liver and pancreas lesions and functional implications of HBV for HLA-G expression

Parisa Khodabandeh Shahraki,[1] Yasmin Zare,[1] Negara Azarpira,[2] Massood Hosseinzadeh,[3] Shirin Farjadian[1]

1. *Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Organ Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
3. *Department of Pathology, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Human leukocyte antigen (HLA)-G is a nonclassical HLA class I molecule with modulatory effects on NK and T cells. Because HLA-G expression is frequently detected in different solid tumors, it may be involved in tumor immune evasion.
**Objectives:** We designed this study to elucidate the prognostic value of HLA-G in hepatocellular carcinoma (HCC) and pancreatic adenocarcinoma (PADC). The influence of hepatitis B virus (HBV) infection on HLA-G expression was also evaluated in patients with HCC.
**Methods:** HLA-G expression was investigated in tumor tissues from patients with HCC (n=74) or PADC (n=42) with immunohistochemical techniques. The presence of HBV genome was also examined in HCC tumor tissues by PCR.
**Results:** HLA-G expression was detected 66% of PADC and in 31% of HCC samples. In contrast to HCC, HLA-G overexpression was associated with advanced stages and grades in PADC. HBV genome was detected in 31% of HCC samples but we found no correlation between HLA-G expression and the presence of HBV genome in these tumors.
**Conclusion:** Our findings showed that HLA-G overexpression in tumor tissue correlated with poor prognosis in PADC. HLA-G expression is apparently affected more by the patient's genetic background and other epigenetic factors than by HBV infection.

**Keywords:** Human leukocyte Antigen-G, hepatocellular carcinoma, hepatitis B virus, pancreatic adenocarcinoma

ICIA2018\Cancer Immunology\Poster\8126
**8126**

# Considering prevalence of B-RAF$^{V600E}$ and PI3KCA genes' mutations in Iranian Colorectal cancer patients: Full COLD PCR and HRMA

Atousa Homafar[1], NaserShagerdi Esmaeli[2], Shahram Samie[3]

1. *Department of nutrition science,TabrizBranch,Islamic Azad university,Tabriz,Iran*
2. *Department of Hematology of ShahidBeheshti University of Medical Science,Tehran,Iran*
3. *Iranian high Institute for Research and Education in Transfusion Medicine, Tehran – Iran.*

**Background:** Colorectal cancer is the third most common cancer in the world. Mutations detection of B-RAF and PI3KCA genes is resolvable for choosing a target Anti-EGFR therapy. The aim of study is considering the mutation prevalence in B-RAF gene of by using Full-COLD PCR method and HRMA and in PI3KCA gene by using HRMA.
**Methods:** DNA of 27 FFPE of Colorectal Cancer was extracted. Therefore, for prevalence in exon 15 of B-RAF gene with using 2 methods combining of Full-COLD and HRMA was determined and for mutations in 2 exons 9 and 20 of PI3KCA gene by HRMA was used. Sensitivity of Full-COLD and HRMA by serial dilution of A-375 cell-line was determined. Sensitivity of this cell-line by serial dilution in Genomic DNA background with ARMS PCR method was considered. HCT-116 cell-line was chosen as positive control for PI3KCA gene mutations.
**Results:** Sensitivity of A-375 and HCT-116 cell-lines was 270 cell/µM. Sensitivity of combining 2 methods of Full-COLD and HRMA was %1. Sensitivity range of A-375 in Genomic DNA background by ARMS PCR was also %1. Although, HCT-116 cell-line was confirmed as positive control for mutations of 2 exons 9 and 20 of PI3KCA gene by using HRMA.
**Conclusion:** No detectable mutation in B-RAF and exon 20 of PI3KCA in extracted DNA from FFPE was found, but in exon 9 of this gene %3.7 was mutated. It seems Full-COLD plus HRMA method is more robust and sensitive method for detection or Screening of PI3KCA and B-RAF mutations.

**Keywords:** Colorectal cancer, B-RAF, PI3KCA, Full-COLD PCR, HRMA

ICIA2018\Cancer Immunology\Poster\8162
**8162**

**Inhibition of the adenosine signaling in 4T1 cell lines has dose-dependent effects**

Zahra Zeinaly, Seyyed Meysam Abtahi Froushani

*Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Adenosine plays an important role in inflammation and tumor progression, and responses to therapies. Some tumor cells like 4T1 (an invasive mice ductal carcinoma) can produce adenosine. Here, we investigated the effects of caffeine, as an adenosine antagonist and a routine drink, in mice peripheral blood mononuclear cells (PBMCs) and 4T1.
**Methods:** $۱\times۱۰^{6}$ live cells of PBMCs of mice and 4T1 were incubated different concentrations of caffeine (0, 0.1, 0.5 and 1 mM) for 48 hours. PBMCs were pulsed with phytohemagglutinin (final concentration of 5 µg/ml). Finally, the survivability of cells was determined by MTT and neutral red uptake method.
**Results:** Attained results indicated that caffeine cannot change the rate of lymphocytes proliferation. Interestingly, caffeine at 0.5 mM significantly reduced the proliferation of 4T1 cells compared to other groups. Nonetheless, caffeine at 1 mM significantly potentiated the proliferation index of 4T1 cells.
**Conclusion:** The moderate dose of caffeine has beneficial anti-proliferative effect against 4T1 cells, Nevertheless higher doses of caffeine have revers and dangerous effects on 4T1 cells.

**Keywords:** 4T1 cell lines, Peripheral blood mononuclear cells, Caffeine.

ICIA2018\Cancer Immunology\Poster\8166
**8166**

# Cytokine production by peripheral blood lymphocytes (PBLs) in the presence of cancer and normal adipose-derived mesenchymal stem cells (ASCs)

Mahboobe Razmkhah [1], Maryam Fakhimi [1,2], Abbas Ghaderi [1,2]

1. *Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Departments of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Tumors are complex tissues of different cell types, including stromal cells, cells of the immune system and mesenchymal stem cells (MSCs). MSCs play a significant role as immunomodulators in the tumor microenvironment through producing different cytokines. The present study aimed to investigate the effects of adipose derived mesenchymal stem cells (ASCs) on IL-10and IL-17 cytokine production by peripheral blood lymphocytes (PBLs).
**Methods:** Human adipose-derived stem cells (ASCs) were obtained from breast adipose tissues of a breast cancer patient and a normal individual in cosmetic mammoplasty surgery. The ASCs were co-cultured with normal PBLs from 5 normal individual for 5 days. The production of cytokines including IL-10 and IL-17 in PBLs were measured by ELISPOT method.
**Results:** Based on the results, the production of IL-10 was increased as PBLs were cocultured with breast cancer ASCs compared to those cultured with normal ASCs or control group although this increased was not statistically significant(P value>0.05). IL-17 production by PBLs decreased in the presence of cancer and normal ASCs compared with control. Comparing the effect of cancer-ASCs with normal-ASCs, the former had a significant effect on IL-17 production (P value=0.043).
**Conclusion:** Based on the results of this study, the cross-talk between ASCs and the immune cells can induce the expression of anti-inflammatory cytokines such as IL-10 and reduce the expression of inflammatory cytokines such as IL-17 in the tumor microenvironment. Consequently, ASCs may promote tumor growth and progression through the immunomodulatory effects on different types of the cells from the immune system.

**Key words:** Adipose-derived mesenchymal stem cell, Breast cancer, Immunomodulation, IL-10, IL-17.

ICIA2018\Cancer Immunology\Poster\8175
**8175**

**Membrane-Type-2 Matrix Metalloproteinase is selectively expressed on malignant myeloma cells**

Shohreh Fakhari[1], Ali Jalili[1], Bahram Nikkhoo[1], BayazidGhaderi[1], SakoMirzaie[2]

1. *Cancer and Immunology Research Center, Kurdistan University of Medical Sciences*
2. *Department of biochemistry ,Sanandaj Branch, Islamic Azad university, Iran*

**Background**: Multiple myeloma (MM) is a plasma cell cancer that is considered 10% of all hematologic malignancies. Despite the development of new drugs for treatment of MM, it remains as one of the incurable cancer. Thus, performing new research is necessary to identify molecules that may play an important role in the biological behavior of myeloma cells. Matrix metalloproteinases (MMPs) are among the key players in migration and metastasis of many cancers. The role of some soluble MMPs (MMP 1,2,7,8,9,13) have been shown in hematologic malignancies, including multiple myeloma, like other cancers. However expression of Membrane Type-MMPs (MT-MMPs) is unknown in hematologic malignancies. Hence, in this study we evaluated expression of 6 MT-MMPs in myeloma cell lines, B cell lines, normal lymphocytes, mononuclear (MNC) and polynuclear cells (PMN).
**Methods:** We first screened expression of 6 MT-MMPs in the myeloma cell lines, B cell lines and normal lymphocytes, MNC and PMN cells by RT-PCR. Then, we performed IHC to evaluate MT2-MMP expression in 10 biopsies of normal bone marrow and 11 biopsies from patients with MM. In addition 7 bone marrow aspirated samples from MM patients MACS into 4 fractions ( PMN, CD19+, CD138- , CD138+ cells) and MT2-MMP expression at mRNA and protein levels were examined by using qRT-PCR and flow cytometry, respectively.
**Results:** Our data showed for the first time that MT2-MMP, from among 6 MT-MMPs, is highly expressed in myeloma cells but not on other cells such as B cell lines and normal lymphocytes, MNC and PMN. Interestingly, IHC data showed that MT2-MMP was expressed in 100% (11 out of 11) MM biopsies and neither of 10 normal biopsies. Additionally, Real-time and flow cytometry data confirmed that expression of MT2-MMP is only expressed on malignant plasma cells (CD138+ cells), but not in normal fractions (CD138-, CD19+ cells and PMN).
**Conclusion**: In conclusion, we have shown for the first time that MT2-MMP is selectively expressed in myeloma cells and that MT2-MMP could be a suitable marker for diagnosis and possibly a novel target for treatment of MM.

**Keywords:** Multiple myeloma, Matrix metalloproteinase

ICIA2018\Cancer Immunology\Poster\8183
**8183**

# H. pylori infection enhances CXCR4 and CXCR7 expression on gastric epithelial cells

Ali Jalili[1], Shohreh Fakhari[2], Zeinab Kavyani[1,3*]

1. *Immunology Department, Medicine faculty, Kurdistan University of Medical Sciences, Sanandaj, Iran*
2. *Cancer and Immunology Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran*
3. *Maternal- Fetal and Neonatal Research Center, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Chemokine SDF-1 is a main cytokine in recruitment and conservation of hematopoietic precursor cells in bone marrow that it's also produced in the site of tissue injury and attracts cells involving in tissue remodeling via its two specific receptors: CXCR4 and CXCR7. *Helicobacter Pylori* as a class-1 carcinogen plays an important role in development of gastric cancer by producing SDF-1 and recruitment of CXCR4/7 presenting cells. These cells can initiate dysplasia and metaplasia in gastric mucosa. In earlier study, the role of CXCR4/CXCR7/SDF-1 axis in gastric cancer and its prognosis in this cancer were evaluated so that enhanced expression of these receptors was related with metastasis; but the role of *H.pylori* in this process has not been investigated. In this study, we examined the effect of *Helicobacter Pylori* on CXCR4 and CXCR7 expression in gastric epithelial cells and gastric biopsy samples.
**Methods:** Gastric epithelial cell line, AGS, was co-cultured with purified colony of *H.pylori* in 100 bacteria/cell for 24h. Expressions of CXCR4 and CXCR7 mRNAs measured by RT-PCR and cell surface expression of these receptors evaluated by flow cytometry. Chemotaxis assay was performed to testing migration of treated and untreated AGS cells with *H.pylori* toward SDF-1consentration gradient. Furthermore, expressions of CXCR4 and CXCR7 in gastric samples were assessed by Real-Time PCR.
**Results:** The results of the study showed that*H.pylori* exposure enhances CXCR4 and CXCR7 receptors expression both in cytoplasm and on cell surface in AGS cells. Migration of *H.pylori* treated cells toward SDF-1 gradient increased in comparison to control group. Gastric samples from *H.pylori* positive patients showed higher levels of CXCR4 and CXCR7.
**Conclusion:** We found that *Helicobacter Pylori* infection enhancesCXCR4 and CXCR7 in gastric epithelial cells and also in gastric samples. Furthermore, enhanced migration capability to SDF-1 may be effective in gastric cancer pathology and metastasis.

**Keywords:** CXCR4, CXCR7, *Helicobacter Pylori*, Gastric cancer

ICIA2018\Cancer Immunology\Poster\8238
**8238**

## IL-17 B expression in peripheral blood mononuclear cells of chronic lymphocytic leukemia patients

Mehdi Barati[1, 2, 3], Mehrnoosh pashaei[2, 3] , Farahnaz Ghahremanfard[3, 4] , Fatemeh Pak[2, 3] , Parviz Kokhaei[2, 3, 5*]

1. *Department of Immunology, Faculty of medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
2. *Department of Immunology, Semnan University of Medical Sciences, Semnan, Iran.*
3. *Cancer Research Center, Semnan University of Medical Sciences, Semnan, Iran*
4. *Department of Internal Medicine, Semnan University of Medical Sciences, Semnan, Iran*
5. *Immune and Gene therapy Lab, Cancer Center Karolinska (CCK), Karolinska University Hospital Solna and Karolinska Institute, Stockholm, Sweden*

**Background:** Interleukin 17B (IL-17B) as a pro-inflammatory cytokine is a member of interleukin 17 (IL-17) family cytokines (IL-17A - IL-17F). IL-17B Signal transduction mediated by a heterodimeric receptor complex that composed of IL-17RA/IL-17RB. Dis-regulation of IL-17B has been associated with a variety of diseases including cancer, autoimmune and inflammation disorders. Over expression of IL-17B in tumor, microenvironment is contributed to progression of several tumors. Therefore, the aim of this study was to investigate the expression of IL-17B in peripheral blood mononuclear cells in chronic lymphocytic leukemia (CLL) patients in compared to healthy subjects.
**Methods:** Initially RNA extraction and cDNA synthesis were performed on PBMCs of CLL patients (n=48) and healthy subject (n=35). RNA expression of IL-17B was quantified using Real-time PCR method.
**Results:** Our data indicated that IL-17B expression in PBMCs of CLL patients were significantly higher than normal subjects.
**Conclusion:** Overexpression of IL-17B may induce tumor progression. Therefore, targeting of IL-17B will be an effective therapeutic target in the future.

**Keywords:** chronic lymphocytic leukemia, IL-17B, tumor microenvironment, Real-time PCR

ICIA2018\Cancer Immunology\Poster\8274
**8274**

# Comparison of Killing effect of synthetic and biologic nano selenium particles with on cell line derived from nervous cells

Shideh Khangholi[1], Mehdi Mahdavi[2], Mohammadhossein Yazdi[3*]

1. *Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran*
2. *Department of Immunology, Pasteur Institute of Iran, Tehran, Iran*
3. *Department of Pharmaceutical Biotechnology and Biotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Today, brain tumors could be lethal without effective treatment especially in case of metastatic form. While many therapeutic approaches such as chemotherapy, radiotherapy and surgery have been utilized to cure this disease. The supplement substances such as selenium can play important role in this matter. As this respect, selenium nano particle is produced in two forms of synthetic and biologic to evaluate cytotoxic effect on nervous cell line in the present study.
**Methods:** A-172 nervous cell line was prepared and cultured in DMEM medium. Selenium nano particle was used in two forms of synthetic and biologic. The cytotoxicity of synthetic and biologic Nano selenium was assayed by MTT test and the result was read by ELISA reader in the wavelength of 570 nanometers.
**Results**: There is significant difference in group treated with biologic Nano selenium as compared to singular cell in concentrations of 100, 300 and 400 µg/ml, 400 µg/ml, 25, 100, 200, 300 and 400 µg/ml after 24, 48 and 72 hrs respectively. Also, there is remarkable difference in group treating with Synthetic Nano selenium in compared to singular cell in certain concentrations after 24, 48 and 72hrs. as a result, biologic Nano selenium has more cytotoxic effect than synthetic.
**Conclusion:** Nano selenium be able to suppress growth of nervous cell line via cytotoxicity function. Also biologic Nano selenium has more killing powerful than synthetic one.

**Keywords**: Selenium, biologic Nano selenium, synthetic Nano selenium, A-172 nervous cell line.

ICIA2018\Cancer Immunology\Poster\8284
**8284**

# Kaempferol effect on telomerase gene expression in hela cervical carcinoma Cell Line

Saiedeh Erfanian[1], Maliheh Moradzadeh[2], Mohsen Farhang[3]

*1. Department of Advanced Medical Sciences and Technologies, School of Medicine, Jahrom University of Medical Sciences (JUMS), Jahrom, Iran*
*2. Golestan Rheumatology Research Center, Golestan University of Medical Sciences, Gorgan, Iran*
*3. Student of Laboratory Sciences, Student Research Committee, Jahrom University of Medical Sciences, Jahrom, Iran*

**Background:** Cervical cancer is one of the most common female cancers, while chemotherapy for this type of cancer is still not satisfactory. In recent years, the use of herbal medicines is expected to rise sharply. The effectiveness of these products of natural origin has urged researchers to discover new medicinal plants. Kaempferol is a natural flavonol which has therapeutic effects. Previous studies have proven the antioxidant and anti-inflammatory effects of kaempferol. In this study, the inhibitory effect of kaempferol on cervical carcinoma (HeLa) cell line proliferation and telomerase genes activity have been studied.
**Method:** After culturing, HeLa cells were treated by 25.50 and 75 micro-molar kaempferol concentrations, and 5-Fluviorasil was treated at a concentration of 80 mM. Also, a combination of kaempferol with concentration of 50mM and 5-Fluyorasil was used for the treatment. HeLa cells viability after proliferation was evaluated by cytotoxicity assay. Also, the polymerase chain reaction quantitative method (real time pcr) was used to evaluate the expression of TRF2 and Htert genes.
**Results:** Treatment with kaempferol significantly inhibited the proliferation of HeLa cells compared to the control group. HTERT and TRF2 gene expression in all studied groups was significantly reduced (P-value <0.05).
**Conclusion:** In this study, for the first time we showed that kaempferol can be a potential inhibitor for the cervical cancer cells. Since kaempferol has lower toxicity compared to chemotherapeutic clinically used agents such as 5-Fluviorasil, it can be used as adjunct therapy in combination with 5-Fluviorasil in patients with cervical cancer.

**Keywords:** kaempferol, cervical cancer, inhibition of cell proliferation

ICIA2018\Cancer Immunology\Poster\8285
**8285**

# Kaempferol effect on cell proliferation inhibition and apoptosis induction in Acute Myeloid Leukemia Cells line (HL60)

Saiedeh Erfanian[1], Maliheh Moradzadeh[2], Mohsen Farhang[3]

*1. Department of Advanced Medical Sciences and Technologies, School of Medicine, Jahrom University of Medical Sciences (JUMS), Jahrom, Iran*
*2. Golestan Rheumatology Research Center, Golestan University of Medical Sciences, Gorgan, Iran*
*3. Student of Laboratory Sciences, Student Research Committee, Jahrom University of Medical Sciences, Jahrom, Iran*

**Background:** Leukemia, one of the most common cancers in Iran, is a progressive and malignant disease of the blood-forming part of the body that is caused by the proliferation and development of white blood cells and its precursors in the blood and bone marrow. Introducing all trans retinoic acid (ATRA) for the treatment of acute promyelocytic leukemia (APL) patients could dramatically improve their survival rate. However, resistance to this drug and their toxicity are major problems in the treatment of APL. Earlier studies suggested that in haematopoietic neoplasms, the green tea polyphenol Epigallocatechin gallate (EGCG) induces cell death without adversely affecting healthy cells. This study was designed to evaluate the effect of EGCG on the level of lysis and expression of genes involved in apoptosis and cellular growth of acute myeloid leukemia cell line.
**Methods:** In the present study, the potential anti-promyelocytic leukemia activity of EGCG and the underlying molecular mechanisms were investigated. Acute Myeloid Leukemia Cells line (HL60) were cultured in RPMI medium and then incubated with different concentrations of EGCG (100-12.5 µM). Cell survival (Viability) was measured by MTT assay. Real time PCR was also used to evaluate the apoptotic expression of genes and confirm the apoptotic mechanism.
**Results:** EGCG (100 µM) significantly inhibited proliferation and induced apoptosis in HL-60 cells. The effect was associated with the expressions of proapoptotic genes p21 and Bax/Bcl-2 ratio were significantly increased.
**Conclusion:** we can conclude that the results of this study, changes in the level of expression of these genes, and the matching of the results with the findings of previous studies, can be argued that the effect of EGCG on acute myeloid leukemia cells is undeniable effects. However, further studies on these compounds are suggested to identify a more precise mechanism.

**Key words:** acute promyelocytic leukemia; EGCG; Apoptosis

ICIA2018\Cancer Immunology\Poster\8307
**8307**

# Reduced Levels of T-helper 17-associated Cytokines in the Serum of Patients with Breast Cancer: Indicators for Following the Course of Disease

Mohammad Reza Atashzar [1], Rasoul Baharlou[1], Abbas Ahmadi Vasmehjani[1], Ebarahim Rahimi[2], Majid Khoshmirsafa[1], Farhad Seif[1], Maryam Mahdiyar[3]

*1. Department of Immunology and Microbiology, School of Medicine, Jahrom University of Medical Sciences, Jahrom, Iran*
*2. Department of Epidemiology, School of Medicine, Jahrom University of Medical Sciences, Jahrom, Iran*
*3. Department of Student Research Committee, School of Medicine, Jahrom University of Medical Sciences, Jahrom, Iran*

**Background:** Interleukin (IL)-17 producing CD4+ T helper (Th17) cells that are known by producing IL-17 have recently been defined as a unique subset of proinflammatory helper cells. IL-17 is an inflammatory cytokine with robust effect on many cells and it can play important roles in pathogenesis of diverse groups of immune-mediated diseases. Therefore, the aim of this case control study was to determine serum levels of IL-17, IL-6, and transforming growth factor beta (TGF-β) in Iranian breast cancer patients.
**Methods:** Blood samples were collected from 55 patients with breast cancer and 34 healthy individuals with no history of malignancies or autoimmune disorders, based on simple sampling. The serum levels of IL-17, IL-6 and TGF-β were measured by the enzyme linked immunosorbent assay (ELISA).
**Results:** The serum level of IL-6 was significantly lower in patients with breast cancer compared with healthy individuals ($p=0.0003$), and also their IL-17 was lower in patient group than controls ($p=0.01$). Interestingly, TGF-β serum level in patients was less than controls ($p<0.0001$).
**Conclusion:** As most of the cases investigated in this study were in early stages, it is concluded that reduced IL-17, IL-6 and TGF-β can be used as predictors for clinical stage and prognosis of cancers such as breast carcinoma.

**Keywords**: Breast cancer, Interleukin-6, Interleukin-17, T-helper 17, Transforming growth factor beta

ICIA2018\Cancer Immunology\Poster\8383
**8383**

# Study of serum IFN-γ and IL-4 levels in diffuse large B cell lymphoma (DLBCL) patients of response and non-response to Rituimab and DLBCL patients of under treatment with Rituximab

Nasrin Zare[1], NahidEskandari[2], Shaghayegh Haghjoo Javanmard[3], Valiollah Mehrzad[4]

*1. Department of Immunology, School of Medicine, Isfahan University of Medical sciences*
*2. Department of Immunology, School of Medicine and Applied Physiology Research Center, Isfahan University of Medical Sciences*
*3. Department of physiology, School of Medicine and Applied Physiology Research Center, Isfahan University of Medical Sciences*
*4. Department of Hematology and Medical Oncology, Isfahan University of Medical Sciences*

**Background:** Patients with DLBCL have variable responses to rituximab. Cytokines might be deregulated after using of rituximab and alsoin DLBCL patients of response and non-response to Rituximab. The current study was designed to investigate and compare serum IFN-γ and IL-4 levels in DLBCL patients of response, non-response to Rituximab and DLBCL patients under treatment with Rituximab.
**Methods:** Serum patients were obtained from the Isfahan's Sayed Al-Shohada Hospital, Iran. Serum IFN-γ and IL-4 were measured using the Human IFN-γ ELISA MAX™ Deluxe (Biolegend) and Human IL-4 ELISA development kit (Mabtech).
**Results:** The IFN-γ and IL-4 were measured in serum of DLBCL patients of response (n=8), non-response (n=8) and under treatment (n=8) with Rituximab. The Mean serum values for these patients, respectively, were: for IFN-γ, 6.14 pg/mL, 8.9 pg/mL and 7.8 pg/mL; for IL-4, 4.3 pg/mL, 2.1 pg/mL and 0.005 pg/mL. DLBCL patients of under treatment with Rituximab exhibited a decreasein IL-4 levels compared to the other two groups (P=0.06). There were no significant difference in IFN-γ (P=0.3).
**Conclusion:** These results indicate that decrease in serum IL-4 and IFN-γ levels may be appropriate biomarkers to identify DLBCL patients with variable responses to rituximab.

**Keywords:** DLBCL, IFN-γ, IL-4, Rituximab

ICIA2018\Cancer Immunology\Poster\8406
**8406**

# IL-17RB expression in peripheral blood mononuclear cells of chronic lymphocytic leukemia

Mehrnoosh pashaei[1, 2], Mehdi Barati[1, 2, 3], Farahnaz Ghahremanfard[2, 4], Fatemeh Pak[1, 2], Parviz Kokhaei[1, 2, 5*]

1. *Department of Immunology, Semnan University of Medical Sciences, Semnan, Iran*
2. *Cancer Research Center, Semnan University of Medical Sciences, Semnan, Iran*
3. *Department of Immunology, Faculty of medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
4. *Department of Internal Medicine, Semnan University of Medical Sciences, Semnan, Iran*
5. *Immune and Gene therapy Lab, Cancer Center Karolinska (CCK), Karolinska University Hospital Solna and Karolinska Institute, Stockholm, Sweden*

**Background:** B Chronic lymphocytic leukemia (B-CLL) is the most common adult leukemia in the western world. The pathogenesis of CLL has been attributed to various forms of immune dysregulations. There are evidence that T cell dysfunction and differentiation shift to Th2 in B-CLL which may contribute to progress of the disease. The IL-17 receptor B (IL-17RB) is a receptor for IL-17E and also able to bind IL-17B. Stimulation of IL-17RB promotes Th2 responses. However, IL-17RB in B-CLL has not been investigated so far. Therefore, this study was performed to evaluate the expression of IL-17RB in peripheral blood mononuclear cells in B-CLL patients compare to healthy subjects.
**Methods:** Initially total RNA was extracted from peripheral Blood Mononuclear Cells (PBMCs) of CLL (n=48) and normal subjects (n=35) and cDNA were synthetized. The mRNA expression of IL-17RB was quantified using Syber Green Real-time PCR method, using ABI 7900 machine.
**Results:** Our data indicated that IL-17RB expression in PBMCs of CLL patients were significantly higher than normal subjects (P value <0.01, P value= 0.0090).
**Conclusion:** High expression of IL-17RB in B-CLL may induce tumor cells viability through its ligands. Tumor cells may express these receptors to evade from apoptosis and promote tumor cells survival. Therefore, blocking of IL-17RB could be new approach for translational investigation and clinical application.

**Keywords:** chronic lymphocytic leukemia, IL-17RB, Peripheral blood mononuclear cells, Real-time PCR

ICIA2018\Cancer Immunology\Poster\8407
**8407**

# The study of Indirubin effect on peripheral blood mononuclear cell of chronic lymphocytic leukemia patientsin invitro

H. Jafarinezhad[1, 2] (M.Sc.), B. Sedighimoghadam[1] (PhD), A. Aghaie[3] (PhD), N. Ghanizadeh[2, 1] (M.Sc.), M. Faranoosh[4] (MD), S. Rostami[5] (PhD) P. Kokhaei[6, 1 *] (Professor-PhD)

*1. Cancer Research Center and Immunology Department, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran*
*2. Student Research Committee, Semnan University of Medical Sciences, Semnan, Iran*
*3. Blood Transfusion Research center, High institute for Research and Education in Transfusion Medicine, Tehran, Iran*
*4. Children's Development Research Center, Endocrinology and Metabolism Research Center, Iran University of Medical Sciences, Tehran, Iran*
*5. Hematology, Oncology and Stem Cell Transplantation Research center, Tehran University of Medical Sciences, Tehran, Iran*
*6. Laboratory of Immunology and Gene Therapy, Karolinska Cancer Center, Karolinska University Hospital, Stockholm, Sweden*

**Background:** Chronic lymphocytic leukemia (CLL) is a malignancy of naive B-cells. Indirubin is an active constituent of a traditional Chinese herbal medicine. Indirubin or its derivatives had the ability to induce apoptosis in cancer cell. Therefore, the aim of this study is evaluation of indirubin effect on peripheral blood mononuclear cell of chronic lymphocytic leukemia patientsin compared with healthy donor in invitro.
**Methods:** Peripheral blood samples (5 mL) obtained from 14 CLL patientsand 8 age-matched healthy subjects. Peripheral blood mononuclear cells (PBMC) were isolated from blood patients and healthy subjects by using Ficoll technique. PBMC cultured with indirubin (in concentration of 0.1 to 80 µM) for 24, 48 and 72 hours. The cytotoxicity percentage of indirubin was measured by MTT technique and also apoptosis percentage was detected by AnnexinV Assay. Finally BAX, Bcl2, CDK1 and CDK2 mRNA expression were investigated by Real time PCR using SYBR Green method.
**Results:** The MTT assay showed that indirubin has high cytotoxicity percentage (20µM, 48h) in the PBMC of CLL patients in compared with control group ($P<0.05$). The Annexin v assay indicated that the indirubin has high apoptosis percentage (20µM, 48h) in the PBMC and B cells (CD19+ cells) in compared with control group ($P<0.05$). The expression level of Bcl2, CDK1 and CDK2 mRNA in PBMC of CLL patients that treated with indirubin in compared with control group decreased significantly ($p<0.05$) and BAX mRNA level showed no significant differences in PBMC of CLL and healthy donors($p>0.05$).
**Conclusion**: It seems indirubin induces apoptosis in tumor cells in dose and time dependent manner. This herbal component causes apoptosis by down regulation of Bcl2 level mRNA and induces cell cycle arrest by down regulation of CDK1 and CDK2 level mRNA in PBMC of CLL patients.

**Keywords**: Chronic lymphocytic leukemia, CDK1, CDK2, Bcl2, BAX

ICIA2018\Cancer Immunology\Poster\8421
**8421**

# Lower circulating levels of interleukin-10 in patients with bladder cancer

Taybeh Tavakoli[1], Hossain Khorramdelazad[1], Mohammad-Taghi Rezayati[1], Somayeh Abolhassani[1], Somayeh Pourtalebi[1], Abdollah Jafarzadeh[2]

1. *Department of Immunology, Medical School, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
2. *Department of Immunology, Medical School, Kerman University of Medical Sciences, Kerman, Iran*

**Background:** Interleukin 10 (IL-10) is considered an immune-regulatory cytokine, exerting both antitumor and pro-tumor properties. The role of IL-10 in tumor development is very controversial. The aim of this study was to investigate the serum levels IL-10 in patients with bladder cancer (BC).
**Methods:** The blood samples were collected from 28 patients with bladder cancer and 23 age and gender-matched healthy subjects as a control group. The serum levels of IL-10 were measured by ELISA.
**Results:** The mean serum levels of IL-10 was 8.39 ± 3.17 Pg/ml in BC group and 10.72 ± 3.60 in healthy subjects. The mean serum levels of IL-10 in BC patients was significantly lower than healthy group ($P<0.02$). The serum levels of IL-10 in BC men was significantly lower than healthy men (7.96 ± 3.44 Pg/ml vs 12.26 ± 3.60 Pg/ml, $P<0.01$). However, IL-10 levels similarly expressed in BC women and healthy women.
**Conclusion:** These results showed lower levels of IL-10 in patients with BC. The IL-10 levels were affected by gender of patients. The clinical values of IL-10 in BC need more consideration.

**Keywords:** Bladder cancer, Interleukin-10, Serum, Gender

ICIA2018\Cancer Immunology\Poster\8424
**8424**

# The Hepatocyte Growth Factor (HGF) can act as immune modulator by increasing IL10, TGFβ and IFNγ cytokines in Mesenchymal Stem Cells: in vitro study

KavehTari[1], Ali Jalili[1]

*1. Cancer and Immunology Research Center, Kurdistan university of medical science*

**Background**: The Hepatocyte Growth Factor (HGF) has important role as a modulator of immune system. This factor produce mainly by Bone Marrow Mesenchymal Stem Cells (BM-MSCs.) The purpose of this study was evaluation of the effect of HGF on human mesenchymal stem cells to produce the gene of IL10, TGFβ and IFNγ cytokines and suppression of the proliferation of Peripheral blood mononuclear cell(PBMCs.)
**Methods:** In this study, the BM-MSCs were Isolated, cultured, characterized and then treated with HGF at dose of 20 ng/ml in different times. IL10, TGFβ and IFNγ gene were measured by qRT-PCR, Also, the ability of the HGF in suppression of the proliferation of PBMNC cells were evaluated by using MLR. Results were analyzed by SPSS software and T-test.
**Results**: The results showed significant production of IL10, TGFβ and IFNγ upon HGF treatment in RNA level ($P<0.05$). Also a significant decrease of PBMNC proliferation co-cultured with HGF treated- MSCs were seen ($P<0.05$).
**Conclusion**: The findings showed that using of HGF could decrease proliferation of HSCs by altering expression of key cytokines in the process of modulating of immune system.

**Keywords**: Hepatocyte Growth Factor, Mesenchymal Stem Cells, Immune Modulating.

ICIA2018\Cancer Immunology\Poster\8439
**8439**

## CD25 expressing B cells in head and neck squamous cell carcinoma draining lymph nodes

Marzie Norouzian[1,2], Fereshteh Mehdipour[2], SimaBalouchi Anaraki[1,2], Bijan Khademi[3], Abbas Ghaderi[1,2]

1. *Department of Immunology, School of Medicine, Shiraz University of Medical Sciences*
2. *Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences*
3. *Department of Otolaryngology, Khalili Hospital, Shiraz University of Medical Sciences*

**Background:** Tumor-draining lymph nodes (TDLNs) are the main site in which tumor-specific immune responses are initiated. B cells are among main residents of TDLNs but compared to T cells, little is known about phenotype and function of B cell subsets repopulated in the TDLN. It has been suggested that B cells expressing CD25have different phenotypical and functional properties as compared to CD25− B cells. CD19+CD25hi B cells have also been reported to have regulatory capacity in humans. The aim of this study was to assess the frequency and phenotype of CD25 expressing B cells in the TDLNs of head and neck squamous cell carcinoma.
**Methods**: Freshly isolated lymph node cells were directly stained with fluorochrome conjugated anti-CD19, -CD25, -CD27, and -IgM antibodies or their isotype controls and analyzed by flow cytometry.
**Results**: Flowcytometric analysis showed that 21.7±17.4% and 6.9±2.1% of CD19+ cells in TDLNs were CD25+ and CD27 hi CD25+, respectively. Moreover assessment of CD27 by CD25+ B cells revealed that 68.4± 20.2% of CD25+ B cells expressed CD27. In addition, 24.1± 16% of CD25+ B cells showed naïve (CD27-IgM+) phenotype while 22.7±15.3% and 40.5± 15.5% of them had unswitched memory (CD27+ IgM+) and switched memory (CD27+ IgM-) phenotypes, respectively.
**Conclusion:** A subset of B cells expressing CD25 in TDLNs of patients with head and neck squamous cell carcinoma was detected. These CD25+ B cells had either memory or naïve phenotypes, most of them however, showed memory phenotype. Further functional and phenotypical investigations are needed to understand the function of these B-cell subsets in TDLNs of head and neck squamous cell carcinoma.

**Keywords:** Head and Neck squamous cell carcinoma, CD25+ B cell, tumor draining lymph nodes

ICIA2018\Cancer Immunology\Poster\8477

**8477**

# Investigation of the gene polymorphism of *CCL22* 16C/A in patients with Basal Cell Carcinoma

Maryam Fakhimi[1], Sara Ghafarpour[3], Mohamad reza Haghshenas[1], Faride Jowkar[3], Nasrollah Erfani[1,2]

1. *Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Departments of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
3. *Department of Dermatology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** The CC-motif ligand 22 (*CCL22*) is a chemokine with crucial role in tumor-associated immunosuppression. *CCL22* is constitutively produced by macrophages, monocytes and dendritic cells and is inducible in many other immune cells such as Th2 and B cells. *CCL22* production has also been reported by several types of tumor cells. This chemokine is a functional ligand for *CCR4* which is commonly expressed on Th2 and Treg cells, and preferably recruits them to tumor microenvironment. The aim of this study was to investigate the association of 16C/A genetic variation (rs4359426) in *CCL22* gene with susceptibility of Iranian patients with Basal Cell Carcinoma (BCC).

**Methods:** 154 pathologically confirmed BCC patients (mean age: 60.67±11.57) and 154 age-sex-matched healthy individuals were recruited. Genomic DNA was extracted from white blood cells, and genotyping was performed using Polymerase Chain Reaction following by Restriction Fragment Length Polymorphism (PCR-RFLP) method.

**Result:** Arlequin analysis showed no deviation of genotype frequencies from Hardy-Weinberg equilibrium among both patients and controls. The frequencies of genotypes among patients and controls were 85.7% vs. 81.8%, 13.6% vs. 16.9%, 0.6% vs. 1.3% for CC, CA, AA genotypes respectively. Statistical analysis revealed no significant differences in the frequencies of genotypes and alleles between patients and controls (P>0.05). Although statistical analysis revealed no significant differences in the frequencies of genotypes and alleles between two groups, the A allele (CA or AA) significantly decreased in male patients comparing to male controls (Pvalue=0.03). No association was also observed between these genotypes and clinicopathological parameters in the patients (P>0.05).

**Conclusion:** Results of this investigation do not support the association of 16 C/A in *CCL22* gene with susceptibility and progression of BCC in Iranian patients. However, investigation of other *CCL22* gene variations and haplotypes may be required to completely roll out the association of *CCL22* gene variants with BCC.

**Keywords:** Basal Cell Carcinoma, Genotypes, Haplotypes, CC Chemokine ligand 22 (*CCL22*).

ICIA2018\Cancer Immunology\Poster\8492
**8492**

# Investigating the effect of interaction between IL-17A, IL-17E, TNF-α and rs3783605 on the activity of VCAM-1 promoter in the human umbilical vein endothelial cells (HUVECs)

Fatemeh Dadgar Pakdel[1], Javad Dadgar Pakdel[3], Mohammad Keramatipour[*2] , Mohammad Vodjgani[*1]

*1. Department of Medical Immunology, School of Medicine, Tehran University of Medical Sciences (TUMS), Tehran, Iran*
*2. Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences (TUMS), Tehran, Iran*
*3. Department of pharmaceutical biotechnology, Pasteur Institute, Tehran, Iran*

**Background:** Vascular Cell Adhesion Molecule-1 has a key role in the cell adhesion and transendothelial movement. Different factors such as gene promoter and its single nucleotide polymorphisms affect the VCAM-1 expression. The previous reports proved an important role of rs378605 in -420 position in the pathogenesis of diseases connecting with VCAM-1 . Present study was designed to investigate the effect of rs3783605 and its interaction with IL-17A, IL-17E and TNF-α cytokines on the activity of VCAM-1 gene promoter in HUVEC.
**Methods :** Two vectors with different alleles of rs3783605 were constructed to express the GFP. GFP expression level was measured by real-time PCR.
**Result :**Results showed that -420 G led to about 2-fold higher transcriptional activity compared with the A allele. HUVECs stimulated with mentioned cytokines alone showed a decrease of expression. The stimulation with IL-17A in addition to TNF-α showed an increase of expression in G allele vector and the stimulation with IL-17E in addition to TNF-α led to an increased expression in the cells containing A allele . The stimulation by IL-17A in addition to IL-17E led to decrease of expression in the cells containing G allele and stimulation with three cytokines simultaneously showed a decrease of expression in G allele vector.
**Conclusion:** In general, the results can be interpreted in three states of stimulation with one, two and three cytokines and on that basis, the results are different. Data from this study can be useful in the treatment of diseases in the future.

**Keywords**: SNP; promoter; VCAM1; HUVEC; IL-17E

ICIA2018\Cancer Immunology\Poster\8546
**8546**

# Expression of the level of CD44 protein in various breast cancer cell lines

*Fatemeh Vahidian[1], Elham Safarzadeh[1], Behzad Baradarn[1]*

*1. Department of Immunology research center, Faculty of medicine, Tabriz university of medical sciences, Iran*

**Background**: Breast cancer is the most common cancer diagnosed in women. Breast cancer can occur in both men and women but it's far more common in women. This cancer is a heterogeneous disease and can be divided into in situ and invasion modes and the most common cause of death in this cancer is metastatic. CD44 is a superficial cellular glycoprotein that plays an important role in the cell-matrix and cell-cell-interaction of the migration and invasion of cancer cells and also is a medium to cell environment, which helps with the survival and migration of cancer cells such as breast cancer. Also the expression of CD44 increases in cancer cells and plays an important role in helping the growth and metastasis of breast cancer cells, and can serve as a therapeutic goal. Interestingly, CD44, as a key marker of cancer on the surface of cancer cells in various malignancies, includes breast cancer.
**Methods**: Breast cancer has different grades and cell lines. In this study we examined the expression of CD44 in four cell lines including MDA-MB231, MDA-MB468, MCF-7 and SKBR3. We cultured each of these cells and then extracted their RNAs and transferred to their related cDNAs then, we did real time PCR on them.
**Results**; By analyzing the data, we noticed that the highest expression of CD44 was in turn in MDA-MB468, SKBR3 and MDA-MB231 which are metastatic cell lines. Further, we recognized that the expression of this protein was very low in MCF-7 which is a non-metastatic cell line.
**Conclusion**: We concluded that the expression of CD44 in metastatic cell lines of breast cancer is more than non-metastatic cell lines, and more importantly, the expression of this protein in MDA-MB468, which is more metastatic than the others, was more.

**Keywords**: breast cancer, CD44, cell lines, metastasis

ICIA2018\Cancer Immunology\Poster\8550
**8550**

# Expression of the inhibitory molecule, Tim-3 on the cells of the breast cancer draining lymph nodes

Fereshteh Mehdipour[1], Atri Ghods[1,2], Sahar Shariati[1,3], Abdol Rasoul Talei[4], Abbas Ghaderi[1, 2]

*1. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
*2. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
*3. Department of Immunology, School of Medicine, ShahidSadoughi University of Medical Sciences, Yazd, Iran*
*4. Breast Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** T cell immunoglobulin and mucin domain-3 (Tim-3) is expressed on different cell types including T cells, dendritic cells, macrophages and natural killer cells. Tim-3 has been suggested to act as an inhibitory molecule and have a negative impact on anti-tumor immunity due to its expression on exhausted or regulatory T cell.The aim of this study was to determine the frequency of Tim-3$^+$ lymphocytes and CD8$^+$T cells in the tumor draining lymph nodes (TDLNs) of breast cancer patients and its relation with disease parameters.
**Methods**: Axillary lymph node specimens were taken from 22 breast cancer patients and mononuclear cells were isolated using Ficoll-Hypaque gradient centrifugation. Cells were then stained for CD8 and Tim-3 and subjected to flow cytometry.
**Results:** The percentages of Tim-3$^+$ cells were determined in lymphocytes and CD8$^+$ T cells respectively. Our results showed that 5.8±2.4 of lymphocytes and 6.1±2.8 of CD8$^+$ T cells expressed Tim-3 without significant differences in the metastatic and non-metastatic lymph nodes, however the geometric Mean Fluorescent Intensity (gMFI) of Tim-3 expression was significantly higher in lymphocytes of the metastatic lymph nodes (P=0.033). The percentage of Tim-3$^+$ lymphocytes were significantly higher in the TDLNs of ER- compared to ER+ patients (P=0.047). A similar non-significant trend was seen in the frequency of TIM-3$^+$CD8$^+$ T cells. Moreover, the frequency of Tim-3$^+$ lymphocytes and CD8$^+$ T cells showed significant and non-significant increases in the TDLNs of patients with grade III as compared with grade II (P=0.020 and P=0.10, respectively). The frequency of Tim-3$^+$ lymphocytes or CD8$^+$ T cells did not show significant associations with tumor size or stage of the disease.
**Conclusion**: Our results suggested a link between higher frequency of Tim-3$^+$ lymphocytes and poor prognosticators like ER- and higher tumor grade. Further functional and phenotypical analysis of this lymphocyte subset is warranted.

**Keywords:** Tim-3, Lymphocyte, CD8$^+$ T cells, Tumor draining lymph nodes, Breast cancer

ICIA2018\Cancer Immunology\Poster\8563
**8563**

# The Increased level of Osteopontin and its receptor in patients with benign and malignant bone tumor

Ameinh Hosseini[1], Alireza Mirzaei[2], Khodamorad Jamshidi[2], Masoumeh Tavakoli Yaraki[1]

*1. Department of Biochemistry, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*
*2. Bone and Joint Reconstruction Research Center, Shafa Orthopedic Hospital, Iran University of Medical Sciences, Tehran, Iran*

**Background and aim**: Osteopontin has emerged as an active player in many physiological and pathological processes, including biomineralization, tissue remodeling and inflammation. As an extracellular matrix protein and proinflammatory cytokine, osteopontin is thought to facilitate the recruitment of monocytes/macrophages and to mediate cytokine secretion in leukocytes. Because OPN acts through several receptor mechanisms including both integrins and CD44, targeting these receptor ligand interactions as already under investigation in cancer therapy. The aim of the present study was to investigate the expression level of OPN and CD44 in tumor tissue of patients with benign and malignant bone tumor comparing to normal bone tissue.
**Materials and Methods**: 50 patients of Shafa Orthopedic Hospital in Tehran with benign and malignant bone tumor have participated in this case-control study. The tumor tissues and margins were used for mRNA extraction and cDNA construction, and to determine the expression level of CD44 gene, a Real-Time PCR-based Cyber Greenmethod was used and data were analyzed using ΔΔCT method. The level of circulating OPN was determined using ELISA. Finally, statistical analysis was performed using Graph Pad Prism software version 5 and independent t-test.
**Results**: Measurement of CD44 expression level in tumor tissues of patients with bone tumor revealed that the level of this gene was significantly increased in patients comparing to healthy subjects and normal tissues. Also, the increased level of CD44 was associated with elevated level of tumor grade and stage ($p < 0.05$). The plasma level of OPN was increased in patients with bone tumor comparing to healthy controls which was correlated with severity of disease.
**Conclusion**: The results of the current study have shown that the OPN and its receptor (CD44) can account as a circulating also local bio marker in patients with bone tumor due to the significant differences of the CD44 and OPN level in patients comparing to controls, it can be noticed as a possible biomarker for controlling disease.

**Keywords**: OPN, CD44, gene expression, Bone Tumor.

ICIA2018\Cancer Immunology\Poster\8601
**8601**

# Evaluation of tissue expression of BTLA and HVEM molecules and soluble form of HVEM in gastric cancerpatients

Maryam Azarafza[1], Mohsen Tehrani[1], Reza Valadan[1], Iradj Maleki[2], Omid Emadian[3], Abolghasem Ajami[1]

1. *Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
2. *Department of Gastroenterology, Gut and Liver Research Center, Mazandaran University of Medical Sciences, Sari, Iran*
3. *Department of Pathology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

**Background:** Gastric cancer is the second leading cause of cancer death and the fourth most commonly diagnosed cancer worldwide. Identifyingand subsequentlyTargeting pathways by which tumors inhibit activity and cytotoxicity of immune cellshas been regarded as a novel strategy to improve clinical approaches and survival outcome.The BTLA/HVEM pathway is one of the inhibitory mechanism, playing an important roleduring the inflammation and tumorgenesis and has been recently recognized as a tumor escape strategy. There is limited information regarding precise level of BTLA and HVEM expression in gastric cancer. The aim of this study was to investigate the expression of BTLA, HVEM and soluble form of HVEM in gastric cancer specimens and serum, respectively.
**Methods:** The expression level of BTLA and HVEM was evaluated by immunohistochemistry and mRNA expression was examined using real-time PCR in gastric tissue specimen of gastric cancer, metaplasia and control groups. sHVEM in serum was assessed by ELISA assay kit.
**Results:** Our results indicated that the expression of BTLA, HVEM in tissue and sHVEM in serum of patients with gastric cancer and metaplasia were high compared to control groups. The increased expression of BTLA, HVEM and serum level of sHVEM was significantly correlated with poor prognosis of gastric cancer and clinicopathological features such as TNM staging and lymph node metastasis.
**Conclusions:** Our data indicated that increase of BTLA and HVEM in patients with gastric cancer may have correlated to tumor progression and might be considered as an independent prognostic factor. Alltogether, this inhibitory pathway could be a promising candidate for immunotherapy of gastric cancer.

**Keyword**: Gastric cancer, B and T lymphocyte attenuator, Herpesvirus entry mediator

ICIA2018\Cancer Immunology\Poster\8606
**8606**

# +49A/G (rs231775) and -318C/T (rs5742909) Polymorphisms of *CTLA-4* Gene Showed No Association with Patients with Prostate Cancer

Mahyar Malekzadeh[1], Parvin Nikaein[1], Ali Ariafar[2], Abbas Ghaderi[1]

1. *Institute for Cancer Research (ICR), Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Department of Urology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:**Prostate cancer is the fourth most common cancer in both sexes and the second most common cancer in men which lethality is normally associated with occurring the metastasis rather than primary tumor. Therefore, targeting the molecular pathways that promote tumor formation and progression could assist better prognosis. CTLA-4 is a protein receptor that functions as an immune checkpoint which its blockage is regarded as an effective mechanism in treating many cancer types.
**Methods:** 5ml peripheral blood from 100 prostate cancer patients and 100 age matched normal men were taken. DNA extraction was done by Proteinase K method. PCR-RFLP and AS-PCR were performed to determine the genotypes of +49A/G (rs231775) and -318C/T (rs5742909) sites, respectively.
**Results:** There was no significant correlation between these sites in patient and control groups. Moreover, no significant relation between clinicopathological findings either the mean age in patient group was found.
**Conclusion:** Our data suggested no association between *CTLA-4* gene variants at positions +49A/G(rs231775) and -318C/T(rs5742909)in patients with prostate cancer compared with healthy controls. To conclude the role of *CTLA-4* gene variants in prostate cancer, investigation of other known polymorphisms reported in *CTLA-4* gene and also reports from other ethnic populations are warranted.

**Keywords:** CTLA-4 Polymorphism, +49A/G, -318C/T, Prostate, Cancer

ICIA2018\Cancer Immunology\Poster\8619
**8619**

# Investigation of CTLA-4 Gene +49A/G (rs231775) and -318C/T (rs5742909) Polymorphisms in Patients with Thyroid Cancers

Shabnam Abtahi[1], Fatemeh Izadi Jahromi[1], Mahyar Malekzadeh[1], Mohammad Javad Fattahi[1], AmirReza Dehghanian[2], Mohammad Hossein Dabbaghmanesh[3], Abbas Ghaderi[1]

1. *Institute for Cancer Research (ICR), Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Department of Pathology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
3. *Department of Endocrinology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Cytotoxic T Lymphocyte Antigen 4 (CTLA-4 or CD152) is among the immune checkpoint molecules and its abnormal expression in genetically and environmentally cancer predisposed individuals might make the person more susceptible to disease initiation and progression. The rs5742909 (-318C/T) and rs231775 (+49A/G) are among the most commonly studied SNPs and have been considered as genetic factors related to thyroid diseases.
**Methods:** We aimed to elucidate the association between these SNPs and susceptibility to various types of thyroid cancers, by comparing the frequency distribution of the genotypes/alleles in these regions with a group of healthy individuals.
**Results:** A statistically significant association was observed between the presence of G allele in rs231775 locus and thyroid carcinoma, when comparing cases and controls (OR=2.4; CI: 1.54 to 3.78; P<0.001) and the frequency of heterozygotes (AG) was higher than homozygotes for allele A (AA), in thyroid cancer patients. However; we observed no difference among allele/genotype frequencies in regards to locus rs5742909.
**Conclusion:** In this study we observed that G allele in rs231775 and possibly lower expression and affinity of mutated CTLA-4 molecule, is associated with higher susceptibility to thyroid carcinoma, especially papillary subtype.

**Keywords:** CTLA-۴, Polymorphism, rs۵۷۴۲۹۰۹, rs۲۳۱۷۷۵, Thyroid, Neoplasms

ICIA2018\Cancer Immunology\Poster\8637
**8637**

# Autophagy, using Atmospheric Cold Plasma (CAP) method, is an alternative therapeutic mechanism for the treatment of melanoma cancer

Mina Alimohammadi[1], Alireza Refeie[1] , Monireh Golpour[1]

*1. Mazandaran University Of Medical Sciences , Mazandaran , Iran*

**Background :** Autophagy is a pathway to tolerate stress that preserves survival under adverse conditions. However, long autophagy prevents the cell from coping with stress due to degradation of cellular structures, leads to autophagy cell death. ROS induction by starvation, involved in stimulating autophagy during the onset and progression of cancer. The new treatment with cold atmospheric plasma (CAP) promotes the production of ROS in the cell and induces oxidative stress. It leads to autophagy in the melanoma cells without affecting normal cells.
**Methods :** In this study, by starving to induce autophagy in B16 melanoma cells and L929 in Invivo and Invitro, the CAP effectiveness measured compared to chemotherapy. Using the MTT assay, we showed the survival rate of CAP-treated cells in comparison with the chemotherapy group and the combination of both treatments significantly decreased.
**Result :** Also, with flowcytometry, we showed that the percentage of cell death in tumor cells in the CAP group was significantly higher than other groups. Also, by examining the mRNA expression of LC3 and ATG5 which involved in autophagic, CAP increased the expression of these proteins. Also, in Invivo study, with B16 inoculation in Balb/C mice, we observed that tumor size in the CAP-treated group was significantly decreased compared to the chemotherapy. Then, to confirm the selective CAP effect on the toxicity and metastasis and the cellular attachments of surrounding healthy tissues, by staining H&E on vital tissues such as the liver, kidneys, lymph nodes and spleen, has showed that CAP does not have any toxicity and deformity in the tissue And cellular connections, while other groups were strongly associated with serious injury, high intracellular toxicity, and loss of intercellular connections.
**Conclusion:** This study showed that CAP did not cause damage to the normal tissue and also caused a high percentage of cell death autophagic in cancer cells .so It can be a good alternative for the treatment of malignant melanoma

**Keywords:**Ros , CAP , Autophagy ,Melanoma

ICIA2018\Cancer Immunology\Poster\8645
**8645**

## Down-regulation of E-Cadherin in Acute Lymphoblastic Leukemia

Mohammad Mahdi Mohammadi[1], Amin Mirzaeian[1], Shahrbanoo Rostami[2], Fatemeh Mirzaeyan[3]

*1. Department of Immunology, Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran*
*2. Department of Hematology-Oncology and Stem Cell Transplantation, Tehran University of Medical Sciences, Tehran, Iran*
*3. Department of Hematology and Blood Banking, Faculty of Allied Medicine, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** E-cadherin is one of the adhesion molecules which contribute to the interaction of haematopoietic progenitors with their niche. While the pivotal role of the loss of E-cadherin expression in tumourigenesis has been well characterized, little is known about its expression level in Acute Lymphoblastic Leukemia.
**Methods:** RT-qPCR was carried out on bone marrow aspirates from 89 ALL patients at the time of diagnosis and 33 age-matched healthy individuals. The ALL patients were categorized into B-ALL (69.7%) and T-ALL (30.3%), and the B-ALL cases were further classified into pro-B, early pre-B, pre-B and immature/mature-B based on immunophenotypic characteristics.
**Results:**Compared with controls, E-cadherin was significantly downregulated in ALL ($p<0.001$). There were no correlation between ALL subtypes or leukocyte count with gene expression level of E-cadherin. However, there was significantly negative correlation between E-cadherin expression and the number of bone marrow blasts as a disease-related prognostic factor ($r=-0.26$, $p=0.01$) in ALL Patients.
**Conclusion:** Our results showed decreased expression of E-Cadherin in ALL patients. Since alterations of adhesion behaviour of haematopoietic stem cells may alter the homoeostasis of haematopoietic cells within bone marrow microenvironment and ultimately lead to transforming ability and vigorous growth of leukaemic cells, we suggest that E-Cadherin signaling pathway should be further explored to reveal disease biology and potential therapeutic targets.

**Keywords:** Acute Lymphoblastic Leukemia, E-cadherin, RT-qPCR.

ICIA2018\Cancer Immunology\Poster\8669
**8669**

# SNP variation of immune system gene in medulloblastoma

FatemeShaabanpour Aghamaleki[1], Shirin Farivar[2]

1. *Department of molecular and cellular Biology School of Biotechnology and Life Science University of ShahidBeheshti*
2. *Department of molecular and cellular Biology School of Biotechnology and Life Science University of ShahidBeheshti*

**Background:**medulloblastomas are the most frequently diagnosed embryonal tumors of the central nervous system. Immunotherapy might be key to improve survival and to avoid morbidity. The efficient killing of tumor cells using immunotherapy requires overcoming cancer-associated strategies to evade cytotoxic immune responses. Single nucleotide polymorphisms have the most effect on immune genes in medulloblastomas that can be used as a specific target in immunotherapy of medulloblastomas.
**Methods:** Genome variation profiling by SNP array of 7 primary medulloblastomas and 10 metastatic medulloblastomas patients was extracted from Geo datasets. SNPs are compared with logfc for their frequency between two groups and sorted by their p-value. Also, the related gene for an SNP, the most related pathway, location, function, and protein networks of this SNPs were identified with NCBI and GeneCard databases.
**Results:** several SNPs were identified for medulloblastomas that may associate with it. Some these SNPs related to an immune system and have a high frequency in metastatic medulloblastoma. One of these SNPs were in PTPRS gene (rs886932). The protein encoded by this gene is a member of the protein tyrosine phosphatase (PTP) family. PTPs are known to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. rs886932 is an intron variant with C/T polymorphism. This SNP has a high frequency in metastatic medulloblastoma.
**Conclusion:** identification of critical SNP of immune system gene in two kinds of medulloblastoma patients can lead to appropriate and specific chemotherapy and immunotherapy by targeting specific SNP.

**Keywords:** medulloblastoma, SNPs, PTPRS gene, cancer immunology and immunotherapy

ICIA2018\Cancer Immunology\Poster\8679
**8679**

# The effects of *Aloe vera* leaf exudate on the proliferation of K562 human tumor cell line

Hamid Shamsi[1], Payman Tavakoli[1], Minoo Shaddel[2], Meysam Abtahi Foroushani[3], RoyaShamsi[4]

1. *Department of parasitology University of Urmia*
2. *Department of parasitology Faculty of Medicine University of Aja University*
3. *Department of Microbiology Faculty of Veterinary University of Urmia*
4. *Department of Clinical Nutrition and Dietetics, Faculty of Nutrition and Food Technology, ShahidBeheshti University of Medical Sciences, Tehran, Iran.*

**Background:** Leukemias are a group of blood malignancies which are classified as lymphoid and myeloid based on the origin of the malignant cells. K562 is a leukemic cell line which was first isolated from a 53 years old woman suffering from chronic leukemia. The present study was carried out to investigate the probable inhibitory effect of *Aloe Vera* on the proliferation of K562 cell line.
**Methods:** The inner parts of the plant including the gelatinous substance were emptied and the outer layer was used for the preparation of the lyophilized extract. The extract was dissolved in DMSO to be used in all experiments. The leukemic cells were cultured in RPMI 1680 medium containing 10% Fetal Bovine Serum (FBS) and were then treated with increasing doses of the extract. The viability of the cells was studied using the MTT test. Peripheral Blood Mononuclear Cells (PBMC) were isolated from the peripheral blood of normal donors using ficoll-hypaque and were subsequently treated with the same doses of the extract. After the incubation time, the IC50 was determined for both the K562 and normal PBMCs using the MTT assay. Doxorubicin and Phosphate-buffered Saline (PBS) were used as the positive and negative controls, respectively. The results were analysed in SPSS software using One-way ANOVA.
**Results**: Statistical analysis of the obtained data showed that the viability of cells in both the leukemic cell line and normal PBMCs decreased following 24, 48, and 72 hours treatment with the extract in a dose-dependent manner.
**Conclusion**: The results of the present study showed that *Aloe Vera* outer layer extract significantly decreases the number of viable cells in both K562 cell line and normal PBMCs with less selectivity in comparison to the conventional anti-leukemic doxorubicin.

**Keywords:** *Aloe Vera* leaf exudate; K562; MTT

ICIA2018\Cancer Immunology\Poster\8681
**8681**

# Expression analysis of activatory and inhibitory receptors of NK cellsin patients with chronic lymphocytic leukemia

Leila Hadadi[1], Ali Akbar Amirzargar[2], Ramazan Ali Sharifian[3], Saied Abediankenari[1,4], Hossein Asgarian Omran[1,4*]

*1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
*2. Department of Immunology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
*3. Clinic of Hematology and Oncology, Vali-Asr Hospital, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
*4. Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

**Background:** Natural killer (NK) cells have pivotal roles in both innate and adaptive immune responses to many inflammatory and chronic infectious diseases. Initial activation and function of NK cells are mediated by signals received from multiple activating and inhibitory receptors. NK cell dysfunction and imbalance in these regulatory receptors have been reported in various solid and hematologic malignancies. In this study, the expression pattern of NKp30 and T cell immunoglobulin and mucin domain containing molecule-3 (Tim-3), as candidates of activating and inhibitory receptors, were evaluated in patients with chronic lymphocytic leukemia (CLL).
**Methods:** Twenty five untreated CLL patients and fifteen sex- and age-matched healthy controls were enrolled in this study. Fresh peripheral blood mononuclear cells were collected from all subjects. $CD56^{+}/CD3^{-}$ cells in lymphoid region were defined as NK cells and the frequency of $CD56^{+}/CD3^{-}/NKp30^{+}$ and $CD56^{+}/CD3^{-}/Tim\text{-}3^{+}$ cells was determined by three-color flow cytometry method.
**Results:** Our Results revealed that Tim-3 expression, as an inhibitory receptor, was significantly increased on NK cells of CLL patients in comparison to normal healthy controls. NK cells from CLL patients showed lower expression of NKp30 activatory receptor compared to controls, but the difference was not statistically significant.
**Conclusion**: Altogether our results confirm that NK cells in CLL patients show dys-regulation in their activatory and inhibitory receptors and may be functionally exhausted.

**Keywords:**Chronic lymphocytic leukemia, NK cell, Tim-3, NKp30

ICIA2018\Cancer Immunology\Poster\8693
**8693**

**Relationship between helicobacter pylori and TIMP-1 in gastric adenocarcinoma**

Rana Ezzeddini[1], Mohammad Taghikhani[1], Mohammad Hossein Somi[2], Amir Salek Farrokhi[3], Amirtaher Eftekharrosadat[4]

1. *Department of Clinical biochemistry, Faculty of Medical Sciences, TarbiatModares University, Tehran, Iran*
2. *Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
3. *Department of Immunology, Faculty of Medical Sciences, TarbiatModares University, Tehran, Iran*
4. *Department of Clinical biochemistry, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Various proteins can be found in adjustment of inflammatory immune response in H. pylori (H.P)-infected in gastric mucosa. Tissue inhibitor matrix metalloproteinase 1 (TIMP-1) plays a vital role in carcinogenesis and is over-expressed in many human malignancies. Although, the value of TIMP-1 is debated as a biomarker in gastric cancer (GC) patients. Overexpression and therefore, increased serum level of TIMP-1 can be related to the pathogenesis of H.pylori-associated stomach adenocarcinoma.The objective of this study is to investigate the association between H.pylori with TIMP-1 in adenocarcinoma cases.
**Methods:** In order to carry out this experiment, 85 gastric adenocarcinoma patients and 130 healthy controls selected. Blood samples collected and TIMP-1 concentration determined by ELISA method. Also, Crystal violet fast (CVF) staining performed for H.pylori detection on endoscopic biopsies. We used T-test for data analysis and P values less than 0.05 considered statistically significant.
**Results:** Our findings indicated significant increased level of TIMP-1 in gastric adenocarcinoma patients comparing control subjects ($P \leq 0.05$). Also, our results showed that 39% of gastric adenocarcinoma patients were positive H.pylori. In addition, we demonstrated that TIMP-1 concentration significantly up regulated in H.pylori positive adenocarcinoma cases compared to negative patients.
**Conclusion:** Altogether, the present survey showed that increased level of TIMP-1 in gastric adenocarcinoma has a correlation with involving H.P in stomach

**Keywords:** Gastric adenocarcinoma, TIMP-1, Helicobacter pylori

ICIA2018\Cancer Immunology\Poster\8712
**8712**

# Correlation among Age and Genetics and Regulatory T cells in Chronic Myeloid Leukemia Patients

Zahra Shaker[1], Zohair Mohammad Hasan[2], Davar Amani[3], Masume Ebtekar[2]

*1. AJA University of Medical Sciences, TarbiatModares University, ShahidBeheshti University of Medical Sciences*
*2. TarbiatModares University*
*3. ShahidBeheshti University of Medical Sciences*

**Background**: Chronic myelogenous leukemia(CML, Chronic Myeloid Leukemia)Can be created at any age, but most adults over age 50 have the disease, and these patients about 70% of patients are included.Hmchnbn the disease occurs in men than in women.Prevalence in children is rare and is reported almost 4 years.Due to the large variable called way of life that is influenced by climate (air, water and soil), economy, customs, traditions and religious beliefs are different age.
**Method**: This is a historical cohort study of 15 patientsCMLFor a period of two years, a peripheral blood sample was taken and testedRT-PCR in real time, To determine the presence of Philadelphia gene and flow cytometerFACS CaliburTo assess the number of cellsTSetting was used .Information obtained by the test-GEE;Generalized Estimation EquationWere analyzed .
**Result:**Some of the results show that when the oncoproteinbcr / ablThe amount of cells has increasedTThe regulation for each year increases the age of the patient by 0.729 , which is significant( Pv = 0.000, Pv<0.05). In this study, it was determined that genetic aging also increased
**Conclusion**: Has a relationship .These findingsDThe prescribed medications and follow up of the treatment, as noted by the experts is very effective.

**Keywords:**CML,Treg,bcrlabl, Age

ICIA2018\Cancer Immunology\Poster\9851
**9851**

**Increased CD8+/PD-1+/TIGIT+ T-cells in Patients with chronic lymphocytic leukemia (CLL)**

Reza Hajiasghar Sharbaf[1], Hossein Asgarian Omran[1,2], Hadi Hossein Nattaj[1], RaminShekarriz[3], Mohsen Tehrani[1,3]

1. *Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.*
2. *Immunogenetic Research Center, Mazandaran University of Medical Sciences, Sari, Iran.*
3. *Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran.*

**Background:** Tumors can escape immune response via uprgulation of immune-checkpoint receptors, such as CTLA-4, PD-1, Tim-3, LAG-3, and TIGIT, leading T-cells to a state of exhaustion. In this study, the frequency of $CD8^+$/ $PD\text{-}1^+$/$TIGIT^+$ T-cells, as exhausted T-cells, was measured in patients with CLL.
**Methods:** PBMCs were isolated from blood samples from CLL patients and healthy controls. Cells were cultured in the presence of phytohemagglutinin (10 µg/mL) for 72 h. Frequency of $CD8^+$/ $PD\text{-}1^+$/$TIGIT^+$ T-cells was then measured using three-colored flow cytometry.
**Results:** Frequency of $CD8^+$/ $PD\text{-}1^+$/$TIGIT^+$ T-cells were higher in patients with CLL (mean, 18.45) than in healthy controls (mean, 7.76 %).
**Conclusions:**Our data indicate that a high number of CD8+ T-cells in CLL patients express immune checkpoint receptors, PD-١ and TIGIT. Therefore, reversal of these inhibitory pathways could be a promising therapeutic approach for CLL.

**Keywords:** Exhausted T-Cell, PD-1, TIGIT, chronic lymphocytic leukemia (CLL)

ICIA2018\Cancer Immunology\Poster\9882
**9882**

# Melphalan cytotoxicity increased in combination with Arsenic trioxode in MT-2 lymphoma cells

Faeze Khodadadi[1], Mohammad Hadi Sadeghian[1]*, HoushangRafatpanah[2] ,Fatemeh B. Rassouli[3], Mehrdad Iranshahi[4]

*1. Cancer Molecular Pathology Research Center, Department of Hematology and Blood Bank, Faculty of Medicine, Mashhad University of Medical Sciences, Iran*
*2. Inflammation and Inflammatory Diseases Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*3. Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran*
*4. Department of Pharmacognosy and Biotechnology, Biotechnology Research Center, Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background** : Adult T cell leukemia lymphoma (ATLL) is a CD4+ lymphoproliferative malignancy resulting from human T-cell leukemia virus type 1 (HTLV1) infection.Iran is known as one of the endemic regions for HTLV-1.Despite advances in treatment of ATLL, the average survival rate of patients is low. Melphalan is one of the alkylating agents, the first types of drugs to be used in chemotherapy of malignant disease, are still an important group of drugs in clinical cancer chemotherapy. To introduce more effective anticancer strategies for the treatment of ATLL, here we investigate whether melphalan could enhance the efficacy of arsenic trioxide, a chemical drug prescribed for ATLL.
**Methods:** In this regard, MT-2 cells, an ATLL cell line, were treated with increasing concentrations of melphalan and Arsenic for 24, 48 and 72 hours, and the IC50 value of drugs was determined. Next, cells were treated with combination of melphalan (0.5 µg/ml) and Arsenic (1 and 2 µM). Then, cell viability was evaluated using Alamar Blue 14 g% reagent.
**Results:** Obtained results indicated that 0.5 µg/ml melphalan increased the toxicity of Arsenic 2 µM, and thus, had synergic effects with this anticancer drug.
**Conclusion:** The results of the present study indicated that combinatorial use of melphalan and arsenic trioxide can be considered in new therapeutic approaches to ATLL.

**Keywords:** melphalan, Adult T-cell leukemia/lymphoma, Arsenic trioxide ,HTLV-1

ICIA2018\Cancer Immunology\Poster\9883
**9883**

# Effects of melphalan on the expression of NF-κB in human lymphoma cells

Faeze Khodadadi[1], Mohammad Hadi Sadeghian[1]*, Houshang Rafatpanah[2], Fatemeh B. Rassouli[3], Mehrdad Iranshahi[4]

*1. Cancer Molecular Pathology Research Center, Department of Hematology and Blood Bank, Faculty of Medicine, Mashhad University of Medical Sciences, Iran*
*2. Inflammation and Inflammatory Diseases Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*3. Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran*
*4. Department of Pharmacognosy and Biotechnology, Biotechnology Research Center, Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background** :Melphalan is a widely used classical chemotherapeutic agent in the group of alkylating nitrogen mustard agents that was developed more than 50 years ago, and substantial clinical experience has been accumulated. Adult T-cell leukemia/lymphoma (ATLL) is an aggressive T-cell malignancy caused by human T-cell leukemia virus type 1 (HTLV-1) infection and often occurs in HTLV-1 endemic areas. Iran especially Khorasan province is known as endemic regions for this virus. In spite of medical advances, the prognosis of ATLL is still very poor. HTLV-1 Tax oncogene activates NF-κB signaling pathway. Herein we studied the effects of melphalan on the expression of NF-κB in ATLL cells.
**Methods:** In this study, we showed the melphalan effects on the expression of NFκB (REL-A) in HTLV-1 infected cell line. Accordingly, MT-2 cells were treated with 0.5 µg/ml melphalan for 72 hours, while control cells were treated with DMSO 0.4% (used as melphalan solvent). Next, total cellular RNA was extracted and treated with DNase I and then reverse transcribed into cDNA. For confirmation of cDNA fidelity PCR using GAPDH primers was conducted. Further Real-time PCR was performed using Taq man probe and specific primers for NF-κB (REL-A).
**Results:** Compared to relevant control, MT-2 cells treated with melphalan (in non-toxic concentration) exhibited significant down regulated levels of NF-κB (REL-A) ($p<0.05$).
**Conclusion:** These *in vitro* results suggest that the alkylating agent melphalan could be considered as an effective agent to alleviate malignant properties of ATLL cells in future *in vivo* studies.

**Keywords:** melphalan, Adult T-cell leukemia/lymphoma, HTLV-1, NF-κB

ICIA2018\Cancer Immunology\Poster\9900
**9900**

## Interaction of Perforin and Grnazyme Band HTLV-1 Viral factors Is Associated with ATLL development

Mohammad Mehdi Akbarin[1], Sadegh Farhadi[2], Abolghasem Allahyari[3], Mohammad Mehdi Koshayar[3], Abbas Shirdel[2], SeyedAbdolrahim Rezaee[1], Zahra Mozaheb[3], Asadollah Mohamadi[1], Alireza bari[2],SeyedehTahereh Mohaddes[2], Hossein Rahimi[2], Houshang Rafatpanah[1]

*1. Inflammation and Inflammatory Diseases Research Centre, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*2. Hematology Department, Ghaem Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*3. Hematology Department, Imam Reza Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background**: Human T lymphotropic virus (HTLV-1) is a human retrovirus which is associated with adult T cell leukaemia (ATLL). ATLL is a malignancy lymphoproliferative disease which infects CD4 T cells. It is not clear why the majority of HTLV-1 infected individual's remains healthy carries and minority develop ATLL. Cellular immune response has a critical role in ATLL and destroys malignant and HTLV-1-infected cells. Perforin and granzyme have important functional role in apoptosis and destruction of infected cells. In the present study we examined the role of perforin and granzyme in ATLL patients and HTLV-1 asymptomatic carries (HAC).
**Method**: Peripheral blood mononuclear cells (PBMCs) were isolated from ATLL patients and asymptomatic carriersby using Ficoll-hytopaque density centrifugation. RNA was extracted and cDNA was synthesised. A real-time PCR TaqMan method was designed and optimized for evaluation of perforin, granzyme, Tax and HBZ gene expression.HTLV-1Proviral load was quantified in patients withATLLand carriers.
**Results**: The mRNA expression of TAX and HBZ was significantly higher in ATLL patients than HACs($p$=0.011andThe HTLV-1 proviralload was higher in ATLL patients compared to HAC group ($p$= There was a significant increases in perforin gene expression in HACs than ATLL patients ($p$=0.002). Furthermore the expression of granzyme was also higher in HTLV-1 carries compared with ATLL patients and significant differences was observed between two groups ($p$=0.036).
**Conclusion**: Low expression of perforin and granzyme in ATLL patients seem influences the efficiency of CTL function and destruction of HTLV-1-infected cells which might contribute to the disease pathogenesis.

**Keywords**: HTLV-1, ATLL, perforin, granzyme, proviral load

ICIA2018\Cancer Immunology\Poster\9947
**9947**

# The survey of Interleukin-35 gene expression in tissue and blood of colon cancer

Milad Ameri[1], Vedad Alkhamis[2]

1. *Department of Pathobiology ,University of Bu Ali Sina Hamedan*
2. *Department of Microbiology University of Shahid Chamran Ahvaz*

**Background:** Colon cancer is the third most common cancer Worldwide. Molecular methods such as Real Time PCR have been used to determine the expression of IL-35 as a screening test for the detection of colon cancer or diagnosis in different stages. The primary purpose of this study was to investigate the expression of IL-35 (IL-35) as a suppressor of activity and increase apoptotic sensitivity in cancer cells in the intestinal cancer tissue.
**Methods:** Due to certain limitations, considering the nature of the IL-35 which have heterodimers of both IL-12 and EBI-3 components, this study was conducted on IL-12α as a marker for the presence and expression of IL-35 In the tissue and peripheral blood of patients with colon cancer. In this study, 20 samples of paraffinaized tissue and 20 blood samples from people with colon cancer and 10 healthy samples were collected from the Khomeini hospital in Tehran. After de-paraffinization, RNA extraction was performed with RNX Plus solution. The cDNA was synthesized by reverse transcription using the MMULV enzyme. Gene expression was measured by the Real time PCR method. Glyceraldehyde phosphate dehydrogenase gene (GAPDH) was also used as internal control.
**Results:** Results showed that only 3 samples with an expression rate (RQ) greater than 1 out of 43 samples, 3 of samples were normal and used to verify the accuracy of the tests, suggesting a decrease in IL 12-R gene expression in most of the samples.
**Conclusion:** Due to staging of the disease, it can be concluded that with the decreased IL-12R expression gene, the colon cancer has progressed.

**Keywords:** Intestinal Cancer, Interleukin 35 Gene, Real Time PCR

ICIA2018\Cancer Immunology\Poster\10054
**10054**

# Highly Reliable Simultaneous Biosensing of CA125 and CA15-3 Tumor Markers and selective tracking and imaging over MCF-7 and OVCAR-3 cells lines via FRET phenomenon based on CDs-$MnO_2$ heterojunction in single Excitation Wavelength

Somayeh Hamd Ghadareh[1], Abdollah Salimi[1,2*], Fardin Fathi[c] , Sara Parsa[1]

1. *Department of Chemistry, University of Kurdistan, 66177-15175, Sanandaj, Iran*
2. *Research Center for Nanotechnology, University of Kurdistan, Sanandaj, Iran*
3. *Cellular and Molecular Research Center, Kurdistan University of Medical Sciences, Sananandaj, Iran*

**Background:** A fluorescence resonance energy transfer (FRET) strategy designed for simultaneous detection of two tumor markers based on coupling carboxylic acid functionalized carbon dots (CDs-COOH) and Rhodamine B (RB) conjugating antibodies. The strong green fluorescent-CDs were synthesized by a simple one-pot hydrothermal method. Monodispersed $MnO_2$ nanosheets were functionalized with ssDNA related aptamers and applied as quencher. More importantly, the solution containing both of the CDs and RB conjugating to CA125 as Ab1 and CA15-3 as Ab2 (CD1-Ab1, RB-Ab2) could be excited by single wavelength excitation of 490 nm. Inspired by these facts, we fabricated a selective and sensitive fluorescence nanosensor for simultaneous targeting of tumor markers.
**Method**: In the absence of CA125 and CA15-3 target antigens the fluorescence of the antibody labeled CD1 and RB, quenched with $MnO_2$ due to energy transfer (FRET) process, while with addition of target antigens, the formation of immunocomplex between CDs-Ab probe, antigen and ssDNA leading to the release of $MnO_2$, resulting in the distinct changes in fluorescence response. The response of the architecture nanoassembly enhanced gradually with the increase in concentration of targets in the range of 80 fg/mL to 5 pg/mL with 15.0 fg/mL as calculated limit of detection for CA125, and the response was found to be linear in the concentration range of 1.0 to 25 pg/mL for CA15-3 and the calculated limit of detection was 0.7 pg/mL. This strategy revealed a stable and good analytical performance for the detection of OVCAR-3 and MCF-7 cells lines ranging from 1000 to 10000 cells/mL with detection limit 2-3 cells in 10 µL of injected sample.
**Result:** Finally, due to low cytotoxicity of the applied CDs, the CDs-antibody hybrids were used in selective tracking and imaging of the cancer cells over MCF-7 and OVCAR-3 cells lines. The selectivity and sensitivity of this fluorescent nanosensor over other markers were carefully investigated. The obtained results implying the potential applications of presented assay for pathological diagnosis, biomedicine research and management of cancer diseases.

**Keywords**: Fluorescence resonance energy transfer, carbon dots, $MnO_2$ nanosheets, Simultaneous detection, CA125, CA15-3, Bioimaging

ICIA2018\Cancer Immunology\Poster\10061
**10061**

# Mesenchymal stem cells derived from stage II of human breast tumor tissue promote the proliferation of the PBMCs :A in vitro assay

Koushansineh sepehr[1], Alireza Razavi[1], Zuhair Mohammad Hassan[2], AbdolReza Fazel[3] ,
Majid Mossahebi Mohammadi[4], Seyed Mahmoud Hashemi [5]

1. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
2. *Department of Immunology, Faculty of Medical Sciences, TarbiatModares University, Tehran, Iran*
3. *Cancer research center, Golestan University of Medical Sciences, Gorgan, Iran*
4. *Sarem Cell Research Center(SCRC),Sarem Women's Hospital,Tehran,Iran*

*5.Department of Immunology, School of Medicine, ShahidBeheshti University of Medical Sciences, Tehran, Iran*

**Background:** Mesenchymal stem cells (MSCs), as a subpopulation of stroma cells found in the tumor microenvironment,are critical in promoting tumor progression and possess immunomodulatory properties. Therefore, the purpose of this study was to isolate MSCs from primary human breast tumor tissue, and to study the effect of soluble factor of MSC on the peripheral blood mononuclear cell (PBMC) proliferation.
**Methods:** The tumor tissues (n=8) obtain from patients with pathological stage II of breast tumor. MSCs were isolated by explant culture method and identified. The Mitogen-induced PBMC was treated with 25 and 50% MSC conditioned medium (CM) for 72h, and changes in proliferation evalueted by BrdU ELISA kit.
**Results:** We successfully isolated and identified MSCs from primary breast tumor tissues. Flowcytometry analysis demonstrated that isolated cells were positive for, CD73, CD44, CD29, CD105 and CD90 but negative for, CD11b, CD45, CD34, and HLADR. In addition, cells possessed the capability of multipotential differentiation into osteoblasts and adipocytes.
The results of theBrdU ELISA assay showed that 50% CM concentrations significantly increased the proliferation of PBMCs.
**Conclusion:** Collectively, our study showed that MSCs were confirmed to exist in stage II of human breast tumor and significantly promote the proliferation of PBMCs In Vitro.

**Keywords:** Breast Cancer, Mesenchymal Stem Cells, Isolation, Immunomodulatory

ICIA2018\Cancer Immunology\Poster\11064
**11064**

# Evaluation of NK(CD16+CD56+CD3-), NKT(CD16+CD56+CD3+), T(CD16-CD56-CD3+) cells cytotoxic activity in PBL of breast cancer patients by using CD107a expression assessment by flow cytometry (before and after co-culture with MCF-7 & PMA/I)

Minoo Adib[1]*, Nasim Samarghandi[1]

*1. Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background:** Invasive ductal carcinoma is the most prevalent type of breast cancer. Because of attention in recent years to innate immunity against tumors, assessment of NK cell function as an important component of the innate immune response is essential to find precise information about these cells. A lack of available assays for the detection of NK cells functional activity is an important limitation in the field of NK cells researches.
**Methods:** There are several methods to estimate the cytotoxic activity of NK cells. A new method has been described by using CD107a expression on the cell surface as a marker of cytotoxic NK & CD8+ T-cell degranulation.
**Results:** There was a significant increase in CD107 expression on NKT cells (with PMA/I stimulation) of control group comparing with patients group. The mean percent of T cells decreased significantly in grade III compared to grade I, II and control group.
**Conclusion:** In this study CD107 expression, the initial marker of cytotoxic activity, on the three populations of cells is investigated in two groups and resulted in expression of this marker on NKT cells is higher than NK and T cells in both groups. Just as many studies indicated a decrease in NK cell cytotoxicity in any grade of disease, this study showed that decrease of NK cells cytotoxicity only occur in grade I & II, but as a striking point , in grade III of disease cell cytotoxicity of these cells increased. Eventually, in this study expression of CD107 on T cells of both groups is equal. Several studies to confirm of our results reported major reasons for the observed decreased cellular immune responses in patients with breast cancer were associated with defective presentation of antigens by dendritic cells, not function of effector T cells.

**Keywords:** NK cell, CD107a ,Breast Cancer, flow cytometry, MCF-7

ICIA2018\Cancer Immunology\Poster\11078
**11078**

**Comparison of miR146a-5p expression in 4T1 and MKN cancer cell lines**

Atefe Alirezaee[1], Ahmad Zavaran Hosseini[1], Sara Soudi[1]

*1. Department of Immunology, Faculty of Medical Sciences, TarbiatModares University, Tehran, Iran*

**Background**: Different studies demonstrated that MicroRNA (*miR*) are participated in tumor formation and progression by regulation of cell proliferation, apoptosis and differentiation. *miR-146a* are among the important microRNAs that control cell apoptosis. Upregulation of *miR-146a will terminated to downregulation of apoptotic pathways. In this* study we want to know if there is a difference in the expression of miR-146a in two cell lines.
**Methods**: For this purpose the amount of miR-146a-5p expression were measured in 4T1 and MKN-45 cancer cell lines as represented the related cells to breast cancer and gastric cancer, respectively. So $3X10^5$ cells of 4T1 and MKN-45 were cultured in 12 well plates. 48 hours after culture, the cells harvested and assessed for miR-146a-5p expression by Real time PCR.
**Result**: According to obtained result, MKN-45 cancer cell line demonstrated two fold decrease in miR-146a-5p expression compared with 4T1.
**Conclusion**: As a result, we confirmed the miR-146a-5p expression in both cell lines. However there is a difference in miR-146a-5p level between different cancer cell lines. So miR-146a-5p, has a different therapeutic value dependent to kind of cancers.

**Keywords**: miR146a-5p, 4T1, MKN-45

ICIA2018\Cancer Immunology\Poster\11097

**11097**

# The anti-tumor effects of arsenic trioxide in combination with an EGFR inhibitor on breast cancer cell lines

Elham Boustanipour [1], Zahra Sadr[1], Ali Zekri[2], Seyed H. Ghaffari [3]

1. *Department of Medical Genetics, Faculty of Medicine, Tehran University of Medical Sciences (TUMS), Tehran, Iran*
2. *Department of Medical Genetics and Molecular Biology, Faculty of Medicine, Iran University of Medical Sciences (IUMS), Tehran, Iran*
3. *Hematology, Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Breast cancer (BC) is the most prevalent malignancy found in women worldwide. Despite extensive advances in diagnosis, surgical techniques, and treatment, BC is among the leading causes of cancer-related deaths in women. Metastasis is the main challenges in the BC management and it accounts for majority of deaths in breast cancer patients. Therefore, understanding the cellular and molecular factors responsible for the metastasis and developing novel therapeutic approaches against metastasis of breast cancer is urgently needed. Previous studies showed that epidermal growth factor receptor (EGFR) plays key roles in breast cancer initiation, growth, dissemination and cancer immunotherapy. Arsenic trioxide (ATO) as a multifunctional drug has been investigated for the treatment of leukemia and several solid tumors. EGFR inhibitor could influence anti-tumor immune responses by changing immune gene expression.
**Methods:** Different breast cancer cell lines were obtained and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum. The effects of ATO as a single and in combination with EGFR inhibitor were assessed on the viability and migration of BC cell lines using MTT, apoptosis and wound-healing assays in different time intervals.
**Results:** ATO or EGFR inhibitor as a single treatment did not have a considerable effect on the viability and wound-healing rate of BC cell lines, but the combination of the ATO with EGFR inhibitor significantly reduced metastasis effect of the cell lines.
**Conclusions:** Present study uncovered that combination of ATO with EGFR inhibitor could overcome metastasis of the breast cancer. Overall, combination of cytotoxic treatment with targeted therapy may represent a promising strategy for treatment of the breast cancer.

**Keywords:** Arsenic trioxide, EGFR inhibitor, Breast cancer, Metastasis.

# Cancer Immunotherapy

# Poster Presentation

ICIA2018\Cancer Immunotherapy\Poster \5236 cancer immunotherapy
**5236**

# Evaluation of Optimal Pattern of Injection and Dosage of Dendritic Cell Cancer Immunotherapy Using the Mathematical Model Based on Artificial Neural Network Model

Zahra Mirsanei[1], Sima Habibi[1], Nassim Kheshtchin[1], JamshidHadjati*[1]

*1. Department of Immunology, School of Medicine, Tehran University of Medical Science, Tehran, Iran*

**Background:** Previous studies have demonstrated that maturation of dendritic cells by *Listeria monocytogenes* lysate or CpG DNA can enhance the efficiency of the cancer vaccines in experimental models. A mathematical model based on artificial neural network of those results, proposed a new exponential pattern for vaccination. According to the model, injection of dendritic cells matured by CPG (CpG-DCs) and *Listeria monocytogenes* (List-DCs) at the same time and with decreasing and increasing dose of each vaccine in a group of mice would induce optimal antitumor immune response and minimum immunosuppression in the tumor microenvironment.
**Method:** 164 cells were implanted subcutaneously in right flank of Balb/C mice in four groups: vaccinated with *Listeria monocytogenes* matured DCs, CpG, both and none as control group. DC vaccines were injected according to proposed mathematical model. Tumor growth in the course of treatment and survival of mice after that was followed by measuring tumor size. GrBElISA test for assessing the activity of CTL, and real time PCR test for evaluation of T-bet ($Th_1$ transcription factor) and $FOXP_3$ (Treg transcription factor) gene expression were used.
**Result:** Results from the group received combination therapy with *Listeria monocytogenes* and CPG matured DCs, compared with other groups that have received only one vaccination alone, showed that growth of tumor is slower and declining, as well as higher activity of CTLs in this group. Gene expression results also confirm the increase of immune response in the tumor microenvironment.
**Conclusion:** Based on the results it can be concluded that mathematical models can be useful tools for predicting the tumor behavior and more efficient treatments, so the effort to improve them should be considered.

**Key word**: Dendritic cells, Cancer vaccine, Mathematical model, Immunotherapy.

ICIA2018\Cancer Immunotherapy\Poster \6330 cancer immunotherapy
**6330**

## MiR-34a Replacement Effect on Growth and Migration Inhibition in HCT 116 Colon Cancer Cell

NazaninJafarpour[1], Habib Onsori[2], Behzad Baradaran[3]

1. *Department of Genetics, East Azarbaijan Science and Research Branch, Islamic Azad University, Tabriz, Iran*
2. *Department of Genetics, Tabriz Branch, Islamic Azad University, Tabriz, Iran*
3. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran (Corresponding Author)*

**Background:** Colorectal cancer is the most common cancer of the digestive tract and is also the fourth leading cause of death in the world. Colorectal cancer is the third most commonly reported case in women, and is the second leading cause of cancer deaths in women. In this study, we increased the miRNA-34a substitution in colon cancer cells by transferring the miRNA-34a gene and expressing this miRNA in these cells and examined the effects of this miRNA on growth and migration inhibition.
**Methods:** Cancer cells were cultured in HCT-116 in RPMI1640 culture medium and all cellular experiments were performed in a logarithmic growth phase. For the transfer of miRNA-34a into colon cancer cells, the JetPAI regent (poly plus) was used. The expression of miR-34a was induced by qRT-PCR after miRNA induction. To investigate the effect of this miRNA on the cellular migration status after induction of Wound healing, ultimately the cells were induced by untreated cells were compared and significant amounts were determined.
**Results:** MTT assay showed that miR-34a induction induced death of colorectal gradient cells. The results of the qRT-PCR test showed a significant increase in the expression of miRNA-34a in transfected HCT116 cells. Ultimately, the results of the Wound Healing test showed reduced migration of transfected cells compared to control cells.
**Conclusion:** The results showed that increase of miRNA-34a induced cell death and decreased metastasis and migration of HCT-116 cell line colon cancer cells.

**Key words:** miRNA, Colon cancer, Metastasis, Transfection

ICIA2018\Cancer Immunotherapy\Poster \7339 cancer immunotherapy
**7339**

# Selection and Evaluation of Human Recombinant Antibodies against ErbB2 Antigen for Breast Cancer Immunotherapy

ElhamNadimi[1, 2], Foroogh Nejatollahi[1, 2], Setareh Moazen[3]

*1. Recombinant Antibody Laboratory, Department of Immunology, Shiraz University of Medical Sciences, Shiraz, IR Iran*
*2. Shiraz HIV/AIDS Research Center, Institute of Health, Shiraz University of Medical Sciences, Shiraz, IR Iran*
*3. Faculty of Science, University of British Columbia, Vancouver, Canada*

**Background:** Breast cancer is the most common cause of cancer-related death in women worldwide. ErBb2/HER2+ breast cancer accounts for 25% - 30% of all cases of breast cancer. Human recombinant single-chain antibodies, which are produced by antibody engineering technologies, are new and effective antibodies in cancer immunotherapy.
**Methods:** Panning process was performed on the clones of a human scFv library using two ErbB2 epitopes and Polymerase chain reaction (PCR) and DNA fingerprinting were done on the selected clones. The clones were evaluated via phage ELISA in terms of reactivity and specificity to epitopes.
**Results:** Single-chain antibodies, scFvI and scFvII, the anti-ErbB2-specific scFvs with 40% and 45% frequencies, were selected against epitopes I and II, respectively. The results of phage ELISA demonstrated a significant difference in the optical density (OD) of scFvs in reaction with the related peptides.
**Conclusions:** Targeted cancer therapy, which acts on a specific molecule in cancer cells, minimizes the side effects of immunotherapy. Due to the unique properties of scFvs, these antibodies have been used in targeted therapy of several cancers. In this study, 2 specific scFvs were selected against 2 ErbB2 epitopes, which contained trastuzumab and pertuzumab binding sites. The results of the panning process demonstrated the selection of 2 specific scFvs (with frequencies of 40% and 45%, respectively), which significantly reacted with the corresponding epitopes in phage ELISA assay. These small, high-affinity, human antibodies, which were selected against regions containing the binding sites of 2 food and drug administration (FDA)-approved monoclonal antibodies for breast cancer immunotherapy, have the potential to be considered for breast cancer targeted therapy. However, in vitro and in vivo tests should be performed to evaluate the antitumor effects of these scFvs.

**Keyword:** Breast cancer, scFv, ErbB2

ICIA2018\Cancer Immunotherapy\Poster \7437 cancer immunotherapy
**7437**

**Anti-tumor activity of Neurokinin 1 receptor inhibitor, in multiple myeloma**

Elham Razani, Davood Bashash*

*Department of Hematology and Blood banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** The substance P/neurokinin 1 receptor (SP/NK1R) cascade has been demonstrated to play a considerable role in development of human cancers. In spite of therapeutic improvements in treatment strategies over the past decades, multiple myeloma (MM) still remains as one of the leading causes of person-years of life lost all over the world. On the basis of the pathogenic role of SP/NK1R pathway in cancer, it was of great interest to investigate the anti-cancer effect of aprepitant, an oral competing non-peptide antagonist of NK1R, in MM cells.
**Methods**: To evaluate the cytotoxic and anti-proliferative effects of aprepitant in multiple myeloma, KMM cells were treated with increasing doses of the inhibitor. Cell viability and metabolic activity were investigated by trypan blue and MTT assays, respectively. Afterwards, Annexin-V/PI staining and DNA content analysis were performed to evaluate whether aprepitant-induced cytotoxic effect could be attributed to either apoptosis induction or cell cycle arrest. Additionally, transcriptional alteration of apoptosis-related target genes was also studied using real-time RT-PCR.
**Results:** We found that treatment of KMM cells with aprepitant resulted in inhibition of both viability and metabolic activity in a concentration-dependent manner. Moreover, flow cytometeric analysis delineated a considerable pro-apoptotic potential of the inhibitor in MM cells, as evidenced by increased externalization of phosphatidylserine, and elevated cell population in sub-G1 phase. Noteworthy, the results of real-time PCR revealed that aprepitant-induced cytotoxicity is mediated through shifting the ratio of death promoters to death repressors via alteration of Bax and Bcl-2 expression.
**Conclusions**: Based on the pharmacologic safety of aprepitant and its broad clinical application in chemotherapy-induced nausea and vomiting prevention, our study suggests this inhibitor as a promising agent for the treatment of multiple myeloma. However, further investigation, including clinical trials will provide valuable clues to add this inhibitor for treatment of MM patients.

**Keywords:** Multiple myeloma, Aprepitant, Neurokinin-1 receptor.

ICIA2018\Cancer Immunotherapy\Poster \7530 cancer immunotherapy
**7530**

# Native human PD1: reducer of $PD1^{+}FOXP3^{-}CD8^{+}$T cells

Samane Mohamadzade[1], Alireza Andalib[1], Nafise Esmael[1], Nahid Eskandari[1], Abbas Rezaei[1], Hosein Khan Ahmad[2], Ilnaz Rahimmanesh[2]

1. *Department of Immunology, School of Isfahan Medical, Isfahan University of Medical Sciences*
2. *Department of Genetics and Molecular Biology, School of Isfahan Medical, Isfahan University of Medical Sciences*

**Background:** PD1 is one of the immune check point receptors which expresses on the activated T cells and suppress stimulatory signaling of TCRs. In the tumor microenvironments, continuous stimulation of $CD8^{+}$T cells increases PD1 expression, $PD1^{+}CD8^{+}$T cells, and PD1 signaling. PD1/PD-L1 engagements induce FOXP3 expression and regulatory T cell differentiation, $FOXP3^{+}PD1^{+}CD8^{+}$T cells, which are inhibitor cells in the tumor microenvironments. Therefore, $FOXP3^{-}PD1^{+}CD8^{+}$T cells are the progenitor population of $FOXP3^{+}PD1^{+}CD8^{+}$T cells. Inhibition of PD1 receptors on the T cells could decrease PD1/PD-Ls interactions and $CD8^{+}$ regulatory T cells induction. In this study we constructed a native human PD-1, shPD1, and assessed its effect on $FOXP3^{-}PD1^{+}CD8^{+}$T cells.
**Methods:** We constructed shPD1 which was contained extracellular domain of human PD1 and GFP.We co-cultured concanavalinA stimulated T cells and MDA-MB-231 cell line with or without shPD1 (groups 1 and 2). After 24 hours, PD1 and FOXP3 expression by $CD8^{+}$T cells was assessed with anti CD8, anti PD1 and anti FOXP3 antibodies using flow cytometry.
**Results:** Population of $FOXP3^{-}PD1^{+}CD8^{+}$T cells in group 1 (6.4%) was significantly, $P<0.05$, lower than group.2 (8.12%).
**Conclusion:** Stimulation of lymphocytes with concanavalinA induced PD1 expression on the surfaces of T cells; therefore PD1 signaling increased $FOXP3^{-}PD1^{+}CD8^{+}$T cells which were progenitors of $FOXP3^{+}PD1^{+}CD8^{+}$T cells. However, shPD1 in co-culture of stimulated T cells with MDA-MB-231 cell line, inhibited PD1/PD-L1 interaction and decreased PD-1 signaling and PD1 expression. Therefore $FOXP3^{-}PD1^{+}CD8^{+}$T cells were decreased and accordingly FOXP3 induction was reduced. We concluded that our shPD1 could be used as a reducer of inhibitory $PD1^{+}CD8^{+}$T cells and preventer of $FOXP3^{+}PD1^{+}CD8^{+}$T cells in the tumor microenvironments. Therefore inhibitory molecules secretion could be decreased using shPD1 in the tumor microenvironments.

**Keywords:** shPD1, $PD1^{+}$ $CD8^{+}$ T cells, FOXP3.

ICIA2018\Cancer Immunotherapy\Poster \7535 cancer immunotherapy
**7535**

# Immunogenicity of Rituximab, Trastuzumab and Bevacizumab Monoclonal Antibodies in patients with malignant diseases

Fatemeh Saffari[1], Abdollah Jafarzadeh[1, 3, 4], Kalantari-Khandani B[3], Farzaneh Saffari[2], Soleimani S[1], Mohammadi MM[1]

1. *Department of Immunology, Medical School, Kerman University of Medical Sciences, Kerman, Iran*
2. *Department of Laboratory Sciences, Para-Medicine School, Shiraz University of Medical Sciences, Shiraz, Iran*
3. *Department of Hematology-Oncology and BMT, Kerman University of Medical Sciences, Kerman, Iran*
4. *Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.*
5. *Department of Immunology, Medical School, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

**Background:** One of the most important limiting efficacy of monoclonal antibodies (mAb) is their immunogenicity that may influence the diagnostic and therapeutic process of them. The aim of this study was determine the presence of antibody against some mAbs including Rituximab, bevacizumab and trastuzumab in malignant patients.
**Methods:** Blood samples were collected from 32 patients with lymphoma and leukemia, 43 patients with breast cancer and 23 patients with adenocarcinoma during treatment with Rituximab, trastuzumab and bevacizumab, respectively. The serum levels of human antibodies against mentioned mAb were determined by designing a standard of sandwich ELISA method.
**Results:** The presence of human antibodies against mentioned antibodies was detected in 4 of 32 (12.5%) of Rituximab-treated patients and 7 of 43 (16.3%) of trastuzumab- treated patients with mean titer of 2.33±0.37 AU/mL and 1.2±0.21 AU/mL, respectively. The seropositivity for the presence of anti-mAb in patients treated with Rituximab alone was significantly higher than patients administrated both Rituximab and chemotherapeutic agents (26.6% versus 0.0%; $P<0.02$). None of bevacizumab-treated patients were developed antibody against administrated mAb.
**Conclusion:** In conclusion, these results indicate the production of antibody against therapeutic mAbs rituximab and trastuzumab were detected in a number of treated patients that may influence their efficacy. The production of the human anti-mAb antibody may influenced by chemotherapeutic drug in rituximab-treated patients. Bevacizumab did not show immunogenicity in our treated patients.

**Keywords:** Immunogenicity, Malignant patients, Rituximab, Trastuzumab, Bevacizumab

ICIA2018\Cancer Immunotherapy\Poster \7555 cancer immunotherapy
**7555**

# Effect of miR-200c replacement on growth and migration of prostate cancer cells (pc3)

Roghayeh Pirzadeh[1], Habib Onsori[2], Behzad Baradaran[3]

1. *Department of Genetics, East Azarbaijan Science and Research Branch, Islamic Azad University, Tabriz, Iran*
2. *Department of Genetics, Tabriz Branch, Islamic Azad University, Tabriz, Iran*
3. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran (Corresponding Author)*

**Background**: Prostate cancer is one of the most common cancers among men around worldwide. In advanced countries, prostate cancer is the most common cancer after skin cancer and the most deadly cancer after lung cancer. Studies show that mir-200c expression in prostate cancer is reduced. In this study, we increased mir-200c level in the prostate cancer cells and examined its effect on cell growth and migration. Also the effect of mir-200c replacement on ZEB1 and ZEB2 expression was evaluated.
**Methods**: The pc3 cells cultivating in RPMI-1640 medium, the IC50 of antibiotic (G418) geneticin was obtained by using the MTT assay. Using jetPEI transfection reagent, miR-200c was transmitted to prostate cancer cells. The qRT-PCR test was used to prove the action of transferring this miRNA into cells. The effect of mir-200c on target genes ZEB1, ZEB2 was evaluated by qRT-PCR. Wound healing assay was performed to examine the migration status of transfected cancer cells.
**Results:** Using the MTT test, an antibiotic Geneticin, IC50 200 µM was obtained .The results of qRT-PCR showed increased expression of miRNA-200c in PCR cells transfected with miRNA-200c. The results of Wound healing showed reduced migration of transfected cells with miRNA-200C compared to untreated control. The result of qRT-PCR showed the expression level of ZEB1 and ZEB2 was decrease after replacement of mir-200c.
**Conclusion**: The results showed that increasing miR-200C plays an important role in inhibiting the growth and migration of PC3 cell line prostate cancer cells.

**Keywords**: miRNA, Prostate Cancer, Immigration, Transfusion

ICIA2018\Cancer Immunotherapy\ Poster \7703 cancer immunotherapy
**7703**

**Leukemia cell growth inhibition by silencing of Mcl-1 gene**

Shaghayegh Askarian amiri[1], Abbas Azadmehr[1], Farzane Ordoni aval[1], Morteza Oladnabi[2]

*1. Cellular and Molecular Biology Research Center,Department of Immunology, Health Research Institute, University of Medical Sciences, Babol, Iran*
*2. Gorgan Congenital Malformations Research Center,Department of Human Genetics, School of Advanced Technologies in Medicine University of Medical Sciences, Gorgon, Iran*

**Background:** T cell acute lymphoblastic leukemia (T-ALL) is one of the most malignant hyperplastic diseases of the hematopoietic system. This tumor is most common tumor diseases in children and youths. Mcl-1 is a pro-survival protein of the Bcl2 family member. Mcl-1 was found as an important factor to conventional cancer therapies. Down regulation of this gene can be potential therapeutic target in multiple types of cancers. **Methods:** The aim of this study was to investigate the effect of specific Mcl-1 small interfering RNAs (siRNAs) on the survival and induction of apoptosis on jurkat cells. Specific Mcl-1 siRNA was transfected. Then, relative Mcl-1 mRNA expression level was measured by quantitative real-time PCR. Moreover, cell survival was determined using colorimetric MTT assay and apoptosis was analyzed using flow cytometry. **Results:** Our findings showed that 48 hours after transfection with Mcl-1 siRNA the expression of mRNA levels was effectively reduced in dose dependent manner. Furthermore, treatment with Mcl-1 siRNA significantly reduced tumor cell survival and significantly increased the extent of T-cell acute leukemia cell apoptosis. **Conclusion:** Our results suggest that down-regulation of Mcl-1 by specific siRNA in T-cell acute leukemia cells can effectively reduce cell survival and induces apoptosis cell death. Therefore, Mcl-1 siRNA may be a complementary agent in treatment of T-ALL.

**Key words:** Mcl-1, siRNA, Jurkat cell line, T-ALL, Apoptosis

ICIA2018\Cancer Immunotherapy\Poster\7704 cancer immunotherapy
**7704**

## BACH1 as inducer marker for prostate cancer cells metastasis

Neda Shajari[1, 2], Behzad Baradaran[1, 2]

*1. Department of Immunology, School of Medicine, University of Tabriz*

**Background:** Metastasis to distant organs is a hallmark of many tumor cells. BACH1 (BTB and CNC homology 1) has been shown to transcriptionally regulate expression of a range of genes that are associated with breast cancer metastasis. The aim of this study was to investigate the effect of BACH1 silencing on prostate cancer cells in vitro.
**Methods:** BACH1 expression was silenced in DU145 prostate cancer cell line using siRNA approach. Quantitative RT-PCR was used to detect BACH1 expression and other metastasis-related genes following siRNA knockdown.
**Results:** Quantitative RT-PCR analysis revealed that the expression levels of BACH1 mRNA in DU145 cells were significantly suppressed after transfection. Also, the CXCR4 and MMP1 expression levels decreased following BACH1 knockdown in DU145 cells.
**Conclusion:** Our results indicated that BACH1 down-regulation in DU145 cells decrease the expression levels of some metastasis-related genes. These results suggest that BACH1 may function as an oncogenic driver in prostate cancer and may represent as a potential therapeutic target for metastatic prostate cancer.

**Keywords:** BACH1, siRNA, Prostate Cancer

ICIA2018\Cancer Immunotherapy\Poster\7745 cancer immunotherapy
**7745**

# Inhibition of glioblastoma cell growth by silencing of TGFβRII

Farzane Ordoni aval[1], Abbas Azadmehr[1], Shaghayegh Askarian amiri[1], Payam Saadat[2], Somayeh Fallahnejad[3], Morteza Oladnabi[4], Behzad Baradaran[5], Behzad Mansoori[5]

*1. Cellular and Molecular Biology Research Center,Department of Immunology, Health Research Institute, University of Medical Sciences, Babol, Iran*
*2. Mobility Impairment Research Center, Health Research Institute, University of Medical Sciences, Babol, Iran*
*3. Obstetrics and Gynecology department, Shahid Beheshti University of Medical sciences, Tehran, Iran*
*4. Gorgan Congenital Malformations Research Center,Department of Human Genetics, School of Advanced Technologies in Medicine University of Medical Sciences, Gorgon, Iran*
*5. Immunology Research Center,University of Medical Sciences, Tabriz, Iran*

**Background:** Glioblastoma (GBM) is the most malignant and aggressive type of glioma, associated with a high rate of mortality. The transforming growth factor-β receptor II (TGFβ RII) is involved in glioma initiation and progression. On the other hand, TGFβ RII blockade is critical to the inhibition of GBM. Therefore, we aimed to determine the effects of specific TGFβ RII siRNA on the survival of U-373MG cells.
**Methods:** TGFβ RII siRNA was transfected and qRT-PCR was performed to examine TGFβ RII mRNA expression. Cell survival was determined using colorimetric MTT assay, and platelet-derived growth factor-BB (PDGF-BB) level was measured in the culture supernatant using ELISA assay.
**Results:** Our findings indicated that specific siRNAs could dose-dependent suppress TGFβ RII mRNA expression after 48 hours. In addition, treatment with TGFβ RII siRNA significantly reduced tumor cell survival and decreased the amount of PDGF-BB proteins in the cell culture supernatant.
**Conclusion:** Our results suggest that TGFβ RII blockade can be a promising complementary treatment for glioma.

**Keywords:** TGF-β RII, siRNA, U-373 MG cell line, PDGF-BB, Glioblastoma

ICIA2018\Cancer Immunotherapy\Poster \7826 cancer immunotherapy
**7826**

# Enhancement of 5-fluorouracil-induced cytotoxicity by LPS in MCF7 human breast cancer cells

Negin Nokhandani[1], Mahdieh Naghavi Alhosseini[2], Seyedeh Naemeh Javid[3], Homa Davoodi[4]

1. *Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran*
2. *Department of Immunology, School of Medicine, University of Golestan Medical Sciences, Gorgan, Iran*
3. *Department of Microbiology, School of Medicine, University of Golestan Medical Sciences, Gorgan, Iran*
4. *Cancer Research Center, University of Golestan Medical Sciences, Gorgan, Iran*

**Background:** 5-Fluorouracil (5-FU) is a widely used anticancer drug in patients with breast and other solid cancers. Despite its many advantages, clinical applications were limited because of the drug resistance. On the other hand, the chemotherapy has shown common side effects include myelosuppression, dermatitis, cardiac toxicity, diarrhea, and mucositis. Therefore, new strategies and tumoricidal agents for therapy and resistance reversal are urgently needed. Lipopolysaccharide (LPS) is one of the major components of the outer layer of gram-negative bacterial membrane. The Stimulation of toll-like receptor 4 (TLR4) through LPS activates immune responses. In the previous study, Davoodi et al. showed that LPS increased the cytotoxic effect of 5-FU on colon cancer cells. In the current study, the effect of LPS pretreatment on cytotoxicity induced by 5-Fu in breast cancer cell line MCF-7 was investigated.
**Methods:** The effect of LPS pretreatment on the cytotoxicity of 5-FU was tested in a breast cancer cell line, MCF7. Cell viability was analyzed using MTT assay. Cancer cells were seeded in 96 well plates. On the following day cells were exposed to LPS (1µg/ml) for 4 hours and then were treated with different concentration of 5-FU (0-500µg/ml). After 24-72 h, the cells were collected and cell viability was determined by MTT assay.
**Results:** The results showed that, 1µg/ml LPS pretreatment increased the cytotoxicity effect of different dose of 5-FU in MCF-7(P<. /05). We showed that 4 hours pretreatment of LPS has desired effect in comparison with simultaneous treatment of LPS and 5-FU.
**Conclusion:** LPS augmented the tumoricidal effects of the chemotherapy by 5-FU in breast cancer cells. LPS can increase the anti-cancer effect of 5-FU and therefore reduce advert side effects of chemotherapy and also reduce the rate of resistance to 5-Fu in patients.

**Keywords:** Breast cancer, Lipopolysaccharide, 5-fluoracil

ICIA2018\Cancer Immunotherapy\Poster \7987 cancer immunotherapy
**7987**

# Overexpression of microRNA-193a-5p, as a novel prognostic marker, inhibits migration of human HT-29 colon cancer cells via down-regulating metastasis-related genes expression

Naghmeh Shirafkan[1, 2], NavidShomali[1,2], Behzad Mansoori[1], Ali Mohamadi[1], Behzad Baradaran[1*]

1. *Immunology Research Center (IRC), Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran*
3. *Correspondence*: Behzad Baradaran, Immunology Research Center, Tabriz University of Medical Sciences*

**Background:** Colorectal cancer (CRC) is among three leading reasons of cancer-related mortality in all over the world. MicroRNAs are important post-transcriptional regulators which dysregulated in cancers and affect the expression of different genes in cancer progression. Down-regulation of miR-193a-5p has been found in some types of cancer. However, effects and potential mechanisms of miR-193a-5p remains elusive in CRC.
**Methods:** Quantitative RT-PCR was performed to detect expression of miR-193a-5p and metastasis-related genes before and after mimic transfection in CRC cell lines. Wound healing assays measured migration capacity of HT-29 cell lines after miRNA transfection. MTT and Annexin V staining were used to determine the role of miR-193a-5p in the regulation of HT-29 proliferation and apoptosis, respectively.
**Results:** In this study, we found that miR-193a-5p was down-regulated in CRC cell lines. And the restoration of miR-193a-5p in human HT-29 cell line could inhibit cell migration. But, reduced viability and apoptosis induction did not occur significantly after miR-193a-5p overexpression. Also, the quantitative RT-PCR analysis showed that the expression levels of miR-193a-5p mRNA were increased after mimic transfection. Furthermore, the Vimentin, E-cadherin and CXCR4 mRNA expression levels decreased after overexpression of miR-193a-5p with mimic in HT-29 cell line.
**Conclusion:** Our results showed that miR-193a-5p may function as a tumor suppressor and play an important role in metastasis and suggest it may be a potential therapeutic target for applying in CRC therapy.

**Keywords:** Colorectal cancer, microRNA, Metastasis, Apoptosis

ICIA2018\Cancer Immunotherapy\Poster \8077 cancer immunotherapy
**8077**

# Investigation of anti-tumor and anti-inflammatory effects of *Nigella sativa* on 4T1 and CT26 cell lines

Fatemeh Hosseini[1], **Hadi Hassannia[2]**

1. *Department of Immunology, School of medicine, Semnan University of Medical Sciences, Semnan, Iran*
2. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Cancer is the second leading cause of death globally and cancer-related inflammation plays an important role in malignant progression of several cancer types. Current therapeutic approaches for cancer therapy are not completely effective and are associated with many side effects. In recent years, there has been growing interest in the application of medicinal herbs, since they have fewer side effects than chemical drugs. Therefore, the present study aimed to investigate of anti-tumor and anti-inflammatory effects of *Nigella sativa* in *in vitro* conditions.
**Methods:** In this study, 4T1 and CT26 cell lines were treated with different concentrations of hydro alcoholic extracts of *Nigella sativa* (50, 250, 500, 1000 µg/mL). After 48h, the effects of the extract on tumor growth and COX-2 gene expression were analyzed by XTT and Real time PCR, respectively.
**Results:** The results revealed that 4T1 and CT26 cell lines growth significantly decreased in a dose dependent manner in compared to the control ($p < 0.05$). Also, Real time PCR results showed that the expression of COX-2 significantly decreased in a dose dependent manner even at the low concentration of extract in compared to the control ($p < 0.05$).
**Conclusion:** Based on these results, *Nigella sativa* extract not only suppressed the growth of cancer cell lines but also significantly reduced cancer-related inflammation. Therefore, it may be utilized as a novel therapeutic in several types of cancer. However, this claim needs further investigations.

**Keywords:** *Nigella sativa*, Cancer, Inflammation, COX-2

ICIA2018\Cancer Immunotherapy\Poster \8163 cancer immunotherapy
**8163**

**Effect of conditioned medium of mesenchymal stem cells pulsed with different doses of caffeine on murine breast cancer cells (4T1).**

Zahra Zeinaly, Nouroz Delerizh, Seyyed Meysam Abtahi Froushani

*Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Conditioned medium of Mesenchymal Stem Cells (MSCs) have potent immunomodulatory and anticancer benefits. It is clear that environmental factors modulate the functions of MSCs. This study was set out to evaluate the effects of the conditioned medium of MSCs (CM) of caffeine (as a very popular drink) treated MSCs on the proliferation of 4T1, a breast adenocarcinoma cell line.
**Methods:** MSCs were isolated by flashing the tibia and femur bones of mice. Third Passage of MSCs incubated with different doses of caffeine (0, 0.1, 0.5 and 1 mM) for 24 hours. Then cells were washed and cultured for 24 hour with fresh medium. The CM was collected and used in the culture medium of 4T1 (50%).
**Results:** Neutral red (NR) uptake and MTT proliferation test indicated that the CM could significantly decrease the proliferation of 4T1 cells. NR uptake and MTT test showed that CM pulsed with caffeine at 0.1 mM and CM pulsed with caffeine at 0.5 mM could significantly increase the anticancer effects of CM, respectively. Data from both tests indicated that CM pulsed with caffeine at high dose (1 mM) could significantly regress these beneficial effects of CM.
**Conclusion:** It seems that caffeine at the low to moderate doses can potentiate the cytotoxic effect of CM. Nevertheless, the high dose had trouble effects on the beneficial effects of CM on the proliferation of cancer cells.

**Keywords:** Caffeine, Conditioned medium, Mesenchymal stem cell.

ICIA2018\Cancer Immunotherapy\Poster \8417 cancer immunotherapy
**8417**

**Immunotherapy in ependymoma brain tumor**

Fateme Shaabanpour Aghamaleki[1], Shirin Farivar[2]

*1. Department of molecular and cellular Biology School of Biotechnology and Life Science University of Shahid Beheshti*
*2. Department of molecular and cellular biology school of biotechnology and Life Science University of Shahid Beheshti*

**Background:** Approximately 50% of children with ependymoma will suffer from tumor recurrences that will ultimately lead to death. Children with the recurrent disease commonly experience cumulative neurological morbidity from repeated surgical and adjuvant therapy interventions, and almost universally suffer from tumor progression. Accordingly, conceptually new treatment approaches are needed, both to decrease the risk of tumor recurrence and to enhance disease control in those children who experience recurrent disease. One of this approaches is immunotherapy, also called biologic therapy, and is a type of cancer treatment that boosts the body's natural defenses to fight cancer. It uses substances made by the body or in a laboratory to improve or restore immune system function.
**Methods:** Gene's expression profiling by array of 20 ependymoma patients with recurrent experience and 45 primary ependymoma patients was extracted from Geo datasets. Genes are compared with logfc for their expression between two kinds of patients and sorted by their p-value. Also, the most related pathway, location, function, and protein networks of this gene were identified with string, GeneCard and DAVID databases.
**Results:** Several important immune genes that associated with recurrent ependymoma tumor are identified such as CCL19, CXCL9, NCF1, and IGSF6. These genes have hyperexpression in ependymoma patients with recurrent experience. Among these genes, CXCL9 is a small cytokine belonging to the CXC chemokine family. CXCL9 is a T-cell chemoattractant, which is induced by IFN-γ. It is associated with CCL19 and CXCL2 and plays roles in Toll-like Receptor Signaling Pathway and ERK Signaling. It has hyperexpression in ependymoma patients with recurrent experience.
**Conclusion:** According to CXCL9 function and expression changes, it can play an important role in ependymoma with recurrent experience. Therefore, it can be used as an immunotherapeutic target for cancer therapy.

**Keywords:** Ependymoma, Immunotherapy,Gene expression,CXCL9

ICIA2018\Cancer Immunotherapy\Poster \8434 cancer immuno
**8434**

**Using CRISPR/Cas9 system for immunotherapy of brain tumors**

Fateme Shaabanpour Aghamaleki[1], Shirin Farivar[2]

*1. Department of cellular and molecular Biology School of biotechnology and life science University of shahid beheshti university*
*2. Department of cellular and molecular biology Faculty of biotechnology and life science University of shahid beheshti*

**Background:** Brain tumors are the leading cause of cancer mortality in children and remain difficult to cure despite advances in surgery and adjuvant therapy. Most current brain tumor research is focused on the molecular and cellular analysis of the tumor mass. There are wide variety of immune genes that play an important roles in brain tumors pathogenesis and progression. Identification of these genes can be used as therapeutic target in CRISPR/Cas9 system. CRISPR/Cas9 system has been modified to edit genomes, by delivering the Cas9 nuclease complexed with a synthetic guide RNA (gRNA) into a cell, the cell's genome can be cut at a desired location, allowing existing genes to be removed and/or new ones added.
**Methods:** Genes expression profiling by array of 117 brain cancer patients and 13 healthy individuals were extracted from Geo datasets. Genes are compared with logfc (fold change) for their expression in healthy and patients group, and then sorted by their p-value. Also the most related pathway, genes function and ontology are identified by DAVID and GeneCard databases. Finally genes with most modification and related to critical pathway in immune system are selected and their best gRNAs (guide RNA) in CRISPR system are identified for editing by CHOPCHOP datasets.
**Results:** TNFRSF19 or TNF receptor superfamily member 19 has hyperexpression among brain tumor patients. It plays an important roles in immune system and inflammatory response. It's important pathway and function are Wnt and TNF Superfamily Pathway. The best gRNAs for this gene is identified through CHOPCHOP database that haven't any secondary structures.
**Conclusion:** Many experiences showed that immune genes play an important roles in cancer progression and metastasis, therefore they can be as a therapeutic target for CRISPR/Cas9 system and gene therapy.

**Keywords:** Brain Tumor, Immune System, CRISPR/Cas9, Gene Therapy

ICIA2018\Cancer Immunotherapy\Poster \8435 cancer immuno
**8435**

# MiR-199a-5p may sensitize the triple-negative breast cancers to paclitaxel chemotherapy

Navideh Haghnavaz[1,2], Faezeh Asghari[1,2], Najibeh Shekari[1,2], Behzad Baradaran[1,2], Tohid Kazemi[1,2*]

1. *Department of Immunology, Faculty of Medicine, Tabriz University of Medical Science, Tabriz, Iran*
2. *Immunology Research Center, Tabriz University of Medical Science, Tabriz, Iran*

**Background:** Paclitaxel is a chemotherapeutic agent that is used widely in the treatment of breast cancer and acts by arresting in cell cycle. We aimed in this study to investigate the effect of paclitaxel treatment on the expression level of miR-199a-5p, cell cycle arresting miRNA in breast cancer cell lines.
**Methods:** MCF-7, MDA-MB-231 and SKBR-3 cell lines were cultured and MTT assay was performed to determine IC50 of paclitaxel. After RNA extraction and cDNA synthesis, expression level of miR-199a-5p was quantitatively evaluated using Real-Time PCR.
**Results:** IC50 concentration for MCF-7, MDA-MB-231 and SKBR-3 was 3.5 µM, 0.3 µM and 4 µM, respectively. Our results showed increased expression level of miR-199a-5p in MDA-MB-231 cell line (1.27 fold, P=0.001) and decreased expression level in two other cell lines including MCF-7 and SKBR-3 (125 fold, P< 0.0001 and 29.4 fold, P< 0.0001, respectively) in response to paclitaxel therapy.
**Conclusion:** Altered expression level of miR-199a-5p could be related to the IC50 concentration of paclitaxel. More sensitive cell line, MDA-MB-231, showed increased expression level of miR-199a-5p after treatment with paclitaxel. In contrast, other two cell lines with higher IC50 concentration showed decreased expression level of miR-199a-5p. In conclusion this miRNA could be involved in resistance/sensitivity of different types of breast cancer to paclitaxel therapy.

**Keywords**: miR-199a-5p, paclitaxel, breast cancer

ICIA2018\Cancer Immunotherapy\Poster \8448 cancer immuno
**8448**

# Fas expression on CD4+ T cells in tumor draining lymph nodes of patients with breast cancer

Zahra Faghih[1], Yasmin Vahidi[1], Abbas Ghaderi[1, 2]

1. *Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background**: Fas receptor, also known as CD95 and APO-1, is a member of tumor necrosis factor α-family of death receptors which modulates T-cell based immune responses. Fas-mediated apoptosis is an important contributor in the contraction phase of antigen driven T cell responses. Fas receptor directly binds to and activates TCR components in a stimulus-dependent manner. Additionally, the interaction of CD95 (Fas) and CD95L (Fas ligand) leads to apoptosis of thymocytes with incorrect TCR rearrangement and of those that recognize self-antigens, a process called negative selection. Moreover, Fas–FasL interaction could also promote activation-induced cell death, a form of apoptosis induced by repeated TCR stimulation, responsible for the peripheral deletion of activated T cells. Recent studies revealed that most cancer cells are resistant to CD95-mediated apoptosis and this resistance play a crucial role in the pathogenesis of several malignancies. In the present study, we aimed to investigate Fas expression on $CD4^+$ helper cells in tumor draining lymph nodes of breast cancer (BC) patients as the main site of immune response formation.
**Methods:** Mononuclear cells were isolated from lymph nodes of 53 untreated BC patients and stained for CD4 and CD95 antibodies to detect Fas expressing cells. The cells were then acquired on four-color flowcytometer and data were analyzed by CellQuest Pro software.
**Results:** More than 43% of $CD4^+$lymphocytes in draining lymph nodes of BC patients expressed CD95 marker though no significant difference was found in the frequency of cells with Fas expression in the patients with different clinicopathological parameters.
**Conclusion:** Our results indicated no direct association between Fas expression on $CD4^+$ lymphocytes and breast cancer prognosis. However, its expression in respect to expression of Fas ligand on tumor cells and other immune cells should also be investigated.

**Key words:** Breast cancer, $CD4^+$ T cells, Fas expression

ICIA2018\Cancer Immunotherapy\Poster \8586 cancer immuno
**8586**

# Evaluation of MicroRNA181a and MicroRNA181b after suppression of PTPN22 gene in T-cell leukemia cell line (Jurkat)

Elham Baghbani[1], Behzad Baradaran[1]

*1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** T-cell acute lymphoblastic leukemia (T-ALL) is one of the most frequent malignancies associated to T-cells. Effectiveness interference in cancer medical care has been characterized by high efficiency and potential, induction of silencing within the advanced stages of growth, low price in compared to the opposite strategies of gene therapy, and high specificity compared to the opposite strategies of cancer therapy like chemotherapy. PTPN22 gene (protein tyrosine phosphatase,non-receptor type 22), encodesa protein, lymphoid tyrosine phosphatase (Lyp) in human. MiR-181 was concerned in regulation of the differentiation of B cells, T cells and natural killer cells during normal hemogenesis. In this study, we specifically investigated the effects of PTPN22 silencing in human acute T-cell leukemia cell line (Jurkat) and it's affect in the expression of miR181a and miR181b. We have shown that PTPN22 can be considered as a potent adjuvant in T-ALL therapy.
**Methods:** Jurkat cells were grown in RPMI-1640 supplemented with 10% (v/ v) FBS, penicillin (100 U/ ml), streptomycin (100 µg/ml), and maintained at 37° C in humidified 5% CO2 atmosphere. PTPN22 siRNA transfection was performed and then total RNA was isolated from cells. Quantitative Real-time PCR was performed by using a standard SYBR Green PCR master mix.
**Results:** Results of Quantitative Real-time PCR showed that downregulation of PTPN22 leads to downregulation of miR-181a and miR181-b in jurkat cell line.
**Conclusion:** The results showed that the expression of miR-181a and miR181-b in jurkat cell line are affected by PTPN22. Deregulation of their expression is related to development of T-cell malignancies and pathological process of leukemia.

**Keywords:** Jurkat, PTPN22, miR181, T-ALL

ICIA2018\Cancer Immunotherapy\Poster \8662 cancer immuno
**8662**

**Restitution of miRNA-145 expression inhibits migration in MKN-45 gastric cancer cell line**

Tahereh Zeinali[1], Dariush Shanehbandi[1], Naiyer Hosseinahli[1], Ali Mohammadi[1], Behzad Mansoori[1], Elham Baghbani[1], Behzad Baradaran[1]

*1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background**: Although gastric cancer (GC) is third most common cause of cancer-related death, major therapeutic advances have not been made, because GC is a polygenic disease arising as the result of multiple gene dysregulations. In addition to other protein-coding oncogenes and tumor suppressor genes, microRNAs play an important role in gastric cancer tumorigenic progression. The tumor suppressor miR-145 is ubiquitously expressed in normal tissues and body fluids but is lost or expressed at reduced levels in various forms of cancer including GC. In this study, we aim to restitute the miR-145 expression in MKN-45 cells and investigate the role of miR-145 in gastric cancer metastasis.
**Methods:** We transfected MKN-45 cells through PCMV-miR-145 plasmid vector delivery. The effects of the ectopic expression of miR-145 were examined by performing wound healing assay in vitro. Also, alterations in K-RAS and MMP-9 mRNA levels, which play significant roles in invasion, were evaluated by qRT-PCR analysis, respectively.
**Results:** Consequently, the results indicated that forced expression of miR-145 decreased cell migration in MKN-45 cells, with concomitant repression of K-RAS and MMP9 as putative targets of miR-145.
**Conclusions:** Replacement of exogenous miR-145 regain control over multiple metastasis pathways that are regulated by the endogenous miR-145, it may have a therapeutic potential to suppress gastric cancer metastasis.

**Keywords**: gastric cancer; restitution; miR-145 replacement therapy; metastasis

ICIA2018\Cancer Immunotherapy\Poster \8664 cancer immuno
**8664**

# UCB CD34+Expansion and Differentiation into NK cells for immunotherapy approaches

Gholamreza Anani Sarab[1], Alireza Shekohifar[2]

1. *Department of Immunology, Birjand University of Medical Sciences. Birjand-Iran*
2. *Department of Molecular Medicine.,Birjand University of Medical Sciences. Birjand-Iran*

**Background:** Human Immune system has some essential reflectors called natural killer cells which are differentiated from CD34+ HSCs and are active in fighting and killing infectious agents and tumor cells. **Methods:** In this study we examined the serum-free expanded CD34+ cells and their differentiation into NKcells. This procedure was 5 weeks long and at the end of this period, the differentiated NK cells which were then characterized through immunophenotyping analysis. IFN-gamma secretion assay was also performed. **Results:** According to the performed analyses the CD34+ differentiated NK cells were reported to be acceptable as normal NK cells based on immunophenotypical properties.
**conclusion:** Concludlingly, the expanded CD34+ cells in the serum-free medium were proved to have the capability of being differentiated into NK cells and providing a good source for anti-cancer immunotherapy.

**Keywords:** NK cells, immunotherapy, UCB CD34+

ICIA2018\Cancer Immunotherapy\Poster \8666 cancer immuno
**8666**

# Restoration of miR-143 expression increases apoptosis in MKN-45 gastric cancer cell line

Naiyer Hosseinahli[1], Dariush Shanehbandi[1], Tahereh Zeinali[1], Nasrin Hosseinahli[2], Ali Mohammadi[1], Behzad Mansoori[1], Elham Baghbani[1], Behzad Baradaran[1]

1. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Azarbaijan Higher Education and Research Complex, Tabriz, Iran*

**Background:** Gastric cancer (GC) with high incidence and mortality is a major health concern especially in the developing countries. Involvement of multiple genes in initiation and progression of GC leads to poor prognosis in this cancer. miRNAs are a class of small non-coding RNAs which negatively control the expression of messenger RNAs (mRNA). MiR-143 is a tumor suppressor miRNA which its aberrant expression has been shown in a variety of human cancers including GC. In the present study, we have tried to restore the miR-143 expression in MKN-45 cells and examine the role of miR-143 in gastric cancer apoptosis.
**Methods:** We transfected MKN-45 cells by pCMV-miR-143 plasmid vectors. Effects of exogenous expression of miR-143 on cell apoptosis were assessed by MTT, respectively. Furthermore, DAPI staining assay was utilized for detection of apoptosis induction. After miR-143 grafting, alterations in Caspase-3, Caspase-9 and Bax mRNA levels, which play key roles in apoptosis, were evaluated by qRT-PCR analysis.
**Results:** According to the outcomes, exogenous expression of miR-143 induced apoptosis in MKN-45 cells. Moreover, increased expressions of Caspase-3, Caspase-9 and Bax, as putative targets of miR-143, were observed.
**Conclusion:** It could be concluded that, reversing the miR-143 expression, by methods such as miRNA replacement, could be considered as an effective strategy to increase apoptosis in gastric cancer.

**Keywords:** gastric cancer, apoptosis, restoration, miR-143

ICIA2018\Cancer Immunotherapy\Poster \8723 cancer immunotherapy
**8723**

## Evaluating the expression of M1 phenotype inducing miRNAs in metastatic breast cancer cell line

Maryam Moradi Chaleshtori[1], Seyed Mahmoud Hashemi[2], Sara Soudi[3], Mojgan Bandehpour[1], Samira Mohammadi Yeganeh[1]

*1. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

**Background:** Breast cancer is the most frequent carcinoma in females and the second most common cause of cancer-related mortalities in women. In recent years, tumor microenviroment (TME) has attracted a lot of attention for developing more effective treatments. Among the immune cells in TME, macrophages are particularly abundant. In breast cancer, more than 50% of the tumor mass includes tumor-associated macrophages (TAMs). In the early stages of tumors, TAMs adopt the antitumor phenotype (M1) and in advanced tumors TAMs shift to a tumorigenic phenotype (M2).The aim of this study was to identify the specific miRNAs in metastatic breast cancer cells carried in exosomes and induce M1 phenotype in macrophages.
**Methods:** We used bioinformatics software and programs such as TargetScan, miRWalk, miRanda, as well as microarray data from GEO to find miRNAs differentially expressed between M1 and M2 macrophages. Real-Time PCR was performed to evaluate the expression of candidate miRNAs in breast cancer cell line which probably carry by exosomes to TAMs.
**Results:** The results of bioinformatic studies proposed candidate miRNAs with differentially expressions between M1 and M2 macrophages and also target the key genes in signaling pathways of macrophage polarization. miR-33 and miR-130 were selected among all candidate miRNAs. The results of Real-Time PCR indicated increased expression of miR-130 and decreased expression of miR-33 in metastatic breast cancer cell line.
**Conclusion:** According to our findings, targeting of these candidate miRNAs can be considered for reprogramming of M2 macrophages into antitumor M1 macrophages as a therapeutic strategy in breast cancer.

**Keywords:** Breast cancer; Tumor-Associated Macrophages; miRNA

ICIA2018\Cancer Immunotherapy\Poster \9746 cancer immuno
**9746**

# The role of aloperopin in expressing the DNMT gene and inhibition of cancer cells

Amirhossein Alimohammadian[1], Negin Maleki[2]

1. *Member of the brilliant talent of young and elite peoples youth club of Ardabil Islamic Azad University, Iran*
2. *Department of medical laboratory, Ardabil branch, Islamic Azad University, Ardabil, Iran*

**Background:** The cytosine methylation in the CpG islets in the promoter region is an important mechanism for regulating the expression of genes, and this arrangement can play a role in the developmental and evolutionary stages and, by binding to TLR9, produces IL12, and results in gamma IFN production, which results in stimulation cytotoxicity of NK cells or can be a factor in silencing the expression of genes, especially in some cancer cells.
**Methods:** In breast cancer cells, the most important marker for methylation is the Her2-neu gene promoter. In this study, different concentrations of nano-aloperopin on the expression of the gene expression in DNMT were investigated using time real time PCR method.
**Results:** The results showed that the relative expression of DNMT1 gene was significantly reduced by applying 30ppm nano-aloperopin in breast cancer cell line.
**Conclusion:** Obviously, by reducing the expression of the MT gene, methylation can be reduced, and the expression of exogenous genes may be expressed again.

**Keywords:** CpG, aloperopin, Cancer, Methylation

ICIA2018\Cancer Immunotherapy\Poster \9827 cancer immuno
**9827**

# Comparative study of the effects of osahexaenoic acid (DHA), *Linoleic acid (LA) and Taxol in decreasing the expression of miR-10b and miR-20a oncomirs in triple-negative metastatic breast cancer cell line*

*Sepideh Maralbashi[1,2] , Farhad Salari[1], Mahsa Javadian[3], Najibeh Shekari[3], Cynthia Aslan[3], Milad Asadi[3], Tohid Kazemi[3]*

1. *Department of Immunology, Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*
2. *Student Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran*
3. *Immunology Research Center,Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Molecular signatures of the effects of widely used omega-3 and omega-6 fatty acids as dietary supplements and even in cancer therapy have been studied. Of them, microRNAs, have been shown to undergo altered expression after treatment with osahexaenoic acid(DHA) and linoleic acid (LA) in different cancer types. In this study, the expression level of two well-known oncomirs, miR-10b and miR-20a, and also MHC class I polypeptide-related sequence A (MICA) as the target for miR-10b was investigated after treatment with DHA, LA, and common therapeutic agent taxol.
**Methods:** MDA-MB-231 cells were cultured and MTT assay was performed to determine IC50 of DHA, LA and taxol. Cells were treated by DHA(100µM), LA(50µM ) and Taxol(3µM) alone or in different combinations. After RNA extraction and cDNA synthesis, the expression level of miR-10b, miR-20a and MICA were determined by quantitative real-time PCR.
**Results**: Our findings showed that DHA, LA, and taxol, and their combinations were led to the significantly decreased expression of miR-10b and miR-20a (all $p<0.0001$). In contrary, MICA as the target of miR-10b was also down-regulated significantly ($p<0.0001$).
**Conclusion**: Useful effects of DHA, LA and their combination in breast cancer could be interpreted in part by decreasing the expression level of oncomirs. Decreased expression of MICA in contrast to the decreased expression of its controlling miRNA, miR-10b, could be the result of altered expression of other miRNAs in treated cells.

**Keywords**: Breast cancer, osahexaenoic acid, miR-10b, miR-20a, MICA

ICIA2018\Cancer Immunotherapy\Poster \9832
**9832**

# Synergistic effect of osahexaenoic acid (DHA) and Linoleic acid (LA) with Taxol in up-regulating the expression of miR-101, but not miR-342 tumor suppressor microRNAs, in HER2-positive breast cancer cell line

Cynthia Aslan[1,2], Mahsa Javadian[1], Najibeh Shekari[1], Sepideh Maralbashi[3], Navid Shomali[1], Tohid Kazemi[1]

*1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
*2. Student research committee, Tabriz University of Medical Sciences, Tabriz, Iran*
*3. Department of Immunology, Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*

**Background:** Breast cancer is the most common malignancy that occurs in women globally. The effect of diet and mainly dietary fat on breast cancer risk has been widely studied. The ω-3 (e.g. DHA) and ω-6 (e.g. LA) polyunsaturated fatty acids (PUFAs) are two key families of PUFAs that their usefulness as dietary supplement especially in cancer has been interested. Several studies have shown that DHA and LA alter breast cancer related microRNAs (miRs). In this study, we investigated the expression of miR-342 and miR-101 as tumor suppressor miRs in human breast cancer cell line BT-474 under treatment with DHA, LA, and also paclitaxel.
**Methods:** Human breast cancer BT-474 cell line was grown and treated by DHA (1, LA (50 and taxol (3 for 24h. Total mRNA was isolated and cDNA was synthesized using standard protocols. Then, expression of miR-101 and miR-342 in all treated and untreated (as control) cells was determined by quantitative real-time PCR.
**Result**: Our results showed that DHA, LA and taxol and also their different combinations caused significant up-regulation of the expression of miR-101 ($p < 0.0001$). In contrary, such treatments led to the significant decrease in the expression of miR-342.
**Conclusion:** Our results showed useful effect of DHA and LA on increasing the expression of tumor-suppressor miRNA, miR-101. As we failed to find similar results about miR-342, another tumor suppressor miRNA, it could be concluded that more studies on the wider range of tumor-suppressor miRNAs and also oncomiRs are needed to find more about molecular signatures of the useful effects of dietary fats in cancer setting.

**Key words:** Breast cancer, DHA, LA, microRNA

ICIA2018\Cancer Immunotherapy\Poster \9848 cancer immuno
**9848**

# Synergistic effect of osahexaenoic acid (DHA) with etaxel in decreasing the expression level of miR-20a, a MICA-regulating oncomiR, in metastatic gastric cancer cell line

Najibeh Shekari[1, 2], Mahsa Javadian[1], Sima Ghaffari[3], Maedeh Moradi[1], Masoud Shirmohammadi[1], Tohid Kazemi[1, 3*]

1. *Immunology Research Center, Tabriz University of Medical Science, Tabriz, Iran*
2. *Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran*
3. *Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background**: Gastric cancer is one of the most prevalent and lethal cancers all over the world. MICA (MHC class I chain-related gene A) is induced upon stress, damage or cancer and recognized by the activating receptor NKG2D on NK cells. MicroRNAs play critical roles in carcinogenesis and their relation with MICA expression has been shown. Here, we aimed to investigate the effect of etaxel, DHA and LA on the expression level of miR-20a oncomir and MICA in gastric cancer cell line, MKN45.
**Methods:** MKN45 cells were cultured and MTT assay was performed to determine IC50 of etaxel, DHA and LA. After RNA extraction and cDNA synthesis, the expression level of miR-20a and its target MICA were determined by quantitative real-time PCR for both treated and untreated cell lines.
**Results:** Analysis of results showed that after treatment, expression level of MICA in all treated cell lines decreased significantly ($p<0.0001$). While, miR-20a expression level significantly decreased in treated cells ($p<0.05$), especially in etaxel/DHA/LA ($p<0.0001$, and fold change = 0.08). LA led to significant up-regulation of miR-20a ($p<0.05$, fold change= 1.12).
**Conclusion:** Decreased expression level of miR-20a as OncomiR in all treatments showed the efficacy of etaxel, DHA and LA therapy, especially when combined together which indicated their synergistic effects. Down-regulation of MICA, independent of miR-20a, may be the effect of other factors which might regulate its expression in MKN45 cell line.

**Keywords:** MICA, miR-20a, etaxel, DHA.

ICIA2018\Cancer Immunotherapy\Poster \9849
**9849**

# Investigating the effects of osahexaenoic acid (DHA), Linoleic Acid (LA) alone or in combination with etaxel on the expression of MMP-2 and Talin-2, and their regulating microRNAs in metastatic gastric cancer cell line

Najibeh Shekari[1, 2], Mahsa Javadian[1], Motahhareh Ghasemi[3], Parviz Farzamifar[1], Masoud Shirmohammadi[1], Tohid Kazemi[1, 3*]

1. *Immunology Research Center, Tabriz University of Medical Science, Tabriz, Iran*
2. *Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran*
3. *Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background**: Gastric cancer (GC), as one of the most lethal cancers, afflicts many people every year. Efforts to improve the current therapies have been continued, and efficacy of using natural supplements including osahexaenoic acid (DHA) and Linoleic Acid (LA) has been suggested. MicroRNAs play critical roles in GC carcinogenesis. Here, we aimed to evaluate the effects of DHA, LA and etaxel on the expression level of metastasis-related genes including; MMP-2 and talin-2, and their controlling miRNAs in GC cell line, MKN45.
**Methods:** MKN45 cells were cultured and determination of IC50 of etaxel, DHA and LA was done by MTT test. After RNA extraction and cDNA synthesis, the expression level of miRNAs and target genes were determined by using quantitative real-time PCR.
**Results:** Analysis of results showed that MMP-2 expression level was decreased significantly in all treated MKN45 cells ($p<0.05$), except of those treated by LA. Expression level of talin-2 was increased significantly ($p<0.05$), but not in cells treated with etaxel and its combinations that showed decreased expression (all $p<0.001$). On the other hand, level of their related tumor-suppressor miRNAs, miR-194 and miR-30 were increased in all cells except of those treated with DHA and LA. Mir-126b increased significantly in cells treated by etaxel, DHA and etaxel/DHA ($p<0.05$).
**Conclusion:** Regardless of the effects of DHA and LA alone, combined usage of DHA, LA and etaxel showed better results and led to the down-regulation of MMP-2 and talin-2 in mRNA level. Also, miRNA expression was significantly up-regulated in combination setting. Increased expression level of miR-194, miR-30 and miR-126b supported useful combinatory effect of DHA, LA and etaxel in inhibition of metastasis.

**Keywords:** Gastric cancer, metastasis, miRNAs, DHA, LA, etaxel.

# Computational Immunology and Systems Biology

# Poster Presentation

ICIA2018\Computational Immunology and Systems Biology\Poster\5190
**5190**

# Application of *In Silico* Approaches in the Development of New Drugs Against Infectious Diseases

Garshasb Rigi[1], Ibrahim Toktaz[2]

1. *Department of Genetics, Faculty of Basic Sciences, University of Shahrekord, Shahrekord, Iran*
2. *National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran*

**Background**: The extension of computational approaches and their usage for the bioinformatics investigation of immunity has prepared new insight into the engineering, variety, and progress of immune repertoires.
**Methods**: In this study we used libraries for ligand screenings were obtained a subset from drug like category from ZINC database. AutoDock vina software was used as the virtual screening software. Top successive hits were then analyzed regarding attraction, dispensation, metabolism, and expulsion confidants by FAFDrugs3 webserver and ADME and toxicity analysis. Moreover probable accessorial human protein target of successive hits and oral toxicity was checked with PROTOX web server.
**Results**: Four new anti-Ebola drugs were introduced by inhibition of VP40 proteins, also four chemical glycoprotein1 (GP1) inhibitors which theoretically prevented Ebola virus entrance were found.
**Conclusion:** We presented a ligand for inhibition of sortaseA (srtA) and two compounds against Listeriolysine-O of *Listeria monocytogenes* using molecular docking based screening.

**Keywords:** Computational Immunology, Drug Design, Anti Infectious Diseases

ICIA2018\Computational Immunology and Systems Biology\Poster\6322
**6322**

# Cloning, expression and purification of humanized format of anti-VEGF single domain antibody

Nastaran Mohseni[1], Fatemeh Kazemi Lomedasht[1*]

*1. Venom &Biotherapeutics Molecules Laboratory of Biotechnology Research Center of Pasteur Institute of Iran, Tehran, Iran*

**Background:** Over expression of Vascular Endothelial Growth Factor in tumor cells angiogenesis has been proven. Therefore, blocking and inhibiting of its function by monoclonal antibodies could be effective way to inhibit angiogenesis. Nanobodies have very high binding affinity and specificity to their target. In addition, nanobodies are able to detect hidden antigen efficiently. The camel origin of nanobodies concerns their use in human therapy. Humanization is effective way to reduce immunogenicity problems.
**Methods:** Here, We used *in silico* approach to design humanized format of nanobody. The 3-Dstructure of camel and humanized nanobody predicted by I-TASSER. The affinity, specificity and bioactivity of both nanobodies were evaluated.
**Results:** Our *in silico* results showed that both nanobodies had similar structures. The significant differences between camel and humanized nanobody was not observed.
**Conclusion:** All in all, the humanized Nanobody could be developed as promising candidate for use in human therapy to target pathologic angiogenesis.

**Keywords:** Nanobody, Antibody engineering, Humanization.

ICIA2018\Computational Immunology and Systems Biology\Poster\6327
**6327**

# Analysis of transcription factors involved in responses of plasmacytoid dendritic cells to influenza virus

Fatemeh Hadi[1,2], Rasoul Godini[1], Hossein Fallahi[1]

*1. Department of Biology, Faculty of Sciences, Razi University, Kermanshah, Iran*
*2. Department of Stem Cells and Developmental Biology, Brain and Cognitive Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran*

**Background:** Flu, caused by several types of influenza virus, is a contagious disease of respiratory organs which infects millions of people worldwide yearly. Plasmacytoid dendritic cells are from defense lines protecting lung against viral infections, including influenza. These cells stimulate immune responses by secretion of substantial concentrations of interferon-α. Herein, to reveal regulatory network governing their defense mechanisms, we analyzed transcriptome profile of plasmacytoid dendritic cells responding to influenza.
**Methods:** To do this study, we used freely available microarray dataset, GSE68849. Differentially Expressed Genes (DEGs) were determined using GEO2R tool of NCBI. We detected TFs governing the DEGs by ChEA, a database for protein-DNA interactions. We also determined all experimentally validated human TFs using TFchekpoint database. Protein-protein interactions (from STRING database) and network analysis were applied for constructing core regulatory network and detecting hub TFs. DAVID database was utilized to reveal biological roles of the TFs.
**Results:** Seeking for all possible TFs, we found 75 DE-TFs in 1127 DEGs, 27 down-regulated and 28 up-regulated. However, ChEA database determined statistically significant protein-DNA interactions for only 11 TFs which based target genes FLI1, SPI1, ATF3, and KLF6 are from most important TFs. By constructing core regulatory network, we determined the TFs, such as JUN, FLI1, and RUNX1, controlling most number of the other TFs. On the other hand, annotation of the TFs showed that immune and inflammatory responses are included for some TFs.
**Conclusion:** Using protein-DNA and protein-protein interactions and rigorous statistical analysis, present study revealed critical TFs involved in plasmacytoid dendritic cells responding to influenza virus. The results can help to a better understanding of defense system against viral infections and might assist to find better way for treatment.

**Keywords:** Influenza, Network, Plasmacytoid dendritic cells, Transcription factors

ICIA2018\Computational Immunology and Systems Biology\Poster\7560
**7560**

**Prediction and Analysis of Antigenic Determinants In HLA-G Molecule**

Motahareh Ehsani[1], Mehdi Shahbazi[1], Mousa Mohammadnia afrrouzi[1]

*1. Department of Immunology, School of Medicine, Babol University of Medical science, Babol, Iran.*

**Background**: Antigenic determinant is an important structural part of an antigen molecule that is also highly accessible to solvent. Generally when an antigen is recognized by immune cells including dendritic cell, its antigenic determinants are processed and presented to T lymphocyte which finally initiates immune response. In this study, Antigenic properties of HLA-G were predicted and the assessment of motifs in the aspect of antigenic potential was done.
**Methods:** using Predicting Antigenic Peptides (http://imed.med.ucm.es/Tools/antigenic.pl), the antigenic properties of HLA-G molecule were assessed and antigenic epitopes were determined. Consequently these epitopes could induce antibody response.
**Results:** The sequence of HLA-G with 414 amino acids has 17 antigenic determinants. According to an antigenic plot, the average antigenic propensity of this molecule was 1.0130 and only a small sequence in the N-terminal region of HLA-G showed a high average antigenic propensity.
**Conclusion:** As abnormal expression of HLA-G on normal renal cells is representative of renal cell carcinoma (RCC), Predicting and analyzing of its antigenic properties could open a way for designing of monoclonal antibodies in order to promote immunotherapy in the field of cancer .

**Key words**: HLA-G, Dendritic cell, T lymphocyte, Antigenic determinant

ICIA2018\Computational Immunology and Systems Biology\Poster\7590
**7590**

## *In silco* study of miRNA involved in TGF-β pathway to prevent tissue fibrosis

Faride Sadeghkhani[1], Ehsan Arefian[2]

1. *Department of Bioengineering, School of Science and Technology, University of Tehran.*
2. *Molecular virology lab, Department of Microbiology, School of Biology, College of Science, University of Tehran*

**Background:** Transforming growth factor-beta (TGF-β) isoforms are one of the most important and well known multifunctional cytokines which play the main role in wound healing and tissue repairing. It is produced by most of the parenchymal cell types but it also released by infiltrating cells such as lymphocytes, monocytes/ macrophages, and platelets after wounding or inflammation. Increased TGF-β can lead to restore normal tissue architecture or may cause tissue fibrosis. Progressive fibrosis in organs like liver, kidney, lung or heart can lead to organs dysfunction and even death. Skin fibrosis caused Scleroderma and other disorders. So development in therapeutic intervention which could involve in this pathway is required to downstream fibrosis-specific genes regulation. One of the most important strategies is study and use miRNAs that have been specialized for regulating this pathway during evolution. In this study, *in silico* methods have been used to find the best interfering miRNAs.
**Methods:** For bioinformatics study, "KEGG"algorithms were used to identify proteins involved in TGF-β pathway including SMAD and then "Target Scan" data help us find miRNAs which have been involved in the TGF-β pathway and sort them in a score table. The most frequent and best ranking miRNAs in score table were selected.
**Results:** according to the score table, *hsa-miR-3613-3p(32times)* and *hsa-miR-4668-5p (14times)* have the most ability to inhibit this pathway.
**Conclusion:** In order to inhibit the progressive fibrosis, current study shows *hsa-miR-3613-3p* and *hsa-miR-4668-5p* could inhibit the pathway with the best total score and highest chance.

**Keywords:** TGF-β pathway, fibrosis, *In silico* study,Target scan.

ICIA2018\Computational Immunology and Systems Biology\Poster\7638
**7638**

# Immuno-informatics basedapproaches to design a novel multi epitope-based vaccine for immune response reinforcement against Leptospirosis

Ahmad Karkhah[1], Majid Validi[2], Hamid Reza Nouri[3]

*1. Student Research Committee, School of Medicine, Babol University of Medical Sciences, Babol, I.R. Iran*
*2. Clinical Biochemistry Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, I.R. Iran*
*3. Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, I.R. Iran*

**Background:** Leptospirosis is known as a zoonotic disease of global importance originated from infection with the spirochete bacterium leptospira. Although several leptospirosis vaccines have been tested, the vaccination is relatively unsuccessful in clinical application despite decades of research. Therefore, this study was conducted to construct a novel multi-epitope based vaccine against leptospirosis by using Hap1, LigA, LAg42, sphH and HSP58 antigens.
**Methods:** T cell and IFN gamma epitopes were predicted from these antigens. In addition, to induce strong CD4+ helper T lymphocytes (HTLs) responses, Pan HLA DR-binding epitope (PADRE) and helper epitopes selected from Tetanus toxin fragment C (TTFrC) were applied. Moreover, for boosting immune response, Heparin-Binding Hemagglutinin (HBHA), a novel TLR4 agonist was added to the construct as an adjuvant. Finally, selected epitopes were linked together using EAAAK, GPGPG, AAY and HEYGAEALERAG linkers.
**Results:** Based on the predicted epitopes, a multi-epitope vaccine was constructed with 490 amino acids. Physico-chemical properties, secondary and tertiary structures, stability, intrinsic protein disorder, solubility, and allergenicity of this vaccine construct were assessed using immunoinformatics analysis. A soluble, and non-allergic protein with a molecular weight of 53.476 kDa was obtained. Further analyses validated the stability of the chimeric protein and the predicted epitopes in the chimeric vaccine indicated strong potential to induce B-cell and T-cell mediated immune response.
**Conclusion:** Immunoinformatics analysis showed that the modeled multi-epitope vaccine can properly stimulate the both T and B cells immune responses and could potentially be used for prophylactic or therapeutic usages.

**Keywords:** Leptospirosis; Immunoinformatics; Multi-epitope vaccine; Reverse vaccinology.

ICIA2018\Computational Immunology and Systems Biology\Poster\7756
**7756**

# Evaluation of Azacitidine-Incorporated SNA as Targeted Delivery to TSDR of FoxP3 Gene by Bioinformatics Tools

Mrs.Zahra Sasanipour[1], Dr.Maryam Darayee[2]

*1. Department of Nanotechnology, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences*
*2. Department of Molecular medicine, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences*

**Background:** FoxP3 is a transcription factor that is responsible for the evolution of regulatory T-cells (Treg cells) and make it to perform better. Treg-specific demethylated region (TSDR) is a highly conserve locus on the FoxP3 gene that is fully demethylated in natural Tregs. Azacitidine is a chemical drug that inhibits DNA methylation. Naturally, the fetus is an external body and must be rejected by the mother's immune system but during the pregnancy, the expression level of this factor is increased in these cells that resulted in fetus keepin.
**Methods:** GC percent of this factor in promotor region was investigated by Endmemo and Sciencebuddies datasets. An azacitidine-incorporated SNA (spherical nucleic acid) was designed through AutoDock to bind to the TSDR of FoxP3 gene specifically. Specific binding of SNA to the TSDR was simulated by PyMOL software.
**Results:** Bioinformatics analysis indicated that TSDR was GC rich (67.5 %). Furthermore, azacitidine incorporation to the SNA particles was possible and this particle could bind to TSDR through targeted delivery.
**Conclusion:** Because of high percent of GC in TSDR, this region is susceptible to methylation and gene suppression and consequently the number of Treg cells should be decreases in *pregnant person's body* and caused to the fetus rejection and abortion as an external body. Therefore through targeted delivery of azacitidine to this region, demethylation would be occurred and lead to prevention of abortion.

**Keywords:** FoxP3, SNA, Azacitidine, TSDR, Drug delivery

ICIA2018\Computational Immunology and Systems Biology\Poster\7918
**7918**

# Designing an *in Silico* chimeric multi epitope-based vaccine targeting LLO, GAPDH and tP60 for robust immune response against Listeria

Maryam Rostamtabar[1], Hamid Reza Nouri [2]

*1. Student Research Committee, School of Medicine, Babol University of Medical Sciences, Babol, I.R. Iran*
*2. Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, I.R. Iran*

**Background:** Listeria monocytogenes as an intracellular pathogen mainly infects pregnant and immunocompromised individuals. Therefore, a subunit vaccine targeting multiple antigens could be considered as an ideal approach for prevention and treatment of listeriosis. Currently, the most effective immunogenic antigens of Listeria are included Listeriolysin-O (LLO), glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) and truncated form of the listerial P60 antigen (tP60).
**Methods:** The $T_{CD8}$+ helper lymphocytes and IFN gamma epitopes were predicted from these antigens. Moreover, Heparin-Binding Hemagglutinin (HBHA), a novel TLR4 agonist was added to the construct as an adjuvant. Then, a multi-epitope chimeric protein was constructed from these dominant epitopes. The chimeric gene structure, its mRNA, and deduced protein were analyzed. Then, the three dimensional (3D) structure was predicted using Modeller. Finally, validation of the predicted protein was assessed by Ramachandran plot statistics.
**Results:** Based on the predicted epitopes, a chimeric construct with 356 amino acids length was prepared by applying GPGPG linker. Physico-chemical properties, stability, intrinsic protein disorder, secondary and tertiary structures, of this construct were assessed. A soluble and non-allergic protein with a molecular weight of 31.4 kDa was obtained. The predicted 3D structure of the chimeric protein showed that most of the dominant epitopes were folded individually. The validation experiment showed that more than 88% residues of chimeric protein are located in favorable regions of the Ramachandran plot. Further analyses indicated T cell epitopes have enough potency for MHC binding and strong potential to induce the T-cell mediated immune response.
**Conclusion:** Immunoinformatics analysis showed that the modeled chimeric vaccine can properly stimulate the $CD4^+$ and $CD8^+$immune responses and could potentially be used for prophylactic or therapeutic usages.

**Keywords:** Immunoinformatics, Listeriosis, Listeriolysin-O, Multi-epitope vaccine.

ICIA2018\Computational Immunology and Systems Biology\Poster\8401
**8401**

## *In silico* determination of superficial clefts in antigen binding fragment of human IgG

Fatemeh Hajighasemi *[1], SoheilaRohani[1], FatemehSefid[2]

1. *Department of Immunology, Faculty of Medicine, Shahed University, Tehran, Iran*
2. *Department of Biology, Faculty of Basic Science, Shahed University, Tehran, Iran*

**Background:** Immunoglobulins (Igs) are a group of plasma proteins consisting of heavy and light chains. Each chain contains variable and constant regions. Idiotypic determinants are epitopes sited on Igs variable region. Private idiotype is a marker exclusive to a singular clone of B cell. As epitopes are commonly positioned close to surface clefts of proteins, recognition of Igs clefts could be very helpful in Igs epitopes determination. In current study superficial clefts in antigen binding fragment of human IgG have been defined by computational immunology.
**Methods**: Amino acid sequence and third structure of reference human IgG were found in PDB databank. The second IgG structure was determined by Phyre 2 software. Surface clefts in IgG antigen binding fragment have been distinguished by Isocleft finder software.
**Results**: Three clefts were recognized by Isocleft finder software. These clefts were located in Fab region of human IgG. First two clefts were in variable and constant domains and third one was in constant domains of Fab region. First, second and third clefts contained 93, 60 and 32 amino acids, respectly. The biggest and deepest cleft was in amino terminal (antigen binding site) of IgG molecule.
**Conclusion**: In present study three clefts in human IgG Fab were identified by computational immunology. The first two clefts contained many amino acids of variable domains. Thus it is very probable that private idiotypes be located in these clefts. So these clefts are useful for private idiotypes determination and generating specific anti-idiotypic monoclonal antibodies to monitor/ target clonally expanded malignant B cells.

**Keywords:** *In silico*, Human IgG, Clefts

ICIA2018\Computational Immunology and Systems Biology\Poster\8423
**8423**

**Assessment of flexible regions in human IgG light chainsby computational analysis**

Fatemeh Hajighasemi *[1], Soheyla Rohani[1], Fatemeh Sefid[2]

1. *Department of Immunology, Faculty of Medicine, Shahed University, Tehran, Iran*
2. *Department of Biology, Faculty of Basic Science, Shahed University, Tehran, Iran*

**Background:** Immunoglobulins (Igs) are glycoproteins produced by plasma cells, play an essential role in protecting the body against infections and destroying them. Immunoglobulin G (IgG) has an important role in defense against pathogens. The serum IgG level differs in several diseases such as infections and immunodeficiencies. Thus IgG has a very high diagnostic importance. For exact measurement of IgG, we need subtle diagnostic tools such as anti IgG- epitope specific monoclonal antibodies (MAbs). Immunogenic epitopes are beneficial for generating very proficient MAbs. More flexible regions in a molecule have more immunogenicity. Computational immunology helps in well identification of immunogenic epitopes through definition of their physiochemical properties such as flexibility. The aim of this study is evaluation of human IgG light chains flexibilityby computational immunology.
**Methods:** The amino acid sequence and third construction of reference human IgG was obtained in PDB database. The second IgG structure was specified by Phyre 2 software. IgG light chain flexible regions were distinguished by IEDB software.
**Results:** The greatest flexible positions were situated in 150 – 160 and in 165-170 amino acid sequences of IgG light chains as was determined by IEDB software.
**Conclusion:** According to results of this study the amino acid sequences located in 150 – 160 and in 165-170 positions which are placed in constant domain of human IgG light chains, organize the most flexible sites and hence are very useful tools for recognition of more immunogenic epitopes to producing greatly sensitive and specific anti- IgG MAbs.

**Keywords:** Human IgG, computational analysis, flexibility

ICIA2018\Computational Immunology and Systems Biology\Poster\8686
**8686**

# Evaluation of operational amino acids in human immunoglobulin G light chain by computational Interpro surf software

Fatemeh Hajighasemi *[1], Soheila Rohani[1], Fatemeh Sefid[2]

1. *Department of Immunology, Faculty of Medicine, Shahed University, Tehran, Iran*
2. *Department of Biology, Faculty of Basic Science, Shahed University, Tehran, Iran*

**Background:** Immunoglobulins (Igs) are bifunctional serum glycoproteins protect the body against foreign substances. Igs consist of light and heavy chains. Human immunoglobulin G light chain involves variable (VL) and constant (CL) domains. The variable domain takes part in antigen recognition and the constant domain influence on the antigen binding site conformation. Operational amino acids have a key role in function of proteins. Computational biology softwares are very useful in solving immunologic problems and well understanding of immune cells responses. Recognition of IgG operational amino acids would be useful in determination of vital amino acids in IgG defensive activities. In this study the human IgG light chain functional amino acids were identified by computational Interpro surf software.
**Methods: A**mino acid sequence and third structure of reference human IgG were attained from PDB database. Second IgG structure was determined via Phyre 2 software. Human IgG light chain operational amino acids were defined using Interpro surf software which is available in http://curie.utmb.edu/prosurf.html database.
**Results:** According to our data **the** operational amino acids of human IgG light chains are generally situated in 1-111 amino acids sequence exist in VL domain.
**Conclusion:** Our results showed that human IgG light chains functional amino acids are mostly situated in VL domain. This is consistent with well-known amino terminal function of IgG (antigen binding). **These** functional amino acids would be helpful in recognition of IgG idiotypic determinants which are located in its variable regions as well as detection of some IgG dysfunction and related immune disorders.

**Key words:** IgG, computational software, operational, amino acids

ICIA2018\Computational Immunology and Systems Biology\Poster\9752
**9752**

## *In silico* analysis of FoxP3's isoforms and its effects

Maryam Darayee[1,2], Amir Rahimi[1,2]

*1. Bioinformatics and Computational Biology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
*2. Department of Molecular Medicine, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** FoxP3 is a transcription factor responsible for the evolution and performance of regulatory T cells (Treg) which have pivotal role in the maintenance of homeostasis. T helper 17 cells (Th17) are a subset of pro-inflammatory T helper cells which related to T regulatory cells and the signals that cause Th١٧s to differentiate actually inhibit Treg differentiation. According to NCBI-Gene database, in human FoxP3 has two different isoforms. One isoform comprises 431 amino acids and the other has 396 amino acids which the smaller form lacking exon 2.
**Methods**: Protein sequencing and related analysis were collected by uniprot database. Pairwise sequence alignment of Foxp3 isoforms was done and compared with each other and the interactions between FoxP3 and other proteins was investigated in STRING database.
**Results:** Collected data from STRING data base showed that FoxP3 interacts with ten proteins including CTLA-4, jun, IL-17A, IL-2RA, NFATC2, IL-2, IL-6, IL-10, TGF-β, RORγt and RORγt among them binds to the second exon of full-length FoxP3. Protein sequence alignment showed that amino acids from 71 to 106 of the full-length (431 amino acids) were excluded in the shorter isoform (396 amino acids).
**Conclusion:** As the biological information revealed, the missed region in shorter isoform is located on the repressor domain of FoxP3 protein and attaches to transcription factors (such as RORγT) that are responsible for T-cells differentiation to Th17 and suppresses them. This occurrences resulted in inflammatory condition and may induce allergy or autoimmune diseases.

**Keywords:** FoxP3 isoforms, Th17, RORγt, *in silico* study

ICIA2018\Computational Immunology and Systems Biology\Poster\9760
**9760**

# An *in silico* assay of the structure and function of HA22 anti-cancer drug

Nadia Manavi[1], Bahareh Hasani Sabzevar[2*], Nazanin Gholampour Faroji[2], Aliakbar Haddad-Mashadrizeh[3], Behrouz Jalali Ghassam[1]

*1. Pharmaceutical Research Group, Khayyam Bioeconomy Institute, Mashhad, Iran*
*2. Structural biology and Bioinformatics Research Group, Khayyam Bioeconomy Institute, Mashhad, Iran*
*3. Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran*

**Background:** The high incidence of lymphoma and associated mortality among societies has led to the development of novel diagnostic and therapeutic methods. In this regard, In 1997 the BL22 was introduced as an immunotoxin drug to treat this type of patients, and in 2002, its optimization resulted in the production of a new form called HA22. Accordingly, structural modeling and functionality assay of this drug in quasi-physiology situation could provide approaches for more optimization which are considered in this study.
**Method:** NCBI, Uniprot, Protein Atlas and RCSB databases were used to retrieve desired protein sequences and structures of drug as well as the amount and hosting tissues which express antigen. Modeller software was used for modeling and assembling the fragment sequences of drug via homology modeling method. ERRAT, Verify 3D and RAMPAGE programs were employed to determine the quality of 3D structures of protein. On the other hand, GROMACS, HADDOCK and IEDB were utilized to assay the structural stability, functionality features and immunogenicity properties of drug, respectively. The PYMOL software was used to visualize structures.
**Result:** Assessment of the primary structure of the HA22 immunotoxin revealed the presence of RFB4 monoclonal antibodies with the ability to target CD22 antigen. Meanwhile, truncated part of *Pseudomonas* exotoxin was revealed in its context. On the other hand, the fragments assembled has led to the production of 7 models with various quality. The best model from a sustainability perspective, showed desirable curves of RMSD and RMSF after being placed in a quasi-physiological condition. However, several epitopic regions were revealed in the structure of the drug which indicating the irritation of the immune response after injection.
**Conclusion:** Totally, the results of this study provide a model of HA22 immunotoxin with stability structure in quasi-physiological condition, which in turn could lead to introduction of a novel isoform of drug, after immunogenicity optimization, which are considered in group.

**Keywords:** lymphoma, BL22, HA22, *In-silico* biology

ICIA2018\Computational Immunology and Systems Biology\Poster\9804
**9804**

**Production of cytokinesuper agonist by mutagenesis (growth hormone as an example)**

Shima Bahador, Sayyed Hamid Zarkesh Esfahani, Rahman Emamzadeh, Samira Mirbaha

*Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran*

**Background:** Human growth hormone (hGH) is a single-chain peptide containing 191 amino acids. Structurally it belongs to class one cytokines. Cytokines are now widely used as modulators of the immune systems and for therapeutic purposes. High does multiple injections are usually needed to achieve and maintain the therapeutic plasma levels of cytokines which incur discomfort and high cost. Long acting cytokine super agonists which need less dose and less administration intervals are highly desirable. The aim of this study was to produce a cytokine super agonist through genetic manipulation. Human growth hormone molecules was used as a model because of its simple and well known cytokine structure and high market value.
**Materials and methods:** Molecule structure and binding site of the growth hormone and its receptor (GHR) and amino acids involved were analyzed using available soft-wares. Eight amino acids in site1of binding site were targeted for replacement. The new construct was designed and synthesized and then the PCR product of mutated gene was cloned into pCold expression plasmid and was transfected in *E. coli*. The mutant rhGH was produced in *E. coli* and purified using its His-tag by Nickel column. The expression and properties of the mutant protein was analyzed using ELISA, SDS page, dot and western blotting techniques and its final concentration was determined by Bradford test and then Biological activity of the mutant rhGH was tested in cell culture.
**Results:** High levels of mutant rhGH were produced using *E.coli* prokaryotic protein production system. Mutant rhGH showed a much higher biological activity compared to wild GH.
**Conclusion:** The affinity of GH to GHR and its biological activity can be increased by introducing appropriate mutations in the GH molecule. This approach can be utilized to make other cytokine super agonists.

**Keywords:** growth hormone, agonist, cytokine, mutation

# Diagnostic Methods in Immunology

# Poster Presentation

ICIA2018\Diagnostic Methods in Immunology\Poster\11085
**11085**

# The Changes of Hepatic Leptin, Resistance, and Adiponectin in Streptozotocin-Induced Diabetic Rats at Translational and Transcriptional Levels.

Hussam Al-Humadi[1*], Rafal Al-Saigh[2], Ahmed Al-Humadi[3]

1. *Pharmacology and Toxicology department, Pharmacy College, University of Babylon, Babylon, Iraq*
2. *Clinical Laboratory sciences department, Pharmacy College, University of Babylon, Babylon, Iraq*
3. *Department of Pharmacology, Medical School, National and Kapodistrian University of Athens, Athens, Greece*

**Background:** Liver is considered as a one of endocrine organs that secrete a variety of metabolically important substances including cytokines and adipokines which may represent a link between the insulin resistance and diabetes (DM). This study was to investigate hepatic leptin (LP), adiponectin (AD), and resistin (RS) in streptozotocin (STZ)-induced DM rats with the immunohistochemical (IHC) expression and real-time polymerase chain reaction (PCR).
**Methods:** Sixteen albino wister male rats with DM induced by single STZ administrated through intra peritoneal injection compared with control group for 30-days duration of experiment and assessing the changes in weight, insulin level (IN), blood sugar (BS), in addition to IHC and gene expression (GEX) of hepatic LP, AD, and RS; (N=8) for each group.
**Results:** There was a non-significant increase in weight with significant increase in BS levels of DM group ($P<0.001$) with a non-significant decrease in IN; serum liver enzymes were demonstrated a significant increase in DM group ($P<0.05$) except in ALT. Non-HDL lipid profile assay (TC, TG and LDL) revealed a significant increase ($P<0.05$) while HDL revealed no significant changes in comparison with control. Hepatic LP and RS by IHCs were significantly increased in DM group ($P<0.05$ vs. control) while LP and GEXs correlated and RS GEX was not vs. control. Although IHC expression of hepatic AD was significantly decreased in DM group ($P<0.05$) with high GEXs correlation.
**Conclusions:** The significant increase in hepatic IHC expression of LP and RS and decreased in AD level with highly correlation with their gene expression in STZ induced DM are augmented the features of insulin resistance with progressive dyslipidemia and abnormal liver function tests. These adipokines have very important role in drawing the scenario of diabetic pathological process and complication and ultimately in choosing an appropriate pharmacological and therapeutic approach.

**Keywords:** Adipocytokines, Leptin, Resistin, Adiponectin, Streptozotocin-Induced Diabetic Rats

ICIA2018\Diagnostic Methods in Immunology\Poster\11085
**7612**

**Ethics in the Laboratory of Immunology**

Tajaddin akbarzadeh khiavi

*Ardabil University of Medical Sciences*

**Background:** With the increasing number and variety of laboratory tests in recent years, the role of the immunology lab in identifying diseases is very important. Accuracy and use of modern methods of the world, as well as attention to the maintenance of rights, respect for lending and human dignity, are among the priorities that have contributed to increasing the satisfaction of clients and the characteristics of an optimal laboratory. In the Laboratory of Immunology, medical ethics is an absolute principle and professional ethics and commitment to the laboratory as expertise are influential. Undoubtedly, scientific advancement and professional ethics lead to the growth and improvement of the treatment process and the prevention of disease and the health of families and society. Morality is like a specialty with a variety of levels that can be used to provide better laboratory services in more effective ways. 1) Pre-test ethics include: patient's well-being and satisfaction, non-discrimination between patients, correct collection and standardization of the sample, and obtaining information and identifying patients. 2) Ethics in carrying out the test: All tests should be based on standard and professional skills and qualifications, and qualitative methods should not be reported in place of quantitative methods. 3) Post-test ethics: The most important principle in the immunology lab is the confidentiality of test results, accurate reporting of results, and the keeping of records and documentation of tests.

**Keywords**: Laboratory, Immunology, Ethics

# Immune Cell Therapy

# Poster Presentation

ICIA2018\Immune Cell Therapy\Poster\7483
**7483**

# The Effect of Lipopolysaccharide on the Expression Level of Immunomodulatory and Immunostimulatory Factors of Human Amniotic Epithelial Cells

Hossein Motedayyen[1], Somayeh Ghotloo[2], Mohsen Masjedi[1], Nariman Mosaffa[3], Abbas Mirshafiey[4], Mahmood Saffari[2*]

**Background**: Human amniotic epithelial cells (hAECs) are a novel source of stem cells and have immunomodulatory effects on both the innate and adoptive immune systems. hAECs can differentiate into multiple cell lineages that make them a suitable cell source for regenerative medicine. These cells express multiple toll-like receptors (TLRs) and respond to various TLR ligands. This study aimed to evaluate the effect of lipopolysaccharide (LPS), a TLR4 ligand, on the level of immunomodulatory and immunostimulatory factors of hAECs.
**Methods:** hAECs were isolated from term placentas obtained from 10 healthy women during uncomplicated elective cesarean deliveries. hAECs were cultured in the presence and absence of LPS (5 µg/ml) at 37 °C with 5% $CO_2$. After 6 hours of incubation, the cells were collected and RNA extraction and cDNA synthesis were performed. Afterwards, the expression level of protagelandin E2 synthase, transforming growth factor-beta1 (TGF-β1), interleukin-10 (IL-10), interleukin-1beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in hAECs were analyzed by q-PCR.
**Results:** Our results indicated that LPS had the ability to up-regulate the expression of protagelandin E2 synthase and TGF-β1 in hAECs. However, there was no change in the level of IL-1β, IL-6 and IL-10 in hAECs when were stimulated with LPS. In addition, we observed TNF-α was only expressed at very low level in some of hAECs samples which its expression was independent of the effects of LPS.
**Conclusion:** These findings suggest that LPS may enhance the immnunomodulatory effects of hAECs through up-regulating immunosuppressive factors and down-regulating pro-inflammatory cytokines in hAECs that may represent an advantageous cell source with potential applications for immunotherapy of disease with immune pathophysiology.

**Keywords:** Human amniotic epithelial cells (hAECs), Immunomodulatory effect, Toll-like receptors (TLRs), Lipopolysaccharide, Regenerative medicine.

ICIA2018\Immune Cell Therapy\Poster\8057
**8057**

## Effect of STAT3 Specific Inhibition on Dendritic Cells Maturation and Function

Jahangiri A.H., Eskandaryan M., Yaftiyan A., Moazzeni S. M.

*Immunology Department, Faculty of Medical Sciences Tarbiat Modares University, Tehran, Iran*

**Background:** Dendritic cells (DCs) are the most potent antigen-presenting cells of the immune systems with apparent influences on outcome of innate and adaptive immune response. Signal transducer and activator of transcription-3 (STAT3) is a transcription factor that modulates the expression of some important genes involved in the immunostimulatory functions of DCs. STAT3 signaling is also essential for NF-κB mediated IL-12 expression. In this study, a small molecule (S31-201) was used as the specific inhibitor of STAT3 signaling to investigate its effects on dendritic cells differentiation and function.
**Methods:** DCs were differentiated from mouse bone marrow (BM) cells in the presence of DCs differentiation cytokines, GM-CSF and IL-4 and their maturation was induced using the LPS as DCs maturation factor. S31-201 was added to select DCs cultures during the maturation period. The DCs maturation state was assessed by the expression of MHCII, CD40 and CD86. The expression of IL-10 and IL-12 cytokines genes were also determined by RT-PCR. The allognic T cell response induction potency of S31-201 treated and non-treated DCs was measured using the MLR assay.
**Results:** Specific inhibition of STAT3, increased the expression of DCs maturation markers. Increment T-cell stimulation was observed in MLR assay using the S31-201 treated DCs. We also observed increase in IL-12 and decrease in IL-10 production after STAT3 inhibition.
**Conclusion:** Our results suggest that specific inhibition of STAT3 cases a clear change in DCs maturation state and produces cells with profound mature DCs phenotypic and functional characteristics which could be useful in DC based vaccination of tumors and infectious diseases.

**Keywords:** Dendritic cells, STAT, Cell signaling, small molecules

ICIA2018\Immune Cell Therapy\Poster\9769
**9769**

**Mycoplasma Contamination in cell based investigation: avoidance, Detection and Eradication**

Hedieh Rahmati[1], Meysam Ganjibakhsh[1], Parvaneh Farzaneh[1], Masoumeh Asadi[1], Zahra Elyasi Gorji[1], Seyed Abolhassan Shahzadeh Fazeli[1, 2]

*1. Human and Animal Cell Bank, Iranian Biological Resource Center (IBRC), ACECR and Tehran, Iran*
*2. Department of Molecular and Cellular Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran*

**Background:** Contaminations in primary cell culture can make so many problems in cell culture. Therefore, an appropriate method for removal of tissue contamination is needed. It is obvious that Mycoplasma contamination causes genetic and epigenetic changes and also disturbances in metabolism pathway of cells. So, prevention and detection of mycoplasma contamination is an important issue in cell culture study.
**Methods:** The tissue specimen obtained and transported under sterile conditions. In this method, the contamination from the tissues (human placenta, chorionic, amnion and human oral cancer) was removed by washing the tissue using the PBS in several times. After that, tissues were immersed in antibiotics including 200 µg/ml Penicillin, 200 µg/ ml Streptomycin, 100 µg/ ml Gentamicin, 100 µg/ ml Ciprofloxacin and 100 µg/ ml Amphotericin B at 4 °C for 1 hour in sterile conditions. Next, the cells were isolated by enzymatic digestion and cultured using ciprofloxacin 100 µg/ ml for 3 weeks. After that, the antibiotic was removed and these cells were analyzed for mycoplasma contamination by DNA staining, PCR and microbial direct culture.
**Results:** Our results showed 69% of oral cancer tissues and 50% of embryonic membranes that transported to lab had mycoplasma contamination. The results obtained from quality control laboratory showed that utilization of this method helped us to decrease the tissue contamination in less than 10 %.
**Conclusion:** Employing proper aseptic technique in laboratory, and occasionally utilization of antibiotic treatments can lead to save valuable cell lines which it should not be lose. Therefore, assessment of the contamination rate before employing of the cells is a practical technique to preserve costly cells.

**Key word:** Contamination, Cell culture, Mycoplasma

ICIA2018\Immune Cell Therapy\Poster\9729
**9729**

# The Effect of Silymarin on the Expression of Chemokine Receptors in T Helper 1 (Th1) Cells

Mohadeseh Toghyani Khorasgani[1]

*1. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background:** Silymarin, a polyphenolic flavonoid derived from milk thistle (Silybum marianum), is known to have anti-inflammatory, hepatoprotective, and anticarcinogenic effects. The aim of this study was to investigate the effect of silymarin on the chemokine receptor of Th1 (T helper 1) cells.
**Methods:** Peripheral blood mononuclear cells (PBMCs) from 8 healthy individuals were activated with Conconavalin 'A' and treated with silymarin (100 µM) or dimethyl sulfoxide (DMSO), as negative control, in a standard condition (RT: 37 and CO2: 5%). Cells were incubated (72 hours) and then examined for the flowcytometric evaluation of chemokine receptor CXCR3 expression. Peripheral blood lymphocyte subpopulations were identified and evaluated by two-color flowcytometric analysis. A non parametric paired samples Wilcoxon test was applied to compare the grouped data. Results were expressed as the mean ± standard deviation (SD). P-values < 0·05 were considered to indicate significant differences.
**Results:** Silymarin increased the expression of CXCR3 on Th1 cells. These results were significant (P = 0.017) in all samples.
**Conclusion:** This study provided evidence of effectiveness for silymarin on the expression of CXCR3. Therefore, in future, silymarin, regarding its lower side effects, could be used instead of other drugs such as immunosuppressive ones in autoimmune diseases.

**Keywords:** Silymarin, Chemokine receptor, T helper cells, Flow cytometry

# Immunogenetics

# Poster Presentation

ICIA2018\Immunogenetics\Poster\5197
**5197**

# Evaluation of the Situation and Function of Immunoglobulin Binding Domains from Full length and Truncated Forms of Protein A

Garshasb Rigi[1], Gholamreza Ahmadian[2]

1. *Department of Genetics, Faculty of Basic Sciences, University of Shahrekord, Shahrekord, Iran*
2. *Department of Industrial and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran*

**Background**: The Staphylococcal protein A (SpA) is widely used in biotechnology to purify polyclonal and monoclonal IgG antibodies. It is also as a very important protein in medicine. The most recent use of protein A is the application of this protein as a drug against autoimmune diseases especially, Rheumatoid Arthritis (RA) and Idiopathic Thrombocytopenic Purpura (ITP).
**Methods**: During our study, recombinant full-length and truncated forms of this protein were expressed in *Escherichia coli*. The bioinformatics review of the http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi and http://www.wwpdb.org databases. Molecular modeling using the I-TASSER software was done. The function of these two forms of protein A was studied using ELISA.
**Results**: *In silico* analyses confirms that protein A is a 42-56 kDa protein containing five homologous domains with about 58 amino acids for binding to immunoglobulin. The Immunoglobulin binding domains are displayed from N terminal of the protein, with the letters E, D, A, B and C. The results of molecular modeling on Protein A represented the IgG-connected domains and the alpha-helix and beta-sheet secondary structures. The ELIZA test showed a higher activity and ability for the truncated form for binding to IgG, compared to the full-length protein. Probably, in the truncated form, due to its proper folding and the lack of extra and hydrophobic regions and the lack of incorrect accumulation, the IgG binding domains are more likely to interact with IgG and thus exhibit more activity.

**Keywords:** Immunoglobulin Binding Domains, Protein A, Autoimmune Diseases

ICIA2018\Immunogenetics\Poster\7368
**7368**

# rs1883025 and rs2230806 Polymorphisms of ABCA1 Gene are Associated with Myocardial Infarction in Southern Iranians

Hamed Fouladseresht[1], Sahar Karimzadeh[1], Shahdad Khosropanah[2], Mehrnoosh Doroudchi[1]

1. *Dept. of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Cardiovascular Research Center, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Coronary artery disease (CAD) is a common disease and a major cause of mortality and morbidity worldwide. Accumulation of lipids in macrophages to become foam cells is a preliminary step in CAD development. ATP-binding cassette protein A1 (ABCA1) is responsible for lipid and cholesterol efflux to extracellular environment, thereby decreasing foam cell formation. The purpose of our study was to investigate the association between ABCA1 genes intronic +1594G/A (rs1803025) and exonic +656G/A (rs2230806) polymorphisms with Myocardial Infarction (MI).
**Methods:** 282 MI patients and 266 healthy blood donors were included in this case-control study. The DNA extracted and the genotype of rs1883025 and rs2230806 SNPs were determined by restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR). The analysis of genetic data was performed using the SPSS19.0 software.
**Results:** We found a significant association between MI and different genotypes of ABCA1 gene. In patients with MI, the frequencies of AA genotype and A allele of rs1883025 were significantly higher as compared to healthy controls (AA vs. GG: OR=2.44, 95%CI=1.146-5.196, p=0.018; A vs. G: OR=1.339, 95%CI=1.028-1.745, p=0.03). Also, the frequencies of GG genotype and G allele of rs2230806 in patients were significantly higher as compared to healthy controls (GG vs. AA: OR=1.865, 95%CI=1.124-3.096, p=0.015; G vs. A: OR=1.329, 95%CI=1.041-1.696, p=0.022).
**Conclusion:** Our study shows for the first time, that intronic polymorphism of ABCA1 gene is associated with Myocardial Infarction. The G allele of the rs1883025 polymorphism is known to result in higher levels of HDL-C in European-American, African-American, American-Indian, Hispanic, Japanese and East-Asia population. Our results also confirms a previous report from Tehran concerning the association of rs1883025 with CAD.

**Keywords:** ABCA1, rs1883025, rs2230806, Myocardial Infarction

ICIA2018\Immunogenetics\Poster\7369
**7369**

# -586G/C and -872C/T Polymorphism of ABCA1 Gene are Associated with Predisposition to Myocardial Infarction in Southern Iranians

Hamed Fouladseresht[1], Kazem Nemati[1], Sahel Khazaee[1], Mohammad Hossein Nikoo[2], Mohammad Javad Zibaeenezhad[2], Mehrnoosh Doroudchi[1]

1. *Dept. of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Cardiovascular Research Center, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** ATP-binding cassette transporter 1 (ABCA1), a member of the ATP-binding cassette family, plays a critical role in the development of atherosclerosis (AS). ABCA1 plays a pivotal role in the reverse cholesterol transport from intra to extra cellular environment. The aim of this study was to evaluate the single nucleotide polymorphisms (SNPs) in ABCA1 gene, [-586G/C (rs1800976) and -872C/T (rs2422493)], in patients with Myocardial Infarction (MI).
**Methods:** Blood samples were collected from 282 individual with MI before receiving medical treatment and 266 age-matched healthy blood donors as a control group. The DNA extracted and the SNPs were determined by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) technique. The analysis of genetic data was performed using the SPSS19.0 software.
**Results:** The rs1800976 and rs2422493 polymorphisms of ABCA1 were statistically associated with increased MI risk in the studied population. In patients with MI, the frequencies of GG genotype and G allele of rs1800976 were significantly higher as compared to healthy controls (GG vs. CC: OR=2.204, 95%CI=1.275-3.809, p=0.005; G vs. C: OR=1.410, 95%CI=1.099-1.809, p=0.007). Also, the frequencies of TT genotype and T allele of rs2422493 were significantly higher as compared to healthy controls (TT vs. TC: OR=1.934, 95%CI=1.217-3.074, p=0.005; T vs. C: OR=1.489, 95%CI=1.009-2.196, p=0.044).
**Conclusion:** This investigation show that G allele of -586G/C position and T allele of -872C/T position of ABCA1 gene are associated with MI in southern Iranian individuals.

**Keywords:** ABCA1**;** rs1800976; rs2422493; Myocardial Infarction

ICIA2018\Immunogenetics\Poster\7383
**7383**

# Decreased levels of Bax and Bcl-2 mRNA Expression in Peripheral Blood from Patients with Multiple Sclerosis

ArezouDabiri[1], Meysam Amidfar[2], Seyed Javad Hasheminia[3], VahidShaygannejad[4]

*1. M.Sc of Immunology, Shahid Sadoughi University of Medical Sciences, International Campus, Yazd, Iran*
*2. Department of neuroscience, school of advanced technologies in medicine, Tehran University of medical sciences, Tehran, Iran*
*3. Department of immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
*4. Isfahan neuroscience research center, Alzahra research institute, Isfahan University of medical sciences, Isfahan, Iran*

**Background:** Apoptosis plays an important role in normal function of immune system and impairments of apoptotic pathways especially Bcl-2 family members could be associated with multiple sclerosis (MS).
**Methods:** In the present study we investigated the mRNA expression of Bax and Bcl-2 in peripheral blood cells of 25 MS patients and 25 control subjects.
**Results:** We found a significant down regulation in the relative gene expression of Bax (p=0.001) and Bcl 2(p=0.02) in white blood cells from MS Patients when compared to healthy individuals. we also observed an increased Bcl-2/Bax expression ratio in peripheral white blood cells from MS patients in compared to healthy subjects but that was not significant(p=0.437).
**Conclusion:** Our data suggest the reduced gene expression patterns of pro-apoptotic Bax and anti-apoptotic Bcl-2 in white blood cells of MS patients as a peripheral marker of MS.

**Keywords:** Multiple sclerosis, Peripheral blood, Gene expression, Bax, Bcl-2

ICIA2018\Immunogenetics\Poster\7395
**7395**

# Association between TRAF3IP2 rs33980500 Polymorphism and Plasma Levels of CXCL1 in Acute Myocardial Infarction

Pordel, S., SajediKhanian, M., Nikoo H., Doroudchi, M.

1. *M.Sc., Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Department of Cardiology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
3. *Karimi, M.H., Associate Professor, Fars Blood Transfusion Center, Shiraz, Iran*
4. *Associate Professor, Cardiovascular Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
5. *Associate Professor, Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** IL-17A and the key adaptor (Act1) in its signaling pathway play important roles in inflammatory responses in Myocardial infarction (MI). CXCL1 chemokine is one of the target genes of the IL-17A signaling pathway. Certain genotypes of rs33980500 from TRAF3IP2, which encodes Act1 adaptor, reinforce the stability of CXCL1 mRNA, which may result in higher production of the protein. We investigated association between plasma levels of CXCL1 and TRAF3IP2-rs33980500 variations in acute myocardial infarction (AMI).
**Methods:** Confirmed AMI patients (n=126) referring to the hospitals affiliated to the Shiraz University of Medical Sciences in a one year period were included in this study. DNA was extracted from PBMCs of venous blood. Control individuals (n=50) were recruited from among healthy blood donors of the same age range and gender. Plasma levels of CXCL1 in patients and controls were measured by ELISA test. AlsoTRAF3IP2-rs33980500variation was identified using PCR-RFLP method.
**Results:** Statistical analysis showed a significant difference in the plasma levels of CXCL1 chemokine between MI patients and healthy controls (P=0.0006). Also we found a significant correlation between plasma contents of CXCL1 and TT genotype of TRAF3IP2-rs33980500 polymorphism in patients (P=0.04).
**Conclusion:** Our results indicated an association of TT genotype of TRAF3IP2-rs33980500 with the plasma levels of CXCL1 in AMI. Alteration of C to T leads to the replacement of Aspartic to Asparagine that increases the activity of TRAF2/TRAF5 pathway which causes augmentation of the stability of CXCL1 mRNA and increase in the plasma levels of CXCL1.

**Keywords:** Myocardial infarction, Single nucleotide polymorphism, TRAF3IP2, CXCL1

ICIA2018\Immunogenetics\Poster\7429
**7429**

**STAT3 Polymorphisms and Acute Kidney Injury After Cardiopulmonary Bypass**

Nadereh Naderi[1, 2], Mahsa Rahimzadeh[3], Sara Aghakhani[3], Mahmoud Khayatian[3], Hossein Montazerghaem[4], Farzaneh Dasturian[3]

1. Department of Immunology, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
2. Food health research center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
3. Department of Biochemistry, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
4. Cardiovascular Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

**Background:** Cardiopulmonary bypass -associated acute kidney injury (CPB -AKI) is clearly linked to increased morbidity and mortality. We explored the association between *inflammation-associated transcription factor,* STAT3, polymorphism and the risk of CPB -AKI.
**Methods:** In this study, STAT3 rs744166 polymorphism were analyzed in 102 patients undergoing coronary artery bypass grafting inJorjani heart center, Bandar Abbas, Iran. The genotypes were determined using sequence-specific primers (PCR-SSP).
**Results:** 39 patients met the criteria for AKI after cardiac surgery (AKI group). The remaining *patients* did not develop AKI (non-AKI group). *Our results did not show any difference* in rs۷۴۴۱۶۶ frequency between AKI and non-AKI groups but there was an association between rs744166 CC genotype and reduced risk of CPB -AKI in older patients (Age≥60).
**Conclusion:** The rs744166 polymorphism is significantly associated with a decreased risk of CPB -AKI in old subjects.

**Key words:** Acute kidney injury; Cardiac surgery; Cardiopulmonary bypass, Polymorphism, Stat3

ICIA2018\Immunogenetics\Poster\7743
**7743**

# Association between Vitamin D Receptor Gene ApaI Polymorphism and Vitamin D Status with Hashimoto's Thyroiditis in Urmia

Amir Hossein Faghfouri[1], Rasoul Zarrin[1], Morteza Bagheri[2], Alireza Mehdizadeh[3], Parvin Ayremlou[4]

1. *Department of Nutrition, School of Medicine, Urmia University of Medical Sciences*
2. *Cellular and Molecular Research center, Faculty of Medicine, Urmia University of Medical Sciences*
3. *Department of Endocrinology, Imam Khomeini Teaching Hospital, Urmia University of Medical Sciences*
4. *Clinical Researches Development Unit of Imam Khomeini Teaching Hospital, Urmia University of Medical Sciences*

**Background:** Hashimoto's Thyroiditis (HT) is one of the most frequent autoimmune diseases cauesd by autoantibodies production against thyroglobulin and thyroid peroxidase resulting in progressive destruction of thyroid gland. Some genetic and environmental backgrounds have been linked to HT. Vitamin D and vitamin D receptor may contribute to the immune system regulation and thereby autoimmune processes in HT. The aim of this study was to examine vitamin D receptor gene ApaI (rs7975232) polymorphism and vitamin D status in HT patients in Urmia population.
**Methods:** A total of 105 patients with HT (16 men and 89 women; mean age=38.7±11.8 years old) and 117 healthy controls (93 men and 24 women; mean age=39.5±10.49 years old) matched by age, sex, vitamin D intakes from foods and supplements and sun-exposure habits were enrolled in this case-control study. ApaI polymorphim were genotyping by polymerase chain reaction and restriction fragment length polymorphism and serum levels of 25-hydroxyvitamin D were measured by electro chemiluminescence immunoassay.
**Results:** The frequencies of AA, AC, CC, A and C genotypes/alleles at ApaI marker were 31.4%, 47.6%, 21% , 55.2% and 44.8% in HT and 35.9%, 41.9%, 22.2%, 55.6% and 44.4% in controls, respectively. Genotype and allelic distribution of ApaI polymorphism were not different in two groups ($P$ = 0.68 and $P$ = 0.95; respectively). Vitamin D status (deficiency= ≤20ng/ml; insufficiency= 20-30 ng/ml; sufficiency= ≥30 ng/ml) did not differ between two groups ($P$=0.06), but vitamin D deficiency were associated with increased risk of HT compared with vitamin D sufficiency ($P$ = 0.02; OR = 2.32 CI 95%, 1.11-4.83). Serum levels of 25-hydroxyvitamin D in patients with "AA" genotype were higher compared with "AC" and "CC" genotype (40.03±15.45 ng/ml vs 30.39±15.43 ng/ml and 35.27±16.53 ng/ml; respectively).
**Conclusion:** Vitamin D status can be associated with HT susceptibility. ApaI may alter 25-hydroxyvitamin D levels in HT patients.

**Keywords:** Hashimoto's Thyroiditis, Vitamin D Receptor,Gene Polymorphism,Vitamin D Status

ICIA2018\Immunogenetics\Poster\7947
**7947**

# Association of Osteopontin Gene Promoter Polymorphism (-66T>G) with Susceptibility to Multiple Sclerosis in Iranian Population.

Fahimeh Dadkhah[1], Fereshteh Alsahebfosoul[2], Nahid Eskandari[3], Mansour Salehi[4], Masoud Etemadifar[5], Mohammad Kazemi[6]

*1. MSc student, Department of Immunology, School of Medicine, Isfahan University of Medical Science, Isfahan, Iran.*
*2. Department of Immunology, school of Medicine, Isfahan University of Medical Science, Isfahan, Iran*
*3. Department of Immunology, school of Medicine, Isfahan University of Medical Science, Isfahan, Iran*
*4. Department of Cellular & Molecular Biology, school of Medicine, Isfahan University of Medical Science, Isfahan, Iran*
*5. Department of Neurosciences, Al-Zahra Hospital, Isfahan University of Medical Science, Isfahan, Iran*
*6. Department of Cellular & Molecular Biology, school of Medicine, Isfahan University of Medical Science, Isfahan, Iran*

**Background:** Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS).Various factors including genetic and environmental factors are involved in the prevalence of MS. The Osteopontin (OPN) has important role in immune response, so its encoding gene could be considered as a candidate for susceptibility to MS diseases. In the present study, we evaluated the frequency of OPN (-66T>G- rs2835709T > G) polymorphism in in MS patients compared with healthy controls in Isfahan, Iran population.
**Methods:** Blood samples were collected from 100 patients with multiple sclerosis and 100 healthy controls. After DNA extraction, OPN (rs28357094) polymorphism was detected with high resolution melt real time polymerase chain reaction (HRM Real Time PCR) technique. The results were analyzed with the SPSS software.
**Result:** In this study, frequency of TT genotype (OPN-rs28357094) (P = 0.020, OR = 1.85) in Patients with multiple sclerosis was higher than the control group significantly.
**Conclusion:** The results of this study show that rs28357094 T>G polymorphism (-66T>G) on OPN gene promoter was associated with susceptibility to multiple sclerosis in Isfahan population.

**Keywords:** Multiple sclerosis, Polymorphism, OPN gene

ICIA2018\Immunogenetics\Poster\8287
**8287**

## -308 G/A Tumor Necrosis Factor Alpha Polymorphism and Coronary Artery Disease risk in Hemodialysis Patients

Saiedeh Erfanian[1], Maliheh Moradzadeh[2], ElhamKashafi Jahromi[3]

*1. Department of Advanced Medical Sciences and Technologies, School of Medicine, Jahrom University of Medical Sciences (JUMS), Jahrom, Iran*
*2. Golestan Rheumatology Research Center, Golestan University of Medical Sciences, Gorgan, Iran*
*3. Food and Drug Department, Jahrom University of Medical Sciences, Jahrom, Iran*

**Background**: Prevalence and incidence of chronic renal failure is increasing and it has been considered as a threading factor in the worldwide. Cardiovascular diseases has been considered as the cause of more than half of death in hemodialysis patient. It has been shown that systemic inflammation has a key role in this issue. TNFα is a mediator cytokine of inflammation response which has a key role in pathophysiology of vascular disease caused by metabolism of lipids and obesity and insulin resistance. The aim of this study was to assess the association of polymorphism -308G/A gene TNF alpha and severity and vulnerability of cardiovascular disease in hemodialysis patient.
**Methods:** In this case control study, 41 hemodialysis patient with cardiovascular risk factors were considered as the case group and then 41 hemodialysis patient without cardiovascular risk factors were considered as the control group. DNA extraction was done with the specific Kit for the extraction of DNA. PCR was used for detection of polymorphism -308G/A and then NocI enzyme was added to each polymorphism for slicing and then electrophoresis was used for confirmation of enzyme slicing. Date were gathered and analyzed with SPSS v 15.
**Results:** there is no significant difference between the 2 group regarding genotype AA distribution and frequency of Allel A (p value>0.05).
**Conclusion:** The results of this study demonstrate that there is not any association between this polymorphism and cardiovascular risk in Iranian population. Other studies with larger samples are beneficent in order to detect the role of this polymorphism.

**Keywords:** Polymorphism -308G/A, TNF alpha, Atherosclerosis, Hemodialysis patient

ICIA2018\Immunogenetics\Poster\8354
**8354**

**Evaluation of MiR-146a and MiR-193a Expression in Colorectal Cancer Cell Lines**

Saeed noorolyai[1,2], Elham baghbani[2], Ahad Mokhtarzadeh[2,1], Behzad baradaran[2]

1. *Department of Biochemistry, Higher Education Institute of Rab-Rashid, Tabriz, Iran*
2. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Colorectal cancer (CRC) is a major cause of cancer related deaths, and the third most commonly diagnosed cancer worldwide. Although the exact mechanisms have not been clearly identified yet, many predisposing conditions are known to be related to the occurrence and progression of CRC, such as the lack of physical activity, diet containing few vegetables and fruits, tobacco smoking, alcohol abuse, overweight, obesity and a family history of cancer. MicroRNAs (miRNAs) are a family of endogenous, small, noncoding RNA molecules that involved in a wide range of biological processes including differentiation, proliferation, and apoptosis. Previous studies have confirmed that miR-193a and miR-146a exhibit pathogenic effects in carcinogenesis, especially in the colorectal adenocarcinoma. In this study, we evaluated the expression of miR-193a and miR-146a levels in three colorectal cancer cell lines.
**Methods:** HCT-116, SW480 and HT-29 cell lines were grown in RPMI-1640 supplemented with 10% (v/v) FBS, penicillin (100 U/ml), streptomycin (100 µg/ml), and maintained at 37°C in humidified5% CO2 atmosphere. Total RNA was extracted from cells. We used a QRT-PCR to evaluate of miR-193a and miR-146a expression levels. Quantitative Real-time PCR was performed by using a standard SYBR Green PCR master mix.
**Results:** Results of Quantitative Real-time PCR showed that the expression levels of miR-193a and miR-146a in HT29 cell line is lower than SW480 and HCT116 cell lines.
**Conclusion:** The results showed that expression of miR-193a and miR-146a in different colorectal cell lines are different and may have diverse effects on the cell lines.

**Keywords**: microRNA, Colorectal cancer, HT29, Quantitative Real-time PCR

ICIA2018\Immunogenetics\Poster\8494
**8494**

**Establishment of Lymphoblastoid Collection from Multiple Sclerosis Patients**

Mehrnaz Izadpanah[1,2], Samaneh Mahmoud Aghdam[1], Masoumeh Asadi[1], Parvaneh Farzaneh[1], Mansoureh Farhangnia[1], seyedAbolhassanShahzadeh Fazeli[1,3]

*1. Human and animal cell bank, Iranian Biological Resource Center (IBRC), ACECR, Tehran, Iran*
*2. School of advanced technologies in medicine, Tehran University of Medical Sciences, Tehran, Iran*
*3. Department of Molecular and Cellular Biology, Faculty of Basic Sciences and Advanced Technologies in biology, University of Science and Culture, Tehran, Iran*

**Background:** Multiple Sclerosis (MS) is an inflammatory disease of the nervous system and pathologic effect of the immune system. Genetic factors play an important role in the development of the disease. The main source for the MS study and treatment is the DNA sequences analysis of related genes and their function. Preparing a human genome for basic and applied studies is not always feasible, and also, these samples do not respond to ongoing research and researchers need to re-sample. The unrestricted way to access the genome sample without re-sampling of human beings is to store unlimited storage and proliferation cells such as immortalized lymphocytes. Thus, large samples of human cells can be replicated and exploited forever, especially for DNA extraction and genetic analysis in following steps.
**Methods:** The blood sample is taken from the patient for MS diseases, and the lymphocyte cells are isolated with Ficholl 1.077g/m. Cells immortalized by the Epstein-Barr virus and sub cultured during 6-8 weeks. Then Lymphoblastoid cells stored at -196 ° C after identity, observing the principles of the cell bank and, if necessary, re-cultivated and exploited.
**Results:** 50 blood samples from MS disease patients were collected. Lymphocyte cells were isolated, immortalized with EBV, expanded and stored finally. MS Lymphoblastoid cells appeared singly or in small and large clusters. All cells were analyzed for quality control tests which are critical in banking process including bacterial, fungal, viral contamination in addition to genetic identification such as Short Tandem Repeat (STR) test.
**Conclusion:** By creating immortalized cell bank from MS diseases in the country, the possibility of further studies and screening will be facilitated even for the discovery of new and not discovered genes involved. In addition, the relationship between different genetic factors and polymorphisms can be reviewed and analyzed by researchers in the field of molecular and medical biology.

**Keywords**: Banking, Lymphoblastoid cell, Multiple Sclerosis

ICIA2018\Immunogenetics\Poster\8544
**8544**

## Association Study between TLR4 Gene Variant (rs4986790) and Toxoplasmosis in Iran

Elnaz Asghari[1], Seyed Shapoor Reza Shojaei[2], Hamid Reza A. Naghash[2]

1. *PhD students of Parasitology, Karaj Branch, Islamic Azad University, Karaj, Iran*
2. *Karaj Branch, Islamic Azad University, Faculty of Veterinary Medicine Karaj, Iran*

**Background:** Variety and genetic variation are very important in the components of the human immune system that recognize and fight against foreign agents and antigens. As a result, humans differ widely in how to detect and respond to pathogens, and some people may be resistant to certain sick strains. Toxoplasma gondii is an obligate intracellular parasite that can invade and replicate in almost all nucleated cells of warm-blooded animals. It has a world-wide geographic distribution and is known to infect many species of birds and mammals, including approximately one third of humans. TLR4 plays a key role in the process of the innate immune response, and activates the inflammatory cell via the NF-κB pathway by inducing the expression of a variety of cytokines and other molecules crucial to immune responses.
T. gondii infection induced the production of interferon (IFN)-β through TLR4 and MyD88 signaling. The glycosyl phosphatidylinositol (GPI) of T. gondii was demonstrated to trigger TLR4 signaling Pathways. The purpose of this investigation was the determination of the distribution of genotypes at single nucleotide polymorphism (SNP) of the toll-like receptor 4 (TLR4) in humans infected with Toxoplasma gondii and the identification of genetic changes predisposing to infection. We decided to describe in this study the prevalence rates of the genotype and alleles at the TLR4, 896 A>G SNP (rs4986790) in women infected with T. gondii in IRAN and compare them to the prevalence rates observed in women uninfected controls.
**Methods:** 35 peripheral blood samples were collected from 35 women with Toxoplasma infection (positive serum test) and the blood of non-sick women was also used as a control group (negative).
**Results and conclusion:** The results were analyzed for SPSS software for statistical analysis. GG genotype (0.070) has been obtained, resulting in a 95% confidence level for this allele, there is no significant difference between the patient and the healthy person.

**Keywords:** Toxoplasma gondii, TLR4, SNP, rs4986790, Genetic variation

ICIA2018\Immunogenetics\Poster\9774
**9774**

# IL-33 Polymorphism rs192992 and its Association with Susceptibility to Different Pattern of Multiple Sclerosis

Mahdavi R[1], Jamali M[2], Rostami Rad M[3]

*1. Department of Immunology, Birjand University of Medical Sciences, Brigand, Iran*
*2. Department of Immunology, Medical School, Kerman University of Medical Sciences, Kerman, Iran*
*3. Department of Microbiology, Medical School, Tehran University of Medical Sciences, Tehran, Iran*

**Background**: Multiple sclerosis is the most common autoimmune demyelinating disease of the central nervous system (CNS) characterized by destruction of the myelin sheath, gliosis with progressive neurological dysfunction. Interleukine-33 (IL-33) is a cytokine with both pro- and anti- inflammatory activities that implicated in the pathogenesis of some auto immune diseases .the aim of this study was to determine single nucleotide polymorphism (SNP) of IL-33, rs1929992, in its gene in patients with multiple sclerosis (MS) and investigation of this polymorphism with susceptibility to multiple sclerosis.
**Methods:** In this case-control study, peripheral blood samples were collected from140 MS patients from January 2016 to February 2017 in the Imam Reza Hospital and Neurology Department of Vali-e-Asr Hospital in Birjand and blood sample of 140 healthy subjects (people referred to the Blood Transfusion Organization) as a control group. Patients with multiple sclerosis and healthy control groups were matched for age and sex. SNP at rs1929992 was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.
**Results:** There was significant difference between both, healthy control group and patient with multiple sclerosis. The frequencies of AA genotype at SNP rs1929992 were significantly higher in patients with SPMS and PPMS as compared with control group ($P<0.05$). However, the frequencies of AG genotype was significantly lower in patients with RRMS in comparison to the healthy group ($P<0.05$). In patients with RRMS, PPMS and SPMS patterns, the frequencies of A allele was significantly higher than that in control group ($P<0.003$, $P<0.001$, $P<0.0001$). In patients with RRMS, PPMS and SPMS pattern, the frequency of G allele was significantly lower than control group ($P<0.003$, $P<0.001$, $P<0.0001$).
**Conclusion:** These results of the present study suggest that the SNP rs1929992 in IL33 gene, may be associated with different pattern of MS susceptibility.

**Keywords**: Multiple sclerosis, Interleukine-33, gene polymorphism

ICIA2018\Immunogenetics\Poster\9802
**9802**

# Bioinformatics Study of Homologous Photolyase Enzyme in Humans and Immune Responses against Skin Cancer

SeyedSaadihosseini[1, 2*], Elmira shiuokhi[2, 3], Hashem Marawne[1], Ehsan Sohrabi[1]

1. *Department of Biotechnology Faculty of Bioscience University of ShahidBeheshti*
2. *Lab. of Regenerative Medicine and Biomedical Innovations, Pasteur institute of Iran*
3. *Department Science and Research .University of Islamic Azad .Central Tehran*

**Background:** With attention to the damage of ozone layer, the impact of UVB rays on DNA is increasing day by day. Ultraviolet (UV) radiation is one of the main causes of skin cancer in the world. The aim of bioinformatics study of Photolyase is to find its homologues in humans and their role in DNA repair and stimulation of immunological responses.
**Methods** :Bioinformatics analyzes with such software F genes H, BioEdit,Mega7, Multi Align, PROTPARM, PROTSCALE, Inter Pro, UNIPROT, Signal P, Inter Pro Scan, MMDB& Cn3D,STRING and R Was performed.
**Results:** Analyzes showed that PHR2 is an enzyme with 579 amino acid and protein PI 8.91, molecular weight of 64304.67. Instability index (71.54) Indicate the low half-life of the protein and its expression in a specific condition in the cell and whit two cofactors, FAD cofactor and diverse classes of antenna chromophores likes 5, 10- methenyltetrahydrofolate (MTHF), 8-hydroxydeazaflavin, FAD is essential for the activity of the enzyme photolyases. Very high homology with the CRY gene in humans , That CRY has more features than PHR2 , which can be used as a genes for the damage caused by ultraviolet beam projection in the future, according to predictions about ozone depletion, and on the other hand, this gene with many genes Key involved in skin diseases and immune responses, The gene has almost all of the characteristics of PHR2, which can be used as a genes in relation to the damage caused by ultraviolet light ejection of the skin in the future, according to predictions about ozone depletion, and this gene with many key genes, including (CLOCK, PER, ARNTL, CSNK1E, FBXL3, CDKN1A, SMARCB (BRG1), which are linked to skin disease and immune responses.
**Conclusion:** This study showed that CRY in human has a high homology in its structure and its properties with PHR in other organisms. Also, these genes were correlated with a number of mechanisms involved in immunological responses.

**Keywords:** Photolyase, Skin cancer, CRY gene, Immunology

ICIA2018\Immunogenetics\Poster\9936
**9936**

# Vitamin D Receptor (VDR) Gene Polymorphisms are not Associated with Susceptibility to Endometriosis in Iranian Women Population

Ali Akbar Delbandi[1, 2], Sepideh Khodaverdi[3], Mohammad Jafari[1, 2], Zahra Moradi[2], Tahereh Mohammadi[2], Zhima Akhavansales[4]

*1. Immunology Research Center, Immunology and Infectious Disease Institute, IRAN University of Medical Sciences*
*2. Immunology Department, School of Medicine, IRAN University of Medical Sciences*
*3. Endometriosis Research Center, IRAN University of Medical Sciences*
*4. Biotechnology Research Center, International Campus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

**Background:** Susceptibility to endometriosis is influenced by a combination of genetic, hormonal, immunological, and environmental factors. Vitamin D is known to reduce inflammatory responses, and it has been shown to have regulating effects on the immune system. This effect may be influenced by genetic polymorphisms in the vitamin D receptor (VDR) gene. The aim of this study was to analyze of association between VDR gene polymorphisms with susceptibility to endometriosis in Iranian women population.
**Methods**: In a case control study, we evaluated the distribution of four genetic polymorphisms of VDR gene [defined by the presence of restriction endonuclease sites for FokI (F/f), BsmI (B/b), TaqI (T/t), and ApaI (A/a)] in 118 women with endometriosis and 116 matched healthy women controls. Simultaneous genotyping of polymorphisms was performed by Polymerase Chain Reaction Sequence-Specific Primers (PCR-SSP) technique that previously reported by our laboratory.
**Results:** No significant difference observed in the distribution of alleles and genotypes between patients and controls for any of the VDR polymorphisms.
**Conclusion:** Our results demonstrated that VDR polymorphisms are not determinative Immunogenetic marker for endometriosis.

**Keywords**: Endometriosis, Vitamin D, Vitamin D receptor, Genetic polymorphism

ICIA2018\Immunogenetics\Poster\9975
**9975**

## The Relationship between *FGF1-1385A/G* Polymorphism and Endometriosis in an Iranian Population

Fariba Mirjalili, Ehsan Zare Mehrjardi, Seyed Morteza Seifati

*Medical Biotechnology Research Center, Ashkezar Branch, Islamic Azad University, Ashkezar, Yazd, Iran*

**Background**: Endometriosis is a chronic disease which endometrial tissue are found outside the uterine cavity. It is a polygenic and multifactorial disease that has a strong genetic base. Angiogenesis appears to be an important event in the pathophysiology of endometriosis (EM). One of the angiogenic factors, fibroblast growth factor (FGF1), play a central role in the initiation of angiogenesis. In this study, the possible association between *FGF1 -1385A/G* polymorphism and susceptibility to endometriosis in an Iranian population was investigated.
**Methods:** Seventy six unrelated premenopausal women with endometriosis and 106 unrelated healthy premenopausal women without endometriosis were enrolled in the study. Genomic DNA was extracted from Peripheral blood in all subjects. After extracting DNA, *FGF1 - 1385A/G* polymorphism was analyzed by PCR-RFLP.
**Results:** There was significant difference between frequencies of *FGF1 -1385A/G* polymorphism in case and control groups (p=0.025). So, this polymorphism was associated with endometriosis in our sample.
**Conclusion:** Further studies involving gene-environment and gene-gene interactions, particularly combination of *FGF1 -1385A/G* polymorphism and other *FGF1* gene family polymorphisms are needed.

**Keywords:** Endometriosis, *FGF1, -1385A/G* polymorphism

ICIA2018\Immunogenetics\Poster\10006
**10006**

## *TSLP* and *TSLPR* Gene Expression Levels in Different Phases of Menstrual Cycle in Women with Endometriosis

SoolmazHabibi[1]; ElhamAmirchaghmaghi[2*]; Fariba Ramezanali.[3]; RezaAflatoonian[3]; ParvanehAfsharian[1]; Maryam Shahhoseini[1*]

*1. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran*
*2. Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran*
*3. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran*

**Background:** Endometriosis is an Estrogen–dependent and inflammatory disease that characterized by the presence of endometriotic tissue outside the uterine cavity. It has been suggested that immunological factors play roles in the pathogenesis of endometriosis. Thymic stromal lymphopoietin (TSLP) is one of cytokines that triggers the dendritic cell-mediated Th2 response and has been suggested to promote Endometriosis. TSLP Receptor, also known as cytokine receptor-like Factor 2 (CRLF2) that forms a functional hetero dimeric complex with Interlukine-7 receptor (IL-7R) to bind TSLP. The present study aimed to elucidate *TSLP* and *TSLPR* genes expression in endometrial tissues of patients with endometriosis compared to women without endometriosis in different phases of menstrual cycle (proliferative phase /secretory phase).
**Methods:** This study consisted of two groups: women with endometriosis (n=15) and healthy fertile women (n=16). Women with any other uterine abnormalities were excluded. Informed written consent was obtained from all women. Endometrial samples were obtained from eutopic and normal endometrial biopsies were obtained by Pipelle ($p < 0.05$).
**Results:** The expression levels of *TSLP* and *TSLPR* (*IL7R* and *CRLF2* ) genes in ectopic and eutopic endometrium of endometriosis group was significantly increased in comparison to normal endometrium in both proliferative and secretory phases of menstrual cycle. In addition, expression levels of *TSLPR* (*IL7R* and *CRLF2)* gene in ectopic and eutopic endometrium was significantly increased in proliferative phase in comparison to secretory phase.
**Conclusion:** Overexpression of *TSLP* and its receptor in endometriotic tissues may have essential role in development of endometriosis through drive Th2 immune response. Also a higher expression of *TSLPR* observed in endometriotic tissues in proliferative phase, may be secondary to higher estrogen level in this phase as endometriosis is estrogen dependent disease.

**Keywords**: Endometriosis, Th2 Immune Response, Thymic Stromal Lymphopoietin (TSLP), Thymic Stromal Lymphopoietin receptor (TSLPR). Menstrual cycle.

# Immunohematology

# Poster Presentation

ICIA2018\Immunohematology\poster\7504
**7504**

# Volumetric Fluid System and Direct Absolute Counting for Platelets Micro Particles Detection in the Patients with Diabetes Mellitus type 2

Razie Mahmoodian[1*], Morteza Salimian[2], Mohsen Hamidpour[3], Ahmad Gharehbaghian[4]

*1. MSc, Department of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. PhD, Paramedical Faculty, Kashan University of Medical Sciences, Kashan, Iran.*
*3. PhD, Department of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*4. PhD, Department of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:**Platelet micro particles (PMPs) are submicron particles released from the membrane of activated platelets through shedding. They are involving in thrombotic problems of the diabetes mellitus type 2. As high clinical relevance, standardization of PMPs detection is required. Flow cytometry methods based on flow-rate and based on volumetric fluidic system are used as calibration factor to turn cytometer events to absolute count.
**Methods:**40 patients with diabetes mellitus type 2 and 35 healthy individuals volunteers were under study. 5 ml of anti-coagulated (sodium citrate 3.8%) blood of patients and controls were collected. After sampling, Platelets Rich Plasma was separated. Absolute counting of $CD41^{+}$- Annexin-$v^{+}$ micro particles was evaluated by bead-based strategies and volumetric system. Isotype controls were used for each flow cytometry run.
**Results:** Direct Absolute Counting for Platelets Micro particles with both methods were recorded and compared. The volumetric Fluid system counts micro particles as accurate as flow-rate beads**.** In comparison with to healthy individuals, Diabetic group showed a significant increase in shedding of platelet micro particles in all of states with ADP stimulation. ($P < 0.001$, SD: ±74.52)
**Conclusion**: The flow cytometric data show that the cytometer armed with a volumetric fluid system provides direct absolute counting and fixing the need for counting beads. The counting of this method may be even more precise and more reliable that it may result in difference in refractive index between beads and membrane vesicles. Platelets in patients with diabetes mellitus type 2 patients are pre active and they are more susceptible to released micro particles that it can be associated to metabolic environment in this patient.

**Keywords:** flow cytometry**,** Volumetric Fluid System, PMPs, diabetes mellitus type 2

ICIA2018\Immunohematology\poster\7523
**7523**

# The effect of *Tribulusterretris* aqueous extract on production and secretion/activity of platelets related factors in healthy Subjects

Soodabeh Aghapour[1,3], Zahra Koolivand[1], Mehrdad Payande[2], Kamran Mansouri[1], Fariba sohrabi[1], Ahmad Tajemiri[1] , Mohammad Alimoradi[1], Ali Mostafaie[1]

*1. Medical Biology Research Center, Kermanshah University of medical sciences, Kermanshah, I.R.Iran.*
*2. Department of Hematology and Medical Oncology, Kermanshah university of medical sciences, Kermanshah, I.R.Iran*
*3. Department of Immunology, Babol university of medical sciences, Babol, I.R.Iran*

**Background**: Platelet has essential role in immune regulation, tissue repair and homeostasis. The plants of the zygophyllacea family have many effects on the immune system. The aim of this study was to investigate the effect of the aqueous extract of Tribulusterrestris on the production and function of platelets in healthy individuals.
**Methods:** Aqueous extract of Tribulusterrestris was prepared and dried and the final powder was distributed twenty healthy subjects to take as pills three times a day for fourteen consecutive days. Blood samples were then taken from individuals on before and days 1, 14 and 28 after stopping taking the pills. The platelet count (in CBC) and secretion of platelet factor-4 (PF4), Von Willebrand factor (VWF) and serotonin (assessed by Enzyme Linked ImmunoSorbent Assay) were used to analyze production and function of platelet, respectively.
**Results:** The Concentration of VWF and serotonin was significantly higher in 1, 14 and 28 days after than before extract usage. Additionally, the concentration of PF4 was significantly lower in 1 day after using extract. However, significant difference was not seen at 14 and 28 days after extract usage. Moreover, there was not any significant difference in platelet count before and after extract usage.
**Conclusion:** Findings of this study suggest that Tribulus Terrestris seems to be remarkable safe choice for prevention and treatment of heart disease and Idiopathic thrombocytopenic purpura (ITP).

**Key words:** *Tribulusterretris*, Platelet, serotonin, VWF, PF4

ICIA2018\Immunohematology\poster\8356
**8356**

**Incidence of Factor VIII Inhibitors in Patients with Hemophilia A**

Akbar Dorgalaleh[1], Shadi Tabibian[1], Yadollah Farshi[2], Esmaeil Saneei Moghaddam[3], Morteza Shamsizadeh[4]

*1. Department of Hematology and Blood transfusion, School of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran*
*2. Department of Hematology and Blood transfusion, School of Allied Medicine, Tehran University of Medical Sciences, Tehran, Iran*
*3. Iranian Blood Transfusion Organization*
*4. Hamedan University of Medical Sciences*

**Background:** Hemophilia A (HA) is an autosomal recessive X-linked disorder resulting from decrease of factor VIII (FVIII) level. In affected patients with replacement therapy, inhibitor development is induced that complicating patients management.
**Methods:** This study was performed in 54 patients with different forms of HA. Patients including 17, 25 and 12 patients with severe, moderate and mild forms of HA, respectively. Activated Partial Thromboplastin Time (aPTT) mixed and Bethesda tests were performed for each patient.
**Results:** Among these 54 patients, 12 patients had high titers FVIII inhibitor and severe form of HA and none of the patients with mild and moderate forms of HA had FVIII inhibitors.
**Conclusion:** It seems that FVIII inhibitor is higher in patients with severe form of HA and it could be the reason behind neutralization of FVIII and development of complications in the treatment process.

**Keywords:** Hemophilia, Factor VIII, Inhibitor, Replacement therapy

ICIA2018\Immunohematology\poster\8420
**8420**

**Introduction of leuko-reduction filters as a biological research source of immunologic cells**

*Maryam Valizadeh[1], Maryam Ajami[1], Ali Akbar Pourfatollah[1,2]*

1. *Department of immunology school of TarbiatModares university of medical faculty*
2. *Department of immunology school of Iranian blood transfusion organization*

**Background:** monocyte cells and monocyte-derived dendritic cells (Mo-DCs) are one of the efficient tools for research purpose**.** Traditionally, peripheral blood mononuclear cells (PBMCs)isolated by density gradient centrifugation on Ficoll-Histopaque**,** and then differentiated to DCs.
Today, because of leukocytes side effects on the blood products, leukocytes remove from blood prior to entering the process of blood products and then use in transfusion. In order to solve this issue, now a day's leukocyte depletion by filtering has been implemented in blood banks.
After filtration process, luekoreduction filters are known as waste. In this study, we intended to retrieve viable peripheral blood leukocytes from a leukoreduction filters that currently are implemented in blood banking. If we succeed to elute high quality leukocyte, introduced to others as a big and efficient source of leukocyte cells for using in research.
**Methods:** After Blood sampling from eligible donors, leukofilters were removed from the blood bag and back-flushed by optimized washing buffer for leukocyte elusion. Preparation of PBMCs from Filter Buffy Coats is the next step; monocyte cells isolation was done using immune-magnetic cell sorting (MACS) method. Differentiation of Mo-DCs accomplished by using cytokine treatment. Finally DCs were characterized for specific surface marker by flow cytometry.
**Results:** leukocyte from filter were recovered at high number (about 5-6 $\times 10^9$) and viability (>95%). Phenotypic and functional assay showed no difference between filter and buffy coat derived Mo-DCs.
**Conclusion:** Leukoreduction Filter can be introduced as a big and efficient source of immunologic cells for using in research.

**Keyword**: Luekoreduction filter, Leukocyte, Dendritic cell, MACS

ICIA2018\Immunohematology\poster\8504
**8504**

**Survey of relationship between the severity of anemia and blood transfusion requirements**

Elham Khalaf Adeli[1], Ali Akbar Pourfathollah[1]

*1. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran*

**Background:** Conventionally, allogeneic blood transfusion is a supportive care for anemic patients in surgical producers. However, that is related to adverse events such as transfusion-related immunomodulation (TRIM), infection, etc. We surveyed transfusion requirement considering with severity of anemia in patients undergoing cardiac surgery.
**Method:** 181 adult patients scheduled for elective cardiac surgery were included in a descriptive study. Preoperative Hb<13g/dl for men and Hb<12g/dl for women considered as anemia. Anemia were classified as mild (11-12.9 g/dl for male, 11-11-11.9 for female) moderate (8-10.9 g/dl), or severe (<8g/dl) based on WHO criteria. Transfusion requirements were recorded during and 24 hours after surgery.
**Results:** Overall, 54(29.8%) patients were anemic. Anemia was mild in 58%, moderate in 28%, and severe in 14% of patients. 81% of patients with mild anemia and all of patients (100%) with severe anemia were transfused. The prevalence of transfusion was 57% in moderate anemia condition. The severity of anemia significantly was correlated with transfusion requirements ($P$=0.03).
**Conclusion:** The need for blood transfusion is prevalent in severe anemia. To avoid of allogeneic blood transfusion, anemia should be correct before surgery. Furthermore using blood conservation strategies and autologous transfusion could be reduce the need for allogeneic blood transfusion.

# Immunology of Chemical Victims and Environmental Pollution

# Poster Presentation

ICIA2018\Immunology of Chemical Victims and Environmental Pollution\Poster\7354
**7354**

# Evaluation of some of the factors associated with immune system following effect of magnetic field in rats

Amin Lafzi[1], Mansour Khakpur[2]

1. *Dvm, Veterinary Medicine Clinician , Tabriz ,Iran*
2. *Department Of Pathobiology, Tabriz Branch, Islamic Azad University, Tabriz, Iran*

**Background:** This study was designed to investigate the effects of electromagnetic fields (EMF) uniformly on the immune system of 12 Norwegian male Wistar rats were used as experimental model.
**Method**: These rats were divided into three control, treatment one and treatment two groups. The first treatment group was subjected to electromagnetic field for four hours and fifteen days. The second group received this treatment for eight hours and fifteen days. At the end of the fifteenth day, blood samples from rats were taken and were prepared to be analyzed. Then, blood cells were counted and after conducting biochemical tests, the data were analyzed using SPSS computer application, one-way ANOVA and Tukey range tests.
**Result:** The results revealed that the level of WBC increased in the first experimental group compared to control group; yet, it decreased in the second experimental group. The level of total protein relatively decreased compared to control group; however, level of albumin decreased significantly and at the same time Gamma globulin increased.
**Conclusion:** Eventually, the increase in the number of lymphocytes and decrease in the number of neutrophils and monocytes are the most significant effects of electromagnetic field on immune system.

**Keywords:** Immune system; Magnetic field; Rats.

ICIA2018\Immunology of Chemical Victims and Environmental Pollution\Poster\7564
**7564**

# The Immune statues of Regulatory T Cells in Chronic Myeloid Leukemia Chemical Victims Exposed to Sulfur Mustard

Zahra Shaker[1], Zohair Mohammad Hasan[2], Masume Ebtekar[2], Davar Amani[3]

*1. Tarbiat Modares University, Shahid Beheshti University of Medical Sciences, AJA University of Medical Sciences*
*2. Tarbiat Modares University*
*3. Shahid Beheshti University of Medical Sciences*

**Background**: The importance of the role of cell balances T in their health and imbalance in various diseases, especially in various types of cancers, it seems that the number of these cells increases in chronic myeloid leukemia, and in different stages of the disease, this increase is different. The suppression of the immune system in chemical injuries causes this increase and exacerbates the disease in these people.
**Methods**: In order to determine the number of T cells, The regulation of flow cytometry and the evaluation of the function of these cells are the amount of production of two cytokines IL-10 And TGF-βBy the same cells using the technique RT-PCR in real time Done.
**Results**: As the findings show, in cases of forming Philadelphia Gene or oncoprotein Bcr / Abl, the number of regulatory T cells has increased significantly(Pv<0.05, Pv = 0.015).In cases of patients who were both philadelphia positive and exposed to chemical gas of mustard, the percentage of T-reg is higher than non-exposed Philadelphia positive cases. This increase is not significant. (Pv= 0.311, Pv> 0. 05)In the exposed group with mustard, the incidence of Bcr / ablonco protein has effect positive on the number of T-regs and this increases significant(Pv = 0.001, Pv<0.05), and in non- exposed group with mustard, the occurrence of Bcr/Ablonco-protein has no meaningful relationship(Pv = 0.398, Pv<0.05).
**Conclusion**: The imbalance of T-regs number CML Can be a reliable indicator in tracking the process of treatment and the disease process. so that, compared with cytogenetic tests, the follow up and comparison of the number of these cells at different times of the disease according to peripheral blood sampling compared with the sampling Bone marrow in genetic tests is both cost-effective and time-consuming and it inflicts fewer illnesses on the patient .

**Keywords**: Chemical Sulfur Mustard, CML, Regulatory T cell

ICIA2018\Immunology of Chemical Victims and Environmental Pollution\Poster\7621
**7621**

**Effect of extremely low frequency electromagnetic fields (ELF-EMFs) on serum levels of IL-6, IL-21 and expression of BCL-6, AID genes in spleens of rats**

Mohammad Gholamian Hamadan,[1,2] Mahdi AlahgholiHajibehzad,[1] Hanie Mahaki[1,3], Zohreh Zareighane,[1] Elaheh Talebi Ghane,[4]Alireza Zamani[1,3]

*1. Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.*
*2. Student Research Committee, Hamadan University of Medical Sciences, Hamadan, Iran*
*3. Molecular Immunology Research Group, Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.*
*4. Department of Biostatistics, School of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

**Background:** The spleen is the important organ in humoral immune system against blood antigens. Some environmental factors such as extremely low frequency electromagnetic fields (ELF-EMFs) may cause harmful effects on it. The purpose of this study was assessing the role of long-term exposure of ELF-EMFs in different intensities on expression of B-cell lymphoma 6 protein (BCL-6) gene, and Activation-induced cytidine deaminase (AID) gene.
**Methods:** Eighty adult male rats were exposed to ELF-EMFs in intensities of 1,100,500 and 2000μT at frequency of 50 Hz for 2 hours daily for up to 60 days.
**Results:** The results showed that AID gene expression was declined in low intensity of 1μT compared with control group, while no changes were observed after ELF-EMFs exposure on expression levels in other intensity and other gene. Serum level of IL-6 increased in 500μT group in compared with other groups. Also, serum level of IL-21 have no significant change in all groups.
**Conclusion:** ELF-EMFs exposure does not alter differentiation of $T_{FH}$ (Tcell Follicular Helper), the most important factor for initiation specific humoral immune system but can effect on Centroblasts.

**Keywords:** BCL-6, AID, IL-6, IL-21, $T_{FH}$, ELF-EMFs.

ICIA2018\Immunology of Chemical Victims and Environmental Pollution\Poster\7725
**7725**

**Maternal exposure to A.lwoffii F78 prevents allergic asthma in offspring**

Najmefrozannia[1]

*1. Department of basic science School of Medicine University of mashhad Islamic azad university*

**Background:** Asthma is a chronic inflammatory disease. Epigenetic factors affecting the asthma phenotype have been lately investigated. Cowshed, farm animals and their products, which are found in farming environment, must have an important role in preventing asthma.
A.lwoffii f78 is a gram-negative cowshed bacterium, which causes allergoprotective effect in prenatal model of transmaternal experimental asthma protection.
**Methods:** For this review we searched for asthma in titles and abstracts and for A.lwoffii F78 in whole papers at PubMed and Elsevier and Google scholar. We found 25 articles 12 of which were irrelevant to our subject or had a low impact.
**Results:** Maternal exposure to A.lwoffii F78 prevents allergic asthma in the mice with homozygous asthmatic mother. The Exposure first causes a transient increase in proinflammatory cytokines and toll like receptor messenger RNA but later it has shown lower amounts in cord. Its effect is dependent on maturnal toll like receptor signaling.
Dendritic cells stimulated by A.lwoffii f78 cause to induce $T_H1$ polarising gene in naïve T-cells .In the mice treated by A.lwoffii F78 $T_H1$ immune response shifting and decrease of $T_H2$ antibodies titration rather than T regulatory cells response were observed.
Exposure to A.lwoffii causes immune suppression and regulatory mechanisms by IL8, TNF, IL-12 cytokine.
**Conclusion:** The articles proved that the strong influence of prenatal exposure to A.lwoffii F78 would prevent allergic asthma as it was claimed in hygiene hypothesis.

**Keywords:** Allergy protection, A.lwoffii F78 Hygiene hypothesis, $T_H1$ response shifting, epigenetic

ICIA2018\Immunology of Chemical Victims and Environmental Pollution\Poster\8460
**8460**

# Study the Effect of Genetics on MMP2 and Regulatory T cell in Chronic Myeloid Leukemia patients who Exposure to Sulfur Mustard

zahra Shaker[1], Zohair Mohammad Hasan [2], EbtekarMasume [3], Davar Amani [4]

*1. Tarbiat Modares University, Shahid Beheshti University of Medical Sciences, AJA University of Medical Sciences*
*2, 3TarbiatModares University*
*4. Shahid Beheshti University of Medical Sciences*

**Background**: Philadelphia chromosome and expression of an encryption Bcr / Abl that happen in stem cells and comet impacts on the production of molecules involved in processes such as matrix metalloproteinase and cancer of blood cells, including lymphoid cell lines and T cells.
The above changes of cells and molecules in some cases even produce an oncoprotein known genetic and is not related to examine the relationship between cancer chronic myeloid leukemia in this study means CML (Chronic Myeloid Leukemia) More than 90% and in many cancers that particular oncogene or cancer is found only extends.
**Methods**: In this study, on patients with CML The two victims exposed to mustard and other chemical exposure cases with mustard has been done, the duration of disease was 8.6 on average during the investigation and treatment of the disease process is followed. Cytogenetic tests include karyotyping, FISH, RT-PCR in real time to obtain findings and test GEE the data used for the analysis.
**Result**: Some of the results from the analysis of the data suggests that in some 55/3707 and 12/3089, such as the concentration of ng Lynr MMP2 That Pv<0.05, MMP2 Along with the occurrence of Philadelphia gene in the mustard exposure group, significantly increased the number of cells T Regulation is effective. The concept of this finding is to increase the number T-reg with variables MMP2. In some experiments, the presence of Philadelphia, will be one of the main causes of cancer metastasis and Mytlayan to CML.
**Conclusion**: Therefore, one of the most important therapies for lowering the concentration MMP2 or deactivating the desired molecule. Approach to the prevention of metastases could be blocking the progression of the disease.

**Keywords:** MMP2, Leukemia, Sulfur Mustard

ICIA2018\Immunology of Chemical Victims and Environmental Pollution\Poster\11171
**11171**

# Evaluation of serum levels of Pro-inflammatory cytokines(TNF-α, IL1-β)in Sulfur Mustard(SM)exposed patients with long term pulmonary complications

Ashraf Vafa, Tooba Ghazanfari, Naghizadeh MM

*Immunoregulation Recearch Center, Shahed University, Tehran, Iran*

**Background:** Exposure to mustard gas leads to acute and chronic toxic effects on the pulmonary system. The results of various studies have been suggested the role of Pro-inflammatory cytokines including IL1-β and TNF-α in the pathogenesis of Pulmonary inflammatory diseases. IL-β and TNF-α are recognized as a key mediator of systemic inflammatory reactions and numerous changes including altered cell signaling, migration, changing on cytokine production and fever. The aim of this study is the evaluation of serum levels of IL-1β and TNFα in SM-exposed patients with long-term pulmonary complications.
**Materials:** In this study, 65 SM-exposed patients as exposed group and 69unexposed people as control group were studied. The both exposed and control groups were homogeneous in terms of gender, age and body mass index. Pulmonary function test was performed by spirometry. The serum levels of IL-1β, TNF-αwere measured by ELISA method.
**Results:** The serum levels of TNF-α was high in SM-exposed group compared to control group. However, there were no significant differences between the serum levels of IL-1β in SM exposed group and the control group.
**Conclusion:** Due to increased serum levels of TNF-α in exposed group compared to the control group, It may play an important role in pulmonary inflammatory complications. It is a proof of TNFα effects on pulmonary system as a mediator of inflammatory reactions. Although there were no significant differences in the serum levels of IL-1β in SM-exposed group compared to the control group, It doesn't rollout the role of IL-1β in pathogenesis of long term pulmonary complications, because it may have local changes in pulmonary system and long term complications. Therefore, locally assessment of IL-1β would be valuable.

**Keywords:** Mustard Gas; TNFα; IL-1β; Pulmonary Complications;

# Immunology of Exercise, Aging, and Nutrition

# Poster Presentation

ICIA2018\Immunology of Exercise, Aging, and Nutrition\Poster\6364
**6364**

# Effects of Endurance Training on the Serum Levels of Tumour Necrosis Factor-α and Interferon-γ in Sedentary Men

Abdolreza Sotoodeh Jahromi[1], Abdossaleh Zar[2], Fatemeh Ahmadi[3], Peter Krustrup[4], Khosrow Ebrahim[5], Friborz Hovanloo[5], Davar Amani[6]

*1. Research Center for Non-communicable Diseases, Immunology Department, Jahrom University of Medical Sciences, Jahrom, Iran*
*2. Department of Physical Education & Sport Science, University of Jahrom, Jahrom, Iran*
*3. Department of Physical Education & Sport Sciences, Shiraz University, Shiraz, Iran*
*4. Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, Exeter, UK*
*5. Department of Exercise Physiology, G.C. Shahid Beheshti University, Tehran, Iran*
*6. Department of Immunology, Medical School, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Physical activity could be considered one of the factors that affect the immune system status and function. To find the relation between exercise and cytokines, we examined the possible effects of an 8-week endurance training program on the serum levels of cytokines, including tumour necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) in sedentary men.
**Methods:** A total of 30 healthy young male volunteers were randomly divided into an endurance training group and a control group. The training group followed a specific exercise protocol (running on a treadmill for 15~30 min at 50~70% maximal heart rate) for 8 weeks and the control group did not participate in any exercise program. Venous blood samples were collected from both the groups 24 h before and 24 h and 48 h after the exercise. Repeated ANOVA was used for statistical purposes. The serum levels of TNF-α and IFN-γ were determined by ELISA.
**Results:** Significant ($p<0.05$) and non-significant ($p>0.05$) decreases were observed in the serum levels of IFN-γ and TNF-α, respectively, after the 8-week endurance training program.
**Conclusion:** Our findings indicated that an 8-week endurance exercise may affect the serum levels of some inflammatory cytokines, suggesting the beneficial role of this training protocol in elderly population and people with certain conditions (inflammation of the vertebrae or other inflammatory diseases).

**Keywords:** Endurance Training, TNF-α, IFN-γ, Sedentary Men

ICIA2018\Immunology of Exercise, Aging, and Nutrition\Poster\7861
**7861**

# Effect of Probiotics on Beta-Interferon Gene Expression

Mohaddase Jarrahi[1], Roya Bishe Kolaei[1]*, Rooholla Abdollahpour[2]

1. *Department of Biology, Islamic Azad University of Qaemshahr, Qaemshahr Iran*
2. *Animal Science Department, Islamic Azad University of Qaemshahr, Qaemshahr Iran*

**Background:** Probiotics, as useful microorganisms for the health of host animals, can affect the function of digestive and immune system. However, the mechanism of beneficial effects of probiotics on immunity is still not fully understood. Since Beta-Interferon gene expression is important for improving immunity function, the present study was designed to investigate the effect of kefir Probiotic compounds on Beta-Interferon gene expression in broiler chickens intestine.
**Methods:** This research was conducted in a completely randomized design in four replications and two stages. The first stage was carried out at a research farm for 80 broiler chickens for 42 days. Treatments in the diet of broiler chickens included the following: 1.The first treatment without any additive in the diet as control.2. The second treatment kefir was added to the diet of broiler chickens. In the second stage, after 42 days of chickens breeding, their samples were taken from the Jejunum tissue of the treated chicks and were transferred to the lab for molecular testing.After that RNA was extracted from the Jejunum tissue sample and the synthesis of cDNA was carried out by PCR. Finally, the samples were applied to examine gene expression by Real-time PCR using the GAPDH gene.
**Result:** The results showed that kefir fed to boiler chickens had significant effect on the immunity system ($P \leq 0.05$) compared with the control group. Application of kefir did not reduce beta- interferon gene expression. Therefore resistance to gastrointestinal infections did not increase.

**Key words**: Beta-Interferon, Probiotics, Kefir, Real time PCR.

ICIA2018\Immunology of Exercise, Aging, and Nutrition\Poster\7863
**7863**

## Effect of Organic Acid on Beta-Interferon Gene Expression

Mohaddase Jarrahi[1], Roya Bishe Kolaei[1]*, Rooholla Abdollahpour[2]

1. *Department of Biology,Islamic Azad University of Qaemshahr ,Qaemshahr Iran*
2. *Animal science Department, Islamic Azad University of Qaemshahr, Qaemshahr Iran*

**Background:** Today healthy breeding of poultry or improving immune system against infectious diseases, antibiotics are used at therapeutic level. At the same time as they are fed, antibiotics are usually applied as growth promoters at lower levels than the treatment dose. Due to immune system resistance of the body to some antibiotics and damages to intestinal microbial balance suitable alternative has been introduced. Feeding some for improving animal immune system resistance is an alternative method. One of the most important protein produced against the immune system is Beta-Interferon, which improves cell resistance to viral infection. The purpose of this project was to investigate the effect of Organic acid on Interferon gene expression.
**Methods:** This research was performance as a completely randomized design in four replications and two steps. The first step was carried out in a research farm for 80 broiler chickens for 42 days; as follow: 1.The first treatment without any additive considered in diet of the control. 2. In the second treatment the organic acid (Acid fires) was added to the broiler chickens diet. In the second step, after 42 days sampling was undertaken from Jejunum tissue of the chickens under treatment and were send to the laboratory for molecular testing. RNA was extracted from the Jejunum tissues and the synthesis of cDNA was carried out by PCR. Finally the samples were applied to examine gene expression by Real-time PCR using GAPDH gene.
**Result:** Result showed that application organic acid (Acid fires) to feeding diet of broiler chickens significantly reduced Beta- Interferon gene expression compared to control group.

**Key words**: Beta-Interferon, Organic acid, Acid fires, Real time PCR

ICIA2018\Immunology of Exercise, Aging, and Nutrition\Poster\8361
**8361**

# Effects of aerobic exercise training on serum Leptin level and insulin resistance in overweight breast cancer women

Mahnaz Sourani[1], Amin Isanejad[1], Mohamadreza Khosravi Farsani[2]

1. *Department of physical education, Shahed University*
2. *Department of Medical Hematology-Oncology, Isfahan University of Medical Sciences*

**Background:** Because both breast cancer and the hormone leptin are associated with obesity and insulin resistance in women and with regard to the positive effects of aerobic exercise in breast cancer patients. The purpose of this study was to investigate the effect of 10 weeks aerobic exercise on serum leptin level and insulin resistance of overweight women with breast cancer.
**Methods**: Twenty- five overweight women with breast cancer based on inclusion criteria for participation in the study selected and randomly assigned to an exercise (n=15) or control (n=10) group. The exercise group trained aerobic exercise three times per week for 10 week at 40 -70 % maximal heart rate. The outcomes were changes in $Vo_{2Max}$, Body composition, fasting blood sugar, insulin, leptin from baseline to post intervention. The serum leptin level was measured by ELISA. Data analyzed by independent t-test and ANCOVA ($P < 0.05$).
**Results**: Body mass was decreased in exercise group ($P = 0.033$), $VO_{2max}$ increased in the exercise group ($P=0.001$). Fasting blood sugar didn't change after 10 week of exercise training, however the insulin resistance was decreased in exercise group ($P = 0.001$). The serum leptin level decreased significantly in exercise training group ($P=0.001$).
**Conclusion:** Aerobic exercise had beneficial effects on $Vo_{2Max}$, insulin resistance, leptin in overweight women with breast cancer. Therefore aerobic exercise recommended as safe rehabilitation for breast cancer survivors.

**Keywords:** breast cancer, aerobic exercise, leptin, insulin resistance

ICIA2018\Immunology of Exercise, Aging, and Nutrition\Poster\8564
**8564**

# Relationship between BMI with IL-17 and IL-23 in renal transplant patients following 10 weeks of exercise training

Somaye pooranfar[1], Mohsen Salesi[2], Omidreza Salehi[3]

1. *Department, Tehran markazi Branch, Islamic Azad University, Tehran, Iran.*
2. *Department of Shiraz Branch, University, shiraz, Iran*
3. *Department of Marvdasht Branch, Islamic Azad University, Marvdasht, Iran*

**Background:** Chronic renal failure has been considered as one of the health problems in world. In recent studies of immunology, exercise has been emphasized on the components of the immune system. The aim of this study was investigation relationship between BMI with IL-17 and IL-23 in renal transplant patients following 10 weeks of exercise training
**Methods:**36 kidney transplant patients were selected and randomly divided into two groups of exercise (n=18) and control (n=18). All the patients took blood and Anthropometric test before and after training. Training group subjects participated in a 10-week training program including a 60-90 minutes training sessions three times a week. Pearson correlation (IL-23) and Spearman (IL-17). Moreover, SPSS software were used for statistical analysis of the findings ($P_{value}$=0/05).
**Results:** Findings showed that there was no significant relationship between the levels of BMI with IL-23 (r = -0.19; r = -0.26) and IL-17 (r = -0.55, p = 0.75) after 10 weeks of selected exercise
**Conclusion:** It can be concluded that there is no significant relationship between BMI with IL-17 and IL-23 following exercise in renal transplant patients.

**Keywords:** BMI, IL-17, IL-23, Kidney transplant patients, Exercise training.

ICIA2018\Immunology of Exercise, Aging, and Nutrition\Poster\8599
**8599**

# An in vitro evaluation of anti-aging effect of guluronic acid (G2013) based on enzymatic oxidative stress gene expression using healthy individuals PBMCs

Mahsa Taeba, Seyed Shahabeddin Mortazavi Jahromib, Abdollah Jafarzadeha, Mohammad Reza Mirzaeic, Abbas Mirshafieyd,

1. *Department of Immunology, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
2. *Department of Cellular and Molecular Biology, Kish International Campus, University of Tehran, Kish, Iran*
3. *Molecular Medicine Research Center, Rafsanjan University of Medical Sciences*
4. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences*

**Background**: Aging is usually associated with increased levels of oxidants, and may result in damages caused by oxidative stress. There is a direct relationship between aging and increased incidence of inflammatory diseases. The present research intended to study the anti-aging and anti-inflammatory effects of the drug G2013 (guluronic acid) at low and high doses on the genes expression of a number of enzymes involved in oxidative stress (including SOD2, GPX1, CAT, GST, iNOS, and MPO) in peripheral blood mononuclear cells (PBMCs) of healthy individuals under in vitro conditions.
**Methods**: Venous blood samples were taken from 20 healthy individuals, the PBMCs were isolated and their RNAs extracted and their cDNAs were synthesized, and the genes expression levels were measured using the qRT-PCR technique.
**Results**: Our results indicated that this drug could, at both low and high doses, significantly reduce the expression of the genes for SOD2, GPX1, CAT, and GST compared to the LPS group ($p < 0.0001$). Moreover, it was noticed that the drug is able to significantly reduce gene expression levels at the high dose and at both doses (low and high), for iNOS and MPO compared to the LPS group ($p < 0.0001$), respectively.
**Conclusions**: The present research showed that G2013, as a novel NSAID drug with immunomodulatory properties, could modulate the expression levels of the genes for SOD2, GPX1, CAT, GST, iNOS, and MPO, to the level of healthy gene expression, and possibly it might reduce the pathological process of aging and age-related inflammatory diseases.

**Keywords**: G2013 Guluronic acid Anti-aging Oxidative stress NSAID Aging

ICIA2018\Immunology of Exercise, Aging, and Nutrition\Poster\9876
**9876**

# The effect of naringin on serum TNF-α level in male rats after swimming

Vahid R Haghaniat[1], Ali R Khoshdel[1], Mohammad R Hajizadeh[1], Mehdi Mahmoodi[1], Giti Farsi[1], Mohammad Ayubi Rad[1], Mohammad Zamanian[2]

1. *Department of Clinical Biochemistry, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
2. *Physiology-Pharmacology Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

**Background:** TNF-α is one of the most known circulatory inflammation marker. Exercise is considered to be a safe activity that causes moderate inflammation, the purpose of this study was to investigate the effects of a moderate-intensity exercise like swimming on the level of TNF-α in serum of male wistar rats with naringin as supplement. Naringin belongs to the flavonoid family the flavonoid naringin is found naturally in citrus fruits, especially in grapefruit, where asnaringin is responsible for its bitter taste.
**Methods:** Twenty- four male wistar rats were randomly divided into 3 groups including; a group of exercised rats with supplement of naringin, and 2 control groups containing non-exercised control without naringin supplement and normal control rats. 160 mg/kg naringinwas used as supplement. Naringin was administered orally (with use of gavage syringe) daily for 20 days. On the 20th day, the rats were killed and the level of TNF-α in the collected serum were measured.
**Results:**The level of TNF-α in exercised control group had Significantly increased compared to non-exercised control group .Naringin supplement significantly decreased serum TNF-α level in treated group in comparison to exercised control group.
**Conclusion:** According to our study, swimming exercise in male rat models increase inflammation and Naringin can significantly delay that by decreasing the level of TNF-α in blood circulation.

**Keywords:** Naringin, Swimming, TNF-α, Inflammation

ICIA2018\Immunology of Exercise, Aging, and Nutrition\Poster\9889
**9889**

# The effect of eight weeks aerobic and anaerobic swimming on some factors of cardiovascular disease in middle men

Mahdi Ghasemiartiyan[1], Seyed Mahmud Hejazi[2]

*1. Assistant professor in sport physiology, Department of physical education, Mashhad branch, Islamic Azad University, Mashhad, Iran*
*2. Institute of Higher Education hakim Nezami, Ghuchan, Iran*
General practitioner Department of Education Mashhad, Area 6, Iran

**Background**: The aim of this study was to compare the effects of aerobic exercise aerobic swimming on the surface of IL-6 and TNF-α and HS.CRP serum in young women.
**Method**: This semi-experimental study population consisted of 100 male swimmer in Mashhad that were in the age range 25 to 30 years and have had sufficient skills in 4 main swimming. 28 patients were randomly assigned to 2 groups (two groups of 14 each aerobic and anaerobic workout swim) and 10 patients in the control group. Blood samples were taken to measure the factors considered and analyzed the data using descriptive and inferential statistics, Kolmogorov-Smirnov test and ANOVA (ANOVA) was performed and the following results were obtained.
**Result**: Serum levels of IL-6 subjects is a significant difference. (019/0 = P). Between the levels of serum TNF-α subject is a significant difference. (005/0 = p). Between the level of HS.CRP subject is a significant difference in serum participants. (001/0 = P)
**Conclusion**: Based on this study, aerobic exercise and anaerobic swimming reduces the level of IL-6 and TNF-α and HS. CRP levels in subjects that this reduction was statistically significant.

**Keywords**: IL-6 and TNF-α and HS CRP

ICIA2018\Immunology of Exercise, Aging, and Nutrition\Poster\9999
**9999**

**Comparison of 6-month endurance and resistance exercise on the balance of TH1, TH2, cortisol and leptin in young people**

Mahdi Ghasemi artiyan[1]

1. *Faculty Member of Sanabad Golbahar Institute of Higher Education.*
2. *Hakim Nezami Institute of Higher Education, Ghuchan, Iran*
3. *teacher of Education Physical Education Department of Education Mashhad, Area 6, Iran*

The aim of this study was to compare the effects of 12 weeks of aerobic and resistance exercise on th1 and th2 balance and serum leptin and cortisol in young men this study used a quasi-experimental data have been collected .Among healthy people and 52 volunteers in carrying out research in the age range 24 to 35 years old, Of the 100 subjects were randomly selected, Demographic information, health history, physical activity and smoking Will complete. The sample consisted of 52 young men, 20 patients who will do aerobic training (Experimental) And 20 other people will doing strength training (Experimental) 12 patients in the control group. Blood samples for measurement of these factors on ELISA (ElISA) of the three groups before and after exercise will take. Moreover, to analyze the data using descriptive statistics and Kolmogorov-Smirnov test and t-test statistical method the student will perform.

# Immunology of Infectious Diseases

# Bacteria & Fungi

# Poster Presentation

ICIA2018\Immunology of Infectious Diseases Bacteria & Fungi\Poster\6357
**6357**

# Studying the Polymorphism in the Gene Encoding Interleukin- 17 [rs4711998 (A/G)] with PCR-RFLP in Patients with Brucellosis Compared with Healthy Subjects: A Case-Control Study

Hamed Farhadi Kohan[1], Fariba Keramat[2,3], Hassan Mahmoudi[4], Alireza Zamani[5], Massoud Saidijam[6], Mohammad Ebrahim Ghaffari[7], Sima Kazemi[4], Mohammad Yousef Alikhani[2,8]

*1. MSc in Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
*2. Brucellosis Research Center, Hamedan University of Medical Sciences, Hamedan, Iran*
*3. Professor, Department of Infectious Diseases, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
*4. PhD Candidate in Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
*5. Professor, Department of Immunology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
*6. Professor, Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
*7. PhD Candidate in Biostatistics, Faculty of Health, Hamadan University of Medical Sciences, Hamadan, Iran*
*8. Professor, Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*

**Background:** Brucellosis is a systemic infection caused by gram-negative coccobacilli and facultative intracellular bacteria of the genus Brucella. Interleukin-17 is one of the important cytokines that plays a role in controlling host immune response in patients with brucellosis. The aim of this study was to investigate polymorphism of the genes encoding IL-17 in patients with brucellosis compared to healthy subjects
**Methods:** This case-control study included 86 patients with brucellosis who were selected based on clinical symptoms, serology, culture and PCR results. The control group composed of 86 healthy people. The polymorphism gene encoding Interleukin-17 was evaluated in both groups by PCR-RELP method.
**Results:** Current study showed that the frequencies of AA [OR=0.047 (95%CI: 0.01-0.12)] and GG [OR=337.20 (95%CI: 20.49-5541.39] were significant at position -1998 (G/A) in both cases and controls [P-values (AA) and (GG) = 0.001]. But, the frequency of AA genotypes in the control group was greater than the frequency of GG genotype in patients. The odds ratio for catching brucellosis in people who have genotype GG was 41 times higher than those who have genotype AA.
**Conclusion:** Findings showed that, AA and AG genotypes at -1998 (A/G) position are more important. So, the risk of brucellosis in people with GG genotype at position -1998 was higher than that in people with AA genotype. In other words, people with AA genotype are more protected against brucellosis at this position

**Keywords:** Interleukin-17, Cytokines, Brucellosis, Polymorphism.

ICIA2018\Immunology of Infectious Diseases Bacteria & Fungi\Poster\7762
**7762**

# The Survey of Typhoid Fever in Suspicious Patients Who Referred to a Medical Diagnostic Laboratory in Ahvaz, Southwest of Iran (2012-2017)

Abdolhussein Shakurnia[1], Sara Iranparast[1], Asadollah Baesi[2], Samaneh Salehi pour[2], Mehri Ghafourian[1]

*1. Department of Immunology, School of Medicine, University of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*
*2. Department of health education, culture and research (ACECR), Branch of Khuzestan, Ahvaz, Iran*

**Background:** Typhoid fever is a systemic infection caused by *Salmonella species* and is an important cause of illness and death, particularly among children and adolescents. Typhoid fever remains an important public health problem in the world especially in the population from developing countries. The aim of this study was to determine the prevalence of Typhoid fever amongst suspicious patients who referred to a medical diagnostic laboratory in Ahvaz, Southwest of Iran in a six years' period from 2012 to 2017.
**Methods:** This study was cross sectional-descriptive. In this epidemiological study, the required information of all patients who were suspected to Typhoid fever by physicians was extracted over the last 6 years by laboratory software. The diagnosis of Typhoid fever was performed using serological Widal test; and raised anti-O antibody level over titer of >1:80 was considered significant of having typhoid fever. The data was analyzed in SPSS 18 software by Chi-square tests.
**Results:** Of 6870 surveyed patients with the mean age of 36.83±16.74 years ،4435(64.6%) were male. The prevalence rate of typhoid fever based on serological test was 2.2%. The trend of prevalence rate of typhoid fever has increased from 0.9% to 5.1% in 2012 and 2017, respectively. (p=0.001). It was observed that prevalence of typhoid fever was higher in females with 106 (2.4%) than males 44 (1.8%) patients (p=0.066). Similarly, that prevalence of typhoid fever was observed higher in spring months (p=0.129). It was also observed that prevalence of typhoid fever was significantly higher among the patients <15 years old (p=0.034). It was concluded that prevalence of typhoid fever has increased among the youths who consume unsafe drinking water and food from outside source.
**Conclusion:** Typhoid fever is an important public health concern in Ahvaz, southwest Iran. This surveillance tool could have wide applications for surveillance for Typhoid fever in developing countries.

**Keywords:** Prevalence, Typhoid fever, Ahvaz, Southwest Iran

ICIA2018\Immunology of Infectious Diseases Bacteria & Fungi\Poster\7798
**7798**

**Assessment of Serum Level of CXCL10 and CCL11 in Patients with Sepsis at Admission and Discharge**

Mahsa Rahgoshay[1], Gholamhossein Hasanshahi[2], Maryam Kafizadeh[3]

*1. Department of Paramedical* Sciences*, School of Paramedical* Sciences*, Kerman University of Medical Sciences*
*2. Department of Medicine, School of Medicine, Rafsanjan University of Medical Sciences*
*3. Department of Medicine, School of Medicine, Rafsanjan University of Medical Sciences*

**Background:** Sepsis is a leading cause of death and the most common cause of death in the intensive care unit.CXCL10 plays a critical role in the recruitment of leukocytes and has angiostatic properties. In addition CCL11 has a potent eosinophil chemo attractant in allergic inflammation and asthma. This study aimed to compare the level of these chemokines in patients with sepsis at admission and discharge based on age and gender studies
**Material and Method:** This study was performed in a cohort of 33 patients with sepsis who were admitted to the Hospital. The peripheral blood sampling was performed (during admission and discharge) twice. Demographic data were collected through clinical history. The serum concentrations of CXCL10 and CXCL11 measured by ELISA. Results obtained along with demographic data were analyzed using the software SPSS, and ANOVA statistical methods
**Results:** The 54 patients participating in the project, 22 were male and 11 females respectively. The minimum age of participants was 45 years and a maximum of 92 years. The mean values of serum chemokines in patients with sepsis at the time of hospital discharge was significantly reduced and the differences were statistically significant (P <0.0001) While CCL11 chemokine showed increased level during discharge. Compared to the overall, mean concentration of serum chemokines in patients with sepsis at admission by sex and age were approximately equal in terms of differences not statistically significant (P =0.193 P =0.816)
**Conclusion:** Our study showed that serum level of CXCL10 and CCL11 increased significantly in septic patients. After the phase of antibiotic therapy, Serum level of CXCL10 was decreased while serum level of CCL11 were remained elevated. Overall, the changes in concentrations of chemokines were not directly associated with age and sex. Finally, our study proposed that chemokines may use for the early diagnosis and treatment of patients with sepsis as a new treatment.

**Keywords:** Sepsis, SDF-1α (Call- inflammatory chemokine and angiogenesis construction)

ICIA2018\Immunology of Infectious Diseases Bacteria & Fungi\Poster\7896
**7896**

# Improving the Delivery of HCV NS3 Protein Using Cady-2 Penetrating Peptide in HEK-293T Cell Line

Sina Alizadeh[1], Azam Bolhassani [2*], Shiva Irani [1], Seyed Mehdi Sadat [2]

*1. Department of Biology, School of Basic Science, Science and Research Branch, Islamic Azad University, Tehran, Iran*
*2. Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran*

**Background:** Hepatitis C virus (HCV) is associated with several hepatic and extrahepatic disorders, including several malignancies. Many studies showed that T-cell responses to HCV nonstructural protein 3 (NS3) play an important role in clearing acute HCV infections, thus NS3 can be considered as a suitable candidate antigen for development of a HCV therapeutic vaccine. However, there are limitations for penetration of NS3 protein into the cells without a suitable delivery system. In this study, we used Cady-2 cell penetrating peptide (CPP) which harbors highly hydrophobic and positively charged residues for the controlled delivery of HCV NS3 antigen, *in vitro*.
**Methods:** The expression of HCV NS3 was performed using prokaryotic pET expression system, and detected by SDS-PAGE and western blotting. Its purification was carried out using affinity chromatography under denaturing condition. The NS3/ Cady-2 complexes with different molar ratios were individually formed at room temperature. The presence of the complexes was confirmed by SDS-PAGE electrophoresis. Moreover, their size and morphology were analyzed with a scanning electron microscope. The efficiency of HCV NS3 transfection using Cady-2 was evaluated by SDS-PAGE and western blotting in HEK-293T cell line.
**Result:** Generally, our data indicated a clear band of ~ 32 kDa for NS3 protein in SDS-PAGE and western blot analysis. SEM data confirmed the formation of discrete nanoparticles with a size of ~200-250 nm. The dominant band of ~ 32 kDa was detected in the transfected cells with NS3/Cady-2 nanoparticles using the anti-NS3 polyclonal antibody. The corresponding band was not detected in the un-transfected cells and transfected with NS3 protein alone.
**Conclusion:** These data suggest Cady-2 CPP as a possible effective protein delivery system for development of therapeutic HCV vaccine.

**Keywords:** Hepatitis C virus, Nonstructural protein 3, Cell penetrating peptide

ICIA2018\Immunology of Infectious Diseases Bacteria & Fungi\Poster\7970
**7970**

# The Survey of Brucellosis in Suspicious Patients Who Referred to a Medical Diagnostic Laboratory in Ahvaz, Southwest of Iran (2012-2017)

Abdolhussein Shakurnia[1], Asadollah Baesi[2], Sara Iranparast[1], Masomeh Tehrani[2]

1. *Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
2. *Department of health education, culture and research (ACECR), Branch of Khuzestan, Ahvaz, Iran*

**Background:** Brucellosis is a bacterial disease caused by pathogenic *Brucella* species. It is a zoonotic disease causing serious public health and economic problems in many parts of the world. To address the lack of recent data on human brucellosis in the region and explore changes in the epidemiology presentation, we conducted a survey on suspected brucellosis patients. The aim of this study was to survey the Brucellosis in Suspicious Patients Who Referred to a Medical Diagnostic Laboratory in Ahvaz, Southwest of Iran in a six years' period from 2012 to 2017.
**Methods:** This study was retrospective cross sectional-descriptive. In this epidemiological study, the required information of all patients who were suspected to Brucellosis by physicians was extracted over the last 6 years by laboratory software. The diagnosis of Brucellosis was performed using serological Wright test; and raised antibody level over titer of >1:80 were considered significant of having Brucellosis. The data was analyzed in SPSS 18 software by Chi-square tests.
**Results:** Of 17872 surveyed patients with the mean age of 39.14±16.43 years, 4938(27.6%) were male. According to Wright's test, 422 patients were diagnosed, the prevalence of brucellosis was 2.42% in this study. Patients had the titers of 1.80, 1.40 and 1.40 for Wright, 2-Mercapto ethanol (2ME) and Coombs tests respectively. Most participants were in the age group of 15-24 years and 69.4% were male. The lowest disease occurrence observed in winter (18%) and the highest frequency observed in autumn (28%). Frequencies of Brucellosis has gradually decreasing trend, so that the percentage of cases recorded during 2012 to 2016 was 4.2% to 1.05%, respectively. This difference was not statistically significant.
**Conclusion:** This study revealed that brucellosis is not prevalent in Ahvaz city. The incidence of brucellosis in the Ahvaz was less than the national average, and Khuzestan province classified to have very low incidence.

**Keywords:** Brucellosis, Prevalence, Epidemiology, Ahvaz, Iran

ICIA2018\Immunology of Infectious Diseases Bacteria & Fungi\Poster\8050
**8050**

# A Survey on Goat and Sheep Milk Contamination with *Brucella Melitensis* from Urmia

Javad Alizadeh[1*], Amir Tukmechi[2]

*1. M.Sc. student, Department of Microbiology, Division of Bacteriology, Faculty of Veterinary Medicine, Urmia University*
*2. Associate Professor, Department of Microbiology, Division of Bacteriology, Faculty of Veterinary Medicine, Urmia University*

**Background:** Brucellosis is an important zoonosis in animal and human. The prevalence of brucellosis is high worldwide and it's endemic in some country such as Iran. Milk, milk products and meat are the most important source for Brucella. The aim of this study was to determine goat and sheep milk contamination with *Brucella melitensis* from different zone of Urmia (North, South, West and East) by milk ring test technique.
**Methods:** So, 202 samples were taken and transferred to the laboratory on ice immediately. At first milk samples were stored at 4 ºC and their contamination with *B. melitensis* were screened by ring test.
**Results:** Results showed all of 202 milk samples (120 samples for sheep and 82 samples for goat) sixty one samples were contaminated with *B. melitensis*. Also, result documented that contamination in sheep and goat milk were 21.6 (26 samples) and 42.6 (35 samples) respectively.
**Conclusion:** It's concluded that the prevalence of *B. melitensis* in goat and sheep milk from Urmia are very high and for confirmation complementary test such as culture and polymerase chain reaction should be used.

**Keywords:** *Brucella melitensis*, Goat, Milk, Sheep, Urmia

ICIA2018\Immunology of Infectious Diseases Bacteria & Fungi\Poster\8107
**8107**

# Effect of Stimulation with Helicobacter pylori and Lactobacillus Acidophilus Antigens on the Production of Various Cytokines by Patient's PBMCs with Abdominal Aortic Aneurysm in Co-culture with Endothelial Cells

Hamid Aria[1,2], Mehdi Kalani[3], Hossein Hodjati[4], Mehrnoosh Doroudchi[1]

1. *Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Bioinformatics and Computational Biology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
3. *Prof. Alborzi Clinical microbiology research center, Nemazee hospital, Shiraz University of Medical Sciences, Shiraz, Iran*
4. *Department of Vascular Surgery, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Probiotics are of great interest in the prevention and treatment of metabolic and inflammatory diseases. In this study we investigated the effect of *Lactobacillus acidophilus* on the pattern of cytokine production by PBMCs in the co-culture with endothelial cells.
**Methods:** PBMCs were isolated from 5 men with diagnosis of AAA and 5 men with normal/insignificant angiography, CT-Scan and Ultrasonography results. EC cells were extracted from umbilical cords by collagenase method (HUVEC). PBMCs were cultured in plates with bacterial extract of CagA+ *Helicobacter pylori with and without Lactobacillus extract* in co-culture with HUVEC cells for 48 hrs. Then, the supernatant were removed to measure IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F, IL-21, IL-22, IFN-ɣ and TNF-α using a commercial fluorescent-labeled bead assay.
**Results:** CagA+ *Helicobacter pylori as well as Lactobacillus acidophilus* induced higher levels of IL-9 by controls' PBMCs in co-culture with HUVECs than patients (P=0.0.5 and P=0.01). *Lactobacillus* induced higher production of IFN-ɣ by PBMCs of controls but co-culture condition and/or presence of CagA increased its production by patients' PBMCs as well. Both CagA+ *and Lactobacillus* extracts decreased IL-21 production by PBMCs' of patients in the co-culture with HUVECs but not in single culture
**Conclusion:** IL-21 is known to enhance survival of endothelial cells but in patients with AAA, its production by stimulated PBMCs was decreased in the co-culture. The significance of the decrease in IL-21 production in AAA development needs further investigation.

**Keywords:** Abdominal aortic aneurysm, Cytokines, Helicobacter pylori, Lactobacillus acidophilus

ICIA2018\Immunology of Infectious Diseases Bacteria & Fungi\Poster\8108
**8108**

# Elevated levels of IL-6 and IL-9 in the Sera of Patients with AAA do not Correspond to their Production by Peripheral Blood Mononuclear Cells

Hamid Aria[1,2], Mehdi Kalani[3], Hossein Hodjati[4], Mehrnoosh Doroudchi[1]

1. *Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Bioinformatics and Computational Biology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
3. *Prof. Alborzi Clinical microbiology research center, Nemazee hospital, Shiraz University of Medical Sciences, Shiraz, Iran*
4. *Department of Vascular Surgery, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Abdominal Aortic Aneurysm (AAA) is the local dilatation of abdominal aorta. AAA is an inflammatory condition in which cytokines may play a pathogenic role.
**Methods:** Peripheral Blood Mononuclear Cells (PBMCs) and serum were isolated from 5 men with diagnosis of AAA and aortic dilation greater than 5.5 centimeters, and 5 men with normal/insignificant angiography, CT-Scan and Ultrasonography results. The supernatant of PBMCs and sera were used to measure IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F, IL-21, IL-22, IFN-γ and TNF-α using a fluorescent-labeled bead assay.
**Results:** The mean serum IL-6 and IL-9 levels were significantly higher in patients than controls (P=0.007 and P=0.007, respectively). While serum TNF-α level was not different between groups, its production by patient's PBMCs was significantly lower than controls (P=0.047). The mean serum levels of IL-10 and IFN-γ in patients were marginally higher than controls (P=0.055, P=0.055, respectively). Mean serum IL-2 level was not different between the groups but its production by PBMCs of patients was significantly higher than the control group (P=0.047).
**Conclusion:** Our study showed alteration in the levels of cytokines from inflammatory, Th1, Th2 and Th17 subtypes in the sera of AAA patients. The production of IL-6, IL-9, IFN-γ and IL-10, however, was not solely attributed to the PBMCs. Therefore, participation of other cells in the tissue or blood should be considered.

**Keywords:** Abdominal aortic aneurysm, IL-9, IL-10, Serum

ICIA2018\Immunology of Infectious Diseases Bacteria & Fungi\Poster\8177
**8177**

# Frequency of *Staphylococcus Aureus* Nasal Carriers in Patients with Allergic Rhinitis and Healthy People

Sahar Mohammadi[1], Habib Soheyli[2], Ehsanollah Ghaznavi Rad[3,1,*]

1. *Department of Microbiology and Immunology, School of Medicine, Arak University of Medical Sciences, Arak, Iran*
2. *Depertment of pediatrics, School of Medicine, Arak University of Medical Sciences, Arak, Iran*
3. *Molecular and Medicine Research Center, Arak University of Medical Sciences, Arak, Iran*

**Background:** Allergic rhinitis is the most common allergic disease in the breathing organ of atopic patients due to an IgE release during inflammatory dysfunction in the nasal mucosa. The most common symptoms of the disease include nasal congestion, itching and nasal congestion, sneezing and runny nose and eye pruritus. There are evidences that some microorganisms, such as Staphylococcus aureus, contribute to the development of allergic rhinitis due to the ability to produce toxins, enzymes and virulence factors. Therefore, the aim of this study is to evaluate the frequency of Staphylococcus aureus nasal carriage in patients with allergic rhinitis and healthy people.
**Methods:** A sample of 100 patients with allergic rhinitis who referred to the allergic clinic in Arak city was subjected to swab specimens that were taken from the anterior part of the nose and then identified by standard methods of microbiology. Also, 100 healthy people who did not have any type of allergy diagnosis were included in the study as control group.
**Results:**Of the 100 patients with allergic rhinitis, 17 (17%) and 20 healthy subjects (20%) were carriers of Staphylococcus aureus.
**Conclusion:** Although there was no difference in the frequency of Staphylococcus aureus in patients with allergic rhinitis and healthy individuals, it is necessary to evaluate the typing and virulence factors of isolated *S. aureus* for any correlation.

**Keywords:** *Staphylococcus aureus*, Nasal carriers, Allergic rhinitis

ICIA2018\Immunology of Infectious Diseases Bacteria & Fungi\Poster\8240
**8240**

# The Survey of Typhoid Fever in Suspicious Patients who Referred to a Medical Diagnostic Laboratory in Ahvaz, Southwest of Iran (2012-2017)

Abdolhussein Shakurnia[1], Sara Iranparast[1], Asadollah Baesi[2], Samaneh Salehi pour[2], Mehri Ghafourian[1]

*1. Department of Immunology, School of Medicine, University of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*
*2. Department of health education, culture and research (ACECR), Branch of Khuzestan, Ahvaz, Iran*

**Background:** Typhoid fever is a systemic infection caused by *Salmonella species* and is an important cause of illness and death, particularly among children and adolescents. Typhoid fever remains an important public health problem in the world especially in the population from developing countries. The aim of this study was to determine the prevalence of Typhoid fever amongst suspicious patients who referred to a medical diagnostic laboratory in Ahvaz, Southwest of Iran in a six years' period from 2012 to 2017.
**Methods:** This study was cross sectional-descriptive. In this epidemiological study, the required information of all patients who were suspected to Typhoid fever by physicians was extracted over the last 6 years by laboratory software. The diagnosis of Typhoid fever was performed using serological Widal test; and raised anti-O antibody level over titer of >1:80 was considered significant of having typhoid fever. The data was analyzed in SPSS 18 software by Chi-square tests.
**Results:** Of 6870 surveyed patients with the mean age of 36.83±16.74 years ،4435(64.6%) were male. The prevalence rate of typhoid fever based on serological test was 2.2%. The trend of prevalence rate of typhoid fever has increased from 0.9% to 5.1% in 2012 and 2017, respectively. (p=0.001). It was observed that prevalence of typhoid fever was higher in females with 106 (2.4%) than males 44 (1.8%) patients (p=0.066). Similarly, that prevalence of typhoid fever was observed higher in spring months (p=0.129). It was also observed that prevalence of typhoid fever was significantly higher among the patients <15 years old (p=0.034). It was concluded that prevalence of typhoid fever has increased among the youths who consume unsafe drinking water and food from outside source.
**Conclusion:** Typhoid fever is an important public health concern in Ahvaz, southwest Iran. This surveillance tool could have wide applications for surveillance for Typhoid fever in developing countries.

**Keywords:** Prevalence, Typhoid fever, Ahvaz, Southwest Iran

ICIA2018\Immunology of Infectious Diseases Bacteria & Fungi\Poster\8265
**8265**

# First-line Treatment of Helicobacter Pylori in Lebanon: Comparison of Bismuth-containing Quadruple Therapy Versus 14-days Sequential Therapy

Mahdi Tarhini*[1], Mohammad Fayyad Kazan*[2], Mahmoud Mokbel[4], Ghada Kchour[3]

*1. Faculty of Health Sciences V, Lebanese University, and director of Clinical Pathology and Blood Banking Department , Raee Hospital, Saida , Lebanon*
*2. Institut de Biologie et de Médecine Moléculaires, Université Libre de Bruxelles, 6041 Gosselies, Belgium*
*3. Laboratory of Cancer Biology and Molecular Immunology, Faculty of Sciences I, Lebanese University, Hadath, Beirut, Lebanon*
*Gastroenterology Department, Raee Hospital, Saida, Lebanon*

**Background:** Helicobacter Pylori (H. Pylori) is the most common cause of peptic ulcer disease (PUD) and represents a strong risk factor for gastric cancer. Treatment of H. Pylori is, therefore, a persistent need to avoid serious medical complications. Resistance to antibiotics remains to be the major challenge for H. Pylori eradication.
**Methods**: In this study, we determined the prevalence of H. pylori infection and evaluated H. pylori eradication efficacy of bismuth-containing quadruple therapy (Pylera) versus 14-days sequential therapy in treatment naïve-Lebanese patients. 1030 patients, showing symptoms of peptic ulcer (PU) and gastritis, underwent 14C-Urea Breath Test and esophagogastroduodenoscopy to examine H. Pylori infection and gastrointestinal disorders. Among the H. Pylori-positive patients 60 individuals were randomly selected, separated into two groups (each consisting of 30 patients) and treated with either bismuth-containing quadruple therapy or 14-days sequential therapy.
**Results:** We show that of the 1050 patients tested: 46.2% were H. pylori-positive, 55% had gastritis, 46.2% had both gastritis and H. pylori infection, 8.8% had gastritis but no H. pylori infection, 44.9% had neither gastritis nor H. pylori infection. Following the 14-days sequential therapy, the eradication rate was significantly higher than that obtained upon using bismuth-containing quadruple therapy [80% (24/30) versus 50% (15/30), $\chi 2=5.93$, $P=0.015$].
**Conclusion:** we determined H. pylori and gastritis prevalence among Lebanese PU-patients and showed that 14-days sequential therapy is more efficient than bismuth-containing quadruple therapy in terms of H. Pylori-eradication.

**Keywords:** H. pylori, Peptic ulcer, Pylera, Sequential Therapy.

ICIA2018\Immunology of Infectious Diseases Bacteria & Fungi\Poster\8270
**8270**

# *Clostridium Difficile* Toxin Assay in Patients Referred to Mahdiyeh Hospital, Tehran, Iran

Ezzati Mehmandust Olya Mohammad[1], Delavari Mahdi[1], Arbabi Mohsen[1], Fatemeh Goudarzi[2], Ebadi Maryam[3]

*1. Department of Parasitology and Mycology, School of Medicine Kashan University of Medical Sciences, Kashan, Esfahan, Iran*
*2. Department of Parasitology and Mycology, School of Medicine Shiraz University of Medical Sciences, Shiraz, Iran*
*3. Department of Parasitology and Mycology, School of Medicine Qazvin University of Medical Sciences, Qazvin, Iran*

***Background:*** *Clostridium difficile* colonizes the large bowel of patients undergoing antibiotic therapy and produces toxin A (Tissue Damaging Enterotoxin) and toxin B (Cytotoxin). *C. difficile* is the leading cause of hospital-acquired diarrhea, known as *C. difficile*-associated disease. Diagnosis of *C. difficile* is based on Glutamate Dehydrogenase (GDH) as antigen and has been shown to be very effective because all strains produce high amount of this enzyme.
**Method**: In this study, *clostridium difficile* toxin assay was performed for 55 patients referred to Mahdiyeh Hospital, Tehran in 2016-2018. Toxin assay conducted by Clostridium K-set (Corisbio-Belgium) using membrane technology with colloidal gold contains antibodies against GDH antigen.
**Results:** Fecal samples from 55 patients (26 females: 29 males) were collected and *clostridium difficile* toxin assay preformed. Mean age of patients was 51.8 years. There was no significant relation between age (P value=0.45), sex (P value: 0.8) and positive results. 20 patients (36.3%) were positive for *clostridium difficile* toxin Antigen vs. 35 patients (63.7%) negative. The most important risk factor associated with the infection was previous use of antibiotics.
**Conclusion:** Positive results for *clostridium difficile* toxin assay is rather high in patients referred to Mahdiyeh Hospital, south of Tehran. Clostridium difficile infection should be considered in patients with diarrhea and traditional risk factors associated with this disease.

**Keywords**: *Clostridium difficile,* Diarrhea, Toxin assay.

ICIA2018\Immunology of Infectious Diseases Bacteria & Fungi\Poster\8545
**8545**

# Induction of Regulatory T and B cells in Patients with Brucellosis

Sanaz Jourghasemi[1], Fariba Keramat[2], Ghasem Solgi[1], Mehrdad Hajilooi[1*]

1. *Immunology Department, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
2. *Department of Infectious Diseases; Department of Pharmacology, Hamedan University of Medical Sciences*

**Background**: Brucellosis is the most common bacterial zoonotic infection which caused by intracellular gram-negative bacteria of the genus *Brucell*a. The aim of this study was to analyze the frequencies of regulatory T cells and regulatory B cells in brucellosis patients compared to healthy controls.
**Method**: Twenty seven patients with brucellosis and twenty healthy controls were included in this case-control study. Frequencies of $CD4^+$ $CD25^+$ $FOXP3^+$ T cells and $CD5^+$ $CD19^+$ $CD38^{Hi}$ $CD1d^{Hi}$ B cells in peripheral blood samples of both groups of the study were determined by flow cytometric analysis.
**Results**: We observed a higher significant frequency of $CD4^+CD25^+FOXP3^+$ T cells in the brucellosis patients compared to healthy controls (2.130 ± 0.1609% vs 0.2123 ± 0.02288%, $P$=0.0001). Similarly, percentage of $CD5^+CD19^+CD38^{Hi}CD1d^{Hi}$ B cells was significantly higher in patients compared to healthy controls (6.313 ± 0.6823% vs 0.7700 ± 0.1139%, $P$=0.0001).
**Conclusion**: Our results suggested that *Brucella Species* may induce regulatory responses to evade immune system and survive in the host.

**Keywords**: Regulatory T cells, Regulatory B cells, *Brucella*

ICIA2018\Immunology of Infectious Diseases Bacteria & Fungi\Poster\8588
**8588**

# Comparison of Naloxone and Propanol on the Immunity Responses of Wistar Rats Challenged with S19 Vaccine

Samira Zand, Meysam Abtahi Foroushani , Masumeh Ahanjan, Sara Golbahari, Nasrin Nourollahzadeh

*Department of Microbiology, Faculty of Veterinary Medicine, University of Urmia*

**Background**: Alum as a most popular human vaccine can only produce humoral immunity. The immunomodulatory effects of Naloxone and Propanol reported in some pervious study. This study was designed to investigate the Adjutant potential of naloxone and propanol on the immunity responses of Wistar Rats challenged with live *brucella abortus bovis* vaccine (S19).
**Materials and Methods**: In this experimental study, 15 Wistar rats were divided into 3 groups according to the following conditions: 1) Naloxone treatment group; 2) Propranolol treatment group; 3) Control group. In the two treatment groups, on day zero, the treatment solution and the vaccine containing 2% brucella was injected into the peritoneum of the mouse. On the 15th day of injection, the vaccine S19 was injected to the foot of each rat. In control group, on day zero, the vaccine containing 2% rubella was injected into the peritoneum of the mouse and on the 15th day of injection, the vaccine S19 was injected to the foot of each rat. Rats were kept for 20 days and the event of symptoms of the disease and their survival were examined.
**Results**: DTH, specific antibody titer and respiratory burst were more prominent in naloxone treated group compared to control group. Nevertheless, there was no significant difference between propranolol and control groups.
**Conclusion:** The use of naloxone is a promising strategy to develop more favorable immune stimulation.

**Keywords**: Respiratory burst, Cell-mediated immunity, Propranolol, Naloxone,

ICIA2018\Immunology of Infectious Diseases Bacteria & Fungi\Poster\8605
**8605**

# Antimicrobial Effects of Acetone Extract Rasha Gavan *(B.M.V)* in Kurdish Ethnomedicine on E. Coli & S. Aureus

Mehrdad Pooyanmehr[1], Amir Amiri Paryan[2]*, Erfan Bahrami[3], Mohammad Reza Khedri[3]

*1. Department of Basic Sciences and Pathobiology, Microbiology section, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran*
*2. Veterinary Student, Pathobiology & Basic Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran*
*3. Veterinary Student, Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran*

**Background:** There are studies on the biological activities of tested plants. Generally, Astragalus (Gavan) species have been used in Kurdish folk medicine to treat various conditions such as diabetes, gastric ulcer , hemorrhoids, inflammation, healing wounds, diuretic ,treatment of upper respiratory, tract infections, improve physical endurance ,lower blood pressure, antioxidative, antiviral, anti-inflammatory , analgesic ,hypotensive , sedative, cardiotonic, hepato protective to , immune resistance and stimulant properties. The aim of this study was to evaluate the antibacterial activities of endemic plants found in Kurdish Ethno medicine.
**Methods:** The aerial parts of the endemic plants rasha gavan (*B.M.V*) were collected from Mountains at Kermanshah Province and air-dried in the shade.solvent acetone, were used for extraction. The plant sample were soxhlet extracted at 60 °C for 12 h. The extract were then vacuum evaporated. The extracts were prepared in different dilutions (800,400, 200,100, 50 and 25mg/ml). After preparing various concentrations of extract the method agar diffusion and Disk diffusion, MIC and MBC of dilution series for E.coli (ATCC10536) and S. aureus (ATCC25923), were used in Mueller Hinton Broth. Tetracycline and Vancomycin as a positive control and 10% of dimethyl sulfoxide (DMSO) was used as a negative Control respectively.
**Results:** Disk diffusion test results showed ,Average Diagonal zone of growth inhibition dilution to 100, 200,400 and 800mg/ml Extract ,were respectively7±0/57,7/33±0/57, 8/66±0/57, 10/66±0/57 mm and Vancomycin 19±0/33mm for S. aureus and7/66±0/57, 10±0/32 mm(400 &800mg/ml) and Tetracycline17±0/39mm for E.Coli. The results showed an increased concentration, the Diagonal zone of growth inhibition increased. The acetone extract was observed the greatest impact inhibition zone diameter 10/66±0/57 mm on S. aureus and 10±0/32 mm on E. coli strain. The results showed MIC value for S. Aureus and for E. coli 800 mg/ml.
**Conclusion:** The results achieved in this study showed that Rasha Gavan Acetonic extract have Antibacterial Activity and growth inhibitory impact on both E. coli and S. aureus (S.aureus is known as more sensitive to the extract than E.Coli).

**Keywords**: Acetonic Extract, Rasha Gavan, E.Coli, S. aureus, MIC, MBC

ICIA2018\Immunology of Infectious Diseases Bacteria & Fungi\Poster\8671
**8671**

**Presentation of Like Melanoma Onychomycosis due to *Fusarium solani* Species Complex**

M. Soltania, A.R. Khosravia, A. Sharifzadeha

*Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

**Background**: Onychomycosis is a fungal infection of the nail caused by molds, yeasts and dermatophytes.
**Method:** In this study, we report a case with unusual fingernail onychomycosis in a 31-year-old Iranian woman suspected having nail bed melanoma. Biopsy was performed from the nail bed and no any evidence of melanoma was reported by the pathologist.
**Result:** In direct microscopic examination (DME) and culture numerous hyaline septated hyphae and canoe-shaped conidia on conidiophores were observed, respectively. Diagnosis was confirmed by molecular DNA sequencing and *Fusarium solani species complex* was identified as etiological microorganism.

**Keywords**: Onychomycosis, Melanoma, Non-dermatophyte fungi, *Fusarium*, DNA sequencing, Itraconazole

ICIA2018\Immunology of Infectious Diseases Bacteria & Fungi\Poster\9932
**9932**

# Detection of IS*903*, IS*26* and IS*Ecp1* Elements in CTX-M-Producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from Leukemic Patients in Iran

Mahdaneh Roshani[1], Hossein Goudarzi[1], Ali Hashemi[1], Abdollah Ardebili[2], Soroor Erfanimanesh [3], Aghil Bahramian[1]

1. *Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
2. *Department of Microbiology, Faculty of Medicine, Golestan University of Medical Sciences, Gorgan, Iran*
3. *Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** The ability of Extended Spectrum Beta-Lactamases (ESBLs) production is one of the main mechanisms for the emergence of antibiotic resistance in *E. coli* and *K. pneumoniae*. The aim of this study was to evaluate the occurrence of IS903, IS26 and ISEcp1 insertion elements among the CTX-M-producing *E. coli* and *K. pneumonia* isolates from leukemic patients in Tehran.
**Methods:** Eighty *E. coli* and *K. pneumoniae* isolates were recovered from patients admitted in hospitals in Tehran. Antibiotic susceptibility tests were performed by Kirby-Bauer disc diffusion and broth microdilution methods. Detection of ESBL producers was evaluated by phenotypic confirmatory test. The presence of IS*903*, IS*26* and IS*Ecp1* insertion elements in CTX-M-positive *E. coli* and *K. pneumoniae* isolates were investigated by PCR-sequencing methods.
**Results:**The rate resistance of 80 *E. coli* and *K. pneumoniae* isolates against the 9 antibiotics was as follows: 100% to ampicillin, 15% to amikacin, 51% to ciprofloxacin, 30% to gentamicin, 58% to ceftriaxone, 10% to imipenem, 63% to cefotaxime, 51% to levofloxacin and 55% to ceftazidime. Using phenotypic confirmatory test, 51 (63.75%) isolates were ESBL producers. The prevalence of CTX-M-1, CTX-M-2, CTX-M-9, CTX-M-8 and CTX-M-25 genes was 87.5%, 13.75%, 23.75%, 10 and 0%, respectively. IS*903*, IS*26* and IS*Ecp1* elements were detected in 93.75%, 71.25% and 100% of isolates, respectively.
**Conclusion:** This study indicates that the occurrence of antibiotic resistance, IS and CTX-M-producing *E. coli* and *K. pneumoniae* isolates could be a major concern and highlights the need of infection control measures.

**Keywords:** Leukemic patients, Escherichia coli, Klebsiella pneumoniae, Extended-spectrum beta-lactamases, Insertion Sequence

ICIA2018\Immunology of Infectious Diseases Bacteria & Fungi\Poster\9934
**9934**

# The First Report of Extended-Spectrum β-Lactamase (ESBL) Genes in an *Escherichia coli* Isolate from a One-Month-Old Infant with Acute Lymphoblastic Leukemia (ALL) in Iran

Mahdaneh Roshani[1], Hossein Goudarzi[1], Ali Hashemi[1]

*Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background** Bloodstream infection is one of the most life-threatening complications and remains the frequent cause of treatment failure in children with acute lymphoblastic leukemia. In this study, we describe the isolation of an extended-spectrum β-lactamase (ESBL) producing *Escherichia coli.*
**Methods:** Antimicrobial susceptibility tests were also determined on the isolate by the Kirby-Bauer disk diffusion method. In addition, ESBL production was examined for the isolate using the Combination Disk Diffusion Test (CDDT). PCR was used to screen the presence of CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25, ISEcp1, $IS_{26}$, and $IS_{903}$ genes in this isolate.
**Results:** We found an ESBL-producing *E. coli* in a 1 month old infant with blood cancer that carried CTX-M-1 group enzymes.
**Conclusion:** Our finding emphasizes the need to have more precise screening methods to identify the causative infectious agent at early stage of infection to choose the appropriate treatment in these severely immunocompromised patients.

**Keywords:** Bloodstream infection, Extended-spectrum β-lactamase, *Escherichia coli,* Acute lymphoblastic leukemia

# Immunology of Infectious Diseases

## Viruses

## Poster Presentation

ICIA2018\Immunology of Infectious Diseases Viruses\poster\5307
**5307**

# Evaluation of hepatitis B viruses serologic markers in thalassemia and dialysis patients of Mazandaran province

Sara Mohamadnezhad[1], Nour Amirmozafari[2], Mahdi Asmar[3], Mohtaram Nasrollahi[4]

1. *Faculty of Medical Sciences, Sari Branch, Islamic Azad university, Sari, Iran*
2. *Department of Microbiology, Iran University of Medical Sciences, Tehran, Iran*
3. *Department of Microbiology, Pasteur Institute of Iran, Tehran, Iran*
4. *Department of Microbiology, Mazandaran University of Medical Sciences, Sari, Iran*

**Background**: Nearly 300 million people worldwide are carriers of the hepatitis B virus (HBV). Thalassemia and dialysis patients are one of the most important occupational groups at risk for the virus. Despite vaccination, in many of them, antibody is reduced and while the exposure to infected materials, there is a possibility of infection to HBV. This study examines some of hepatitis B serologic markers among special patients of Mazandaran province.
**Methods**: 94 special patients were enrolled to our study. For all patients in the study, a questionnaire including demographic information, history of the dose of the vaccination for hepatitis B, blood transfusion and blood products and surgical history were completed. 5ml of venous blood was taken from each student and serum was separated and stored in -20°C. For all samples, ELISA test operated for anti-HBs and anti-HBc and HBsAg.
**Results:** Among 94 under studied thalassemia and dialysis patients, 55 people (58.5%) were male and 39 people (41.5%) were female. In these patients 57 people (60.6%) were dialysis and 37 people (39.4%) were thalassemia, in which 4 people (7%) of dialysis patients and 8 people (21.6%) of thalassemia were infected by HBV. 12 people (22.8%) of samples were positive by the presence of HBsAg, 54 people (57.4%) of samples were positive by the presence of Anti-HBs and 13 people (13.8%) of samples were positive by the presence of anti-HBc.
**Conclusion:** About 12.8% of understudied patients were HBV positive and also 42.6% of them were sensitive to HBV. Therefore, screening of them for HBV antibody is recommended before any blood products usage. Also, in order to adequate protection of the patients, the serologic studies is necessary during blood production usage.

**Keywords**: Hepatitis B Viruses, Serologic makers, Vaccinations, Thalassemia and Dialysis.

ICIA2018\Immunology of Infectious Diseases Viruses\poster\6315
**6315**

# Increased of PDGF-β gene expression is associated with ATLL development in HTLV-1 carriers

Atabati Hadi [1, 2], Rafatpanah Houshang [1, 2]*, Allahyari Abualqasem[3], Davoud Ghaffarian[1, 2]

*1. Department of Immunology, School of Medicine, Mashhad University of Medical Sciences(MUMS)*
*2. HTLV-1 Center and related diseases, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*3. Department of Hematology, Ghaem Hospital ,Mashhad, School of Medicine, University of Medical, Sciences(MUMS)*

**Background:** Human T lymphotropic virus (HTLV-1) is a human retrovirus which is associated with adult T cell leukaemia (ATLL). ATLL is a malignancy lymphoproliferative disease which infects CD4+ T cells. It is not clear why the majority of HTLV-1 infected individuals remain healthy carries and minority develop ATLL. It has been shown that angiogenesis is increased in ATL patients and the plasma levels of angiogenesis factors such as VEFG are upregulated in the patients. In the present study, we examined the mRNA expression of another angiogenesis factor, PDGF (platelet derived growth factor) and its receptors in patients with ATL and HTLV-1 healthy carriers (HACs).
**Materials and Methods:** Peripheral blood mononuclear cells (PBMCs) were isolated from ATLL patients and asymptomatic carriers by using ficoll-hytopaque density centrifugation. RNA was extracted and cDNA was synthesised. A real-time PCR TaqMan method was designed and optimized for evaluation of PDGF, PDGF-R and Tax gene expression. HTLV-1 proviral load was also quantified in patients with ATLL and carriers.
**Results:** The mRNA expression of Tax was significantly higher in ATLL patients than HACs ($p$=0.01). The HTLV-1 proviral load was higher in ATLL patients compared to HTLV-1 carriers ($p$= There was significant increases in PDGF gene expression in HACs than ATLL patients ($p$=0.002). The expression of PDGF was higher in HTLV-1 carries compared with ATLL patients and significant differences was observed between two groups ($p$<0.001). There was no significant differences in PDGF-R gene expression between ATL patients and HACs (p>0.05).
**Conclusion:** High expression of PDGF-R in HTLV-1 carriers might block the PDGF signalling pathway and prevent ATLL development.

**Keywords:** HTLV-1, PDGFβ, PDGFβ-R

ICIA2018\Immunology of Infectious Diseases Viruses\poster\6328
**6328**

# Dissection of plasmacytoid dendritic cell's responses to influenza virus based network analysis

Fatemeh Hadi[1, 2], Rasoul Godini[1], Hossein Fallahi[1]

*1. Department of Biology, Faculty of Sciences, Razi University, Kermanshah, Iran*
*2. Department of Stem Cells and Developmental Biology, Brain and Cognitive Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran*

**Background:** Plasmacytoid dendritic cells are responsible for defense against viral infection in respiratory organs. These cells secrete massive amount of interferon-α through innate immune responses. Flu, as a life threating respiratory disease through the world, is caused by influenza virus. It has been known that plasmacytoid dendritic cells are responsible for protecting lung against flue. In here, using network analysis, we dissected transcriptome profile of plasmacytoid dendritic cells affected by influenza virus to reveal underlying molecular mechanisms.
**Methods:** In order to do this study, we used freely available microarray dataset numbered GSE68849. Differentially expressed genes were determined using GEO2R tool of NCBI. Protein-protein interactions were constructed by validated data from STRING database. Annotations for gene ontology and disease were obtained from DAVID database. The networks were visualized and analyzed by Cytoskape and Gephi software programs. Protein complexes were determined by ClusterONE plug-in of Cytoscape. Network centrality factors including degree and betweenness were used to detect hub genes.
**Results:** 1127 differentially expressed genes were detected, 605 and 522 down- and up-regulated, respectively. Using medium confidence of STRING database, a network of protein-protein interaction with 707 nodes and 2552 edge was constructed. Overlapping neighborhood expansion algorithm detected 15 modules. Centrality factors analysis showed 33 hub genes including JUN, HSP90AA1, SYK, MAPK3 and IL8. Annotation of the genes showed defense response to virus, innate immune response, and type I interferon signaling pathway are from processes which the genes are involved in.
**Conclusion:** These results reveal principal information about molecular mechanisms involved in response to influenza virus that can help to development a better treatment of this disease.

**Keywords:** Immune response, Influenza, Network, Protein-protein interactions

ICIA2018\Immunology of Infectious Diseases Viruses\poster\6333
**6333**

# Relative Frequency of Cytomegalovirus (CMV) in Tissue Samples of Women with Breast Cancer in Sanandaj, Iran

Maryam karimi[1, 2], Seyede Zeinab Hosseini[3*], Bahram Nikkhoo[4], Farid Soleimani Mohammadi[5]

1. *M.SC Microbiology, School of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran*
2. *Student Research Committee, Kurdistan University of Medical Sciences, Sanandaj, Iran*
3. *virology Department, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
4. *MD Pathologist, Kurdistan University of Medical Sciences, Sanandaj, Iran*
5. *Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Despite the researchers' efforts, the cause and development of breast cancer is still incompletely understood. Currently, in some reports, human cytomegalovirus has been referred as a risk factor for breast cancer. This study aimed to determine relative frequency of cytomegalovirus in tissue samples of women with breast cancer in Sanandaj county.
**Methods:** To determine the relative frequency of the human cytomegalovirus (CMV), 50 formalin-fixed tissues of breast cancer, which all were invasive ductal carcinoma, were studied using the nested-polymerase chain reaction.
**Results:** In 26 cases of breast cancer tissues (26/50), human cytomegalovirus was detected. Seventeen cases of breast cancer tissues were in a moderately differentiated stage, and nine cases had poor-differentiated stage tissues that were positive for viral DNA. At older ages (>45 years) the rate of human cytomegalovirus DNA was higher, but no significant association was seen (p=0.16). In general, due to the high prevalence of the DNA of human cytomegalovirus (58%).
**Conclusion:** in this study it is assumed that human cytomegalovirus (HCMV) has a contributing role in breast cancer; although more study is required to clearly define its part in this type of cancer.

**Keywords:** Human Cytomegalovirus (HCMV), Breast Cancer, Nested PCR, ductal carcinoma

ICIA2018\Immunology of Infectious Diseases Viruses\poster\7614
**7614**

**Evaluation of camel serum immunoglobulin on inhibition of hepatitis C virus in cell culture**

Fariba ghorbanzadeh[1, 2], Jalil Tavakkol Afshar[1, 2], Amin Reza Nikpoor[1, 2]

*1. Department of Immunology, Faculty of Medicine, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran*
*2. Immunogenetic and Cell Culture Department, Immunology Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** Hepatitis C virus (HCV) represents a major public health problem, affecting 3% of the world's population. Although the development of new therapeutic approaches to combat HCV infection has taken place, no efficient vaccines exist yet. Therefore this study aimed to evaluate the role of immunoglobulins extracted from camel serum, the first in cell culture, then in future research in animal models and in clinical phase done as a pilot for the treatment of patients with HCV infection. Camels are special animals in antibody production as they possess a unique type of IgG antibody in their serum which lack light chains as well as the heavy chain constant domain "CH1", so-called heavy chain antibodies or nanobodies.
**Methods:** First immunoglobulins were extracted from camel serum with ammonium sulfate. Then three types of experiments were performed on HuH7.5 cell. In the first process, the HCV pre-infected cells were treated with the immunoglobulins and in the second process HCV was incubated with the purified immunoglobulins and then mixed with cell at the end of the process the immunoglobulins were incubated with the cells and then exposed to HCV. Eventually with Real time-PCR method reduction or no reduction in viral titers were measured.
**Results:** The results indicate that there is a direct interaction between HCV and camel IgGs, which led to inhibition of HCV binding to the cells. Camel IgGs were capable of inhibiting the intracellular HCV replication at concentrations of 18.5 mg/ml. But IgGs could not inhibit entry the HCV into the cell.
**Conclusion:** Camel serum immunoglobulins isolated from camel serum could inhibit the HCV infectivity. Immunoglobulins inhibit the HCV infectivity through inhibiting proliferation and binding the virus to the cell but immunoglobulins could not prevent the HCV from entering the cell.

**Keywords:** Hepatitis C Virus, Camel Antibody, Infectivity

ICIA2018\Immunology of Infectious Diseases Viruses\poster\7771
**7771**

# The effects of curcumin on gene expression and serum levels of Toll like receptor -2 &4 (TLR-2 and TLR-4) in patients with myelopathy associated with HTLV-1 (HAM/TSP) and virus carriers

Marjan Zamani[1], Dr. Houshang Rafatpanah[2], Dr. Reza Boostani[3]

*1. Department of Allergy and Immunology, faculty of medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*2. Department of Allergy and Immunology, Inflammation and inflammatory Diseases Research Centre, faculty of medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*3. Department of Neurology, Ghaem Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** TLRs are innate immune receptors that are able to respond to pathogens and derived molecules of a host. Curcumin has anti-inflammatory and anti-oxidant effect which can be used for the treatment inflammatory diseases. In the present study the anti-inflammatory effects of curcumin in HAM/TSP patients by evaluation of TLR-2 and TLR-4 which have pivotal role in inflammatory response were examined.
**Materials and Methods:** The population studied included ۲۱ HAM/TSP patients before and after treatment with curcumin, 21 asymptomatic carriers, and 21 healthy controls. Peripheral blood mononuclear cells were separated by using ficoll and cDNA was synthesized. The genes expression and serum protein levels of TLR-2 and TLR-4 by real time PCR and ELISA was evaluated.
**Results:** The expression of TLR-2 was increased in treated group compared with untreated group ($p<0.05$), however no significant differences in TLR-2 serum levels was observed between two groups ($p>0.05$). Furthermore, the expression of TLR-4 was increased after treatment with curcumin compared with untreated group ($p<0.05$). No significant differences in TLR-4 serum levels was observed between two groups. The mRNA expression of TLR-4 was higher in asymptomatic carriers compared to HAM/TSP patients ($p<0.05$), however no significant differences in the serum levels of TLR-4 was observed between HAM/TSP patients and asymptomatic carriers ($p>0.05$). There were significant difference in TLR-2 and TLR-4 gene and protein expression between healthy controls and HAM/TSP, and healthy controls and carriers ($p<0.05$).
**Conclusion:** In conclusion the results of the present study might suggest that viral factors interference with curcumin in order to maintain the balance between the virus and inflammatory immune response. It seems that the expression of viral factors might also contribute in the difference between TLR-2 and TLR-4 gene expression between two groups. Further studied are needed to clarify this issue.

**Keywords:** HTLV-1, HAM/TSP, TLR-2, TLR-4.

ICIA2018\Immunology of Infectious Diseases Viruses\poster\7782
**7782**

# Evaluation of relationship between HBs Ab titer with birth weight and mode Of delivery in 3-5 years old children

Motahareh Ehsani[1], Mehdi Shahbazi[1], Mousa Mohammadnia afrouzi[1], Kowsar Smaeilnejad, Mojgan Bagherzade[1]

*Department of Immunology, School of Medicine, Babol University of Medical Sciences, Babol, I.R.Iran*

**Background:** Vaccination of hepatitis B infection has been known as a crucial step in prevention of HBV. Both environmental and genetic factors affect immune response to vaccination. Environmental factors in children include birth weight, mother age, birth season, place of residence, Feeding status and mode of birth. In this study the relationship between HBs Ab titer with birth weight and mode of delivery was assessed.
**Methods:** Peripheral blood samples were collected from 100 children between the ages of 3-5. Sera were tested for anti-HBS antibody by enzyme linked immunosorbent assay (ELISA).
**Results:** Findings demonstrated that there is no significant difference (P value>0.05) between HBs-antibody titer in caesarean-born children and vaginally-born ones and also in children with low birth weight (<2500) and high birth weight (>2500), this means that, both birth weight and mode of delivery couldn't affect on HBs-antibody titer in children with the age of 3-5.
**Conclusion:** Vaccination has been considered as an attempt in inducing immunity and protection against HBV infection. According to these findings, vaccine immune responses and Anti-HBs antibody titer in children are not affected by weight and mode of delivery, However there is not any assured conclusion and more researches is needed in this field.

**Keywords:** Vaccination, HBs Ab, Mode of delivery, Birth weight

ICIA2018\Immunology of Infectious Diseases Viruses\poster\7901
**7901**

## Sequencing of HTLV1 p12 region in HAM/TSP, ATL patients and HTLV1 carriers in Mashhad, Iran

MehranMoharreri[1],SeyedAliMirhosseini[1],SeyedRohollahArab[1],Baratali Mashkani[2],SeyedAbdolrahimRezaee[3], RezaFaridHosseini[4],HoushangRafatpanah[1]

*1. Inflammation and Inflammatory Diseases Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*2. Biochemistry and Nutrition Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*3. Immunology Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*4. Allergy Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** HTLV1-virus is associated with two important diseases ATL & HAM/TSP while most of the infected cases remain as asymptomatic carriers (ACs). Considering Mashhad is one of the HTLV-1 endemic areas in the world, here, we report genomic variability of p12 region of positive HTLV-1 patients. Our aim in this study was increase our understanding about similarities and differences of p12 genome in 3 groups of individuals with distinctive sign and symptoms.
**Methods:** Blood samples were collected from 15 individuals including 5 HTLV-1 asymptomatic carriers, 5 HTLV-1 associated with HAM/TSP and 5 patients with adult T-cell leukemia lymphoma (ATLL). DNA was extracted from the peripheral blood mononuclear cells (PBMCs) then PCR was done in p12 genes. Finally, PCR products were cloned and sequenced.
**Results:** P12 nucleotides and amino acids in 15 HTLV1 infected patients who participated in our study were strikingly similar. According to molecular analytic considerations P34L mutation in P12 gene of Mashhad HTLV1 infected patients does exist.
**Conclusion:** The results presented in this study suggest that some p12 natural mutations may be associated to HAM/TSP development and to the proviral load of HTLV-1-infected individual. These results indicate that genetic variations among HTLV-1 strains are very low. It is probable that these genetic variations, result of geographical differences among origins of HTLV-1 strains. We will hope that this valuable genomic data facilitates vaccine design against HTLV1. Moreover, there are a lot of controversial debates about function of p12 protein, so sequencing of p12 region could help researchers to explain whether different p12 genomic make up have specific effect on manifestation disorders in HTLV-1 infected people.

**Keywords**: HTLV-1, HAM/TSP, ATL, P12, DNA sequencing

ICIA2018\Immunology of Infectious Diseases Viruses\poster\7913
**7913**

## Cytotoxic and inflammatory markers determined the outcome of HTLV-1 infection

Houshang Rafatpanah[1], Asadollah Mohammadi[1], Reza Boostani[2]

*1. Immunology Research centre, Division of Inflammation and Inflammatory Diseases, Mashhad University of Medical sciences, Mashhad, Iran*
*2. Department of Neurology, Mashhad University of Medical sciences, Mashhad, Iran*

**Background:** Human T-lymphotropic virus type I (HTLV-1) is a type C retrovirus which is associated with two main types of diseases known; adult T-cell leukemia/lymphoma (ATLL) and HTLV-1 associated myelopathy/ tropical spastic paraparesis (HAM/TSP). We have previously showed that north east of Iran, Mashhad and Sabzevar, have been recognized as an endemic region of HTLV-1. It is not fully known why a small portion of HTLV-1-infected individuals develop HAM/TSP and majority remains lifelong asymptomatic carriers. The different outcome of HTLV-1 infection may be explained by the existence of viral agents, genetic background or even viral factors. High titers of HTLV-I antibody, high proviral load and high frequency of HTLV-1 specific CTL in HAM/TSP patients distinguish them from HTLV-I carriers. Among these factors, the CTL response is known to be a key factor in HTLV-I infection. Furthermore proinflammatory markers are also involved in the outcome of HTLV-1 infection.
**Methods:** In the present study, we examined the role a panel of cytotoxic markers including levels of mRNA and protein to clarify their roles in HAM/TSP patients and HTLV-1 carriers. Furthermore, we also investigated the role of inflammatory markers such as IL-6, Toll like receptors (TLR) and high mobility group box1 (HMGB1) and viral markers such as proviral load in studied groups.
**Results & Conclusion:** The results of the study showed that cytotoxic response is failed in HAM/TSP patients. Additionally, increased inflammatory markers and viral load is shown to be associated with HAM.

**Keywords**: HTLV-1, HAM/TSP, Cytotoxic markers, inflammatory markers

ICIA2018\Immunology of Infectious Diseases Viruses\poster\8051
**8051**

**Evaluation of anti-HBS titer of vaccinated students of Guilan University of medical science (2013-2014)**

Sara Rasouli[1], Masumeh Avari[2]

1. *Department of Microbiology, College of Science, Rasht Branch, Islamic Azad University, Rasht, Iran*
2. *Department of Microbiology, College of Science, Rasht Branch, Islamic Azad University, Rasht, Iran*

**Background:** Hepatitis B is a viral disease with high prevalence. Unfortunately, immunologic response to the vaccines is not sometimes perfect and it is necessary to examine the immune response.
**Methods:** In a cross-sectional study of 181 students that have been vaccinated against hepatitis B, blood samples were obtained for assessing antibody titer of HBV by ELISA. After collecting demographic data at the University of Medical Sciences, Guilan in 2014, data were analyzed by SPSS software and presented with chi-square test.
**Results:** Among 181 participants completed the three stages of vaccination against HBV, 64/7% had adequate response against hepatitis. There was no significant correlation between the immunity response and disease history, gender and other variables except on duration of vaccination ($p< 0.001$).
**Conclusion:** According to our findings health education and evaluation of immune responses against hepatitis in medical students is necessary. Also, it should be considered revaccination for persons with low titers of antibody.

Keywords: Hepatitis B, vaccination, antibodies

ICIA2018\Immunology of Infectious Diseases Viruses\poster\8098
**8098**

**A comparison of Phylogenetic of Human Immunodeficiency Virus Type 1(HIV-1) from Khorasan Razavi**

Faeze Fakour.k[1], Dr. Abdolrahim Rezaee [1]

*1. Department of Mashhad University of Medical Sciences*

**Background:** HIV is the virus with high genetic diversity, this genetic diversity because of the mutations of this virus that is produced due to the rapid proliferation and characterization of recombinant of reverse transcriptase. HIV-1 divided into four phylogenetic groups based on gag gene sequences, which are M, N, O and P groups. M Group is pandemic factor and divided to 9 subtypes (A to K) and 61 circulating recombinant forms (CRF). The HIV-1 infection in our country has been increased significantly in recent years.
**Methods:** Risk of HIV infection of the patients in this project confirmed by using ELISA and immunoblot. For this purpose, first samples were collect and then send them to the laboratory of immunology, by Sciences inflammation and inflammatory diseases Research Center in Mashhad University of Medical, genetic material (DNA) was extracted in accordance with international standard method. Then with NESTED -PCR method of gene sequences areas p17 of gag and c2-v5 env gene was amplified by using specific Primers and then was sent to Denazist Asia company for sequencing. The nucleotide sequences of gag and env genes reviewed and edited in Macrogen korea company.
**Results:** The data obtained from sequencing was arranged by MEGA6 software and phylogenetic trees where drawn with maximum-likelihood. 80% of patients who entered the study were male and 20% were female. 65% of them were in the age group 20-39 years. 65% of them were infected through intravenous drug use and 19% through sex. In terms of co-infections with other viruses, 45% co-infection were observed with hepatitis B and 45% co-infection with hepatitis C, and 10% co-infection with HTLV1.
**Conclusion:** Samples formed a significant cluster with subtype CRF35-AD from Afghanistan, subtype A1 from Pakistan, subtype F from South America, subtype A and C and of East Africa and subtype B of Eastern Europe.

**Keywords:** HIV, Phylogeny, Subtype, Iran

ICIA2018\Immunology of Infectious Diseases Viruses\poster\8171
**8171**

**Altered expression of Human Leukocyte Antigen G (HLA-G) in HIV+ drug naïve patients**

Versa OmraniNava[1], Saeid Abediankenari[1], Jamshid Yazdani[2]

*1. Department of Immunology, Immunogenetics research center, School of Medicine University of Mazandaran medical Science*
*2. Department of Biostatics School of Health, University of Mazandaran Medical Science*

**Background:** HLA-G is an immunomodulatory protein that alteration in its expression has been reported in several pathologic conditions. For as much as the results of HLA-G expression in HIV+ patients have been in consistent and some reports suggest the effects of antiretroviral Therapy (ART) on HLA-G, we investigate HLA-G expression and its clinical relevance with disease status in drug naïve patients.
**Methods:** We enrolled a total of 20 HIV+ drug naïve patients referring to the Behavior Disease Consultation Center of Mazandaran and 20 sex-Age matched healthy volunteers in this study. Blood mononuclear cells were isolated by ficoll method. After RNA isolation and cDNA synthesis, Real time PCR was carried out to assess gene expression in both groups. CD4+ cells count were determined in cases using flowcytometry. Data were analyzed by SPSS and REST softwares.
**Results:** The mean age of case and control groups were 34.6 (CI 95% 30.83-38.37) and 33.9 (CI 95% 30.66-37.14), respectively. Median CD4 count in patient was 515 (CI 95% 395-606). HLA-G expression was statistically different between groups and patients showed lower expression. There was no relation between disease status, CD4 Count and HLA-G expression in patients.
**Conclusion:** Our findings suggest that HLA-G expression is disregulated in HIV+ patients. Lower expression in patients may influence Antigen recognition process. Further follow up are needed to clarify interactions between HIV and non-classical HLA molecules.

**Keywords:** HLA-G, HIV, CD4, gene expression

ICIA2018\Immunology of Infectious Diseases Viruses\poster\8173
**8173**

# Relationship between Up-regulation of Interleukin-10 (IL-10), Regeneration and Tolerance Factor (RTF) and CD4+ cell count in HIV+ patients

Versa Omraninava[1], Saeid Abediankenari[1], Jamshid Yazdani[2]

*1. Department of Immunology, Immunogenetics research center, School of medicine University of Mazandaran medical science*
*2. Department of Biostatics, school of Health, University of Mazandaran medical science*

**Background:** Regeneration and Tolerance Factor (RTF), is a potential Th2 response marker with two forms of membrane bound and soluble. RTF can induce IL-10 expression and block tumor cell lysis by NK cells, thus it has been considered as an immune suppressor. Considering contrary in literatures regarding Th1-Th2 response pattern in HIV infection and effects of antiviral therapy on mentioned molecules, we determined IL-10 and RTF expression in HIV+ Patients before treatment and possible association with CD4 levels.
**Methods:** We enrolled 40 subjects in this study, 20 of whom were HIV+ patients without treatment history and 20 were healthy individuals. RNA extraction and cDNA synthesis were done on isolated blood mononuclear cells of all participants. We used Real time PCR to assess gene expression of RTF. Plasma IL-10 levels were measured by ELISA test. SPSS was used to analyze data.
**Results:** In case group, 14 male and 6 female subjects (mean age, 34.6) and in control group 15 male and 5 female (mean age, 33.9) were studied. RTF and IL-10 were increased in HIV+ patients in comparison to healthy subjects. A negative correlation between plasma IL-10 and CD4 cell count were observed in patients (r: -0.04).
**Conclusion:** Increased expression of Th2 associated markers in HIV+ and its negative correlation with patient immune status may explain immune evasion mechanisms employed by HIV. Potential therapeutic objectives of mentioned molecules needs to be investigated.

**Keywords:** HLA-G, RTF, IL-10, HIV

ICIA2018\Immunology of Infectious Diseases Viruses\poster\8204
**8204**

**Hepatitis C virus viremia among first time Iranian blood donors**

Fahimeh Ranjbar Kermani[1], Sedigheh Amini Kafi Abad[1], Kamran Mousavi Hosseini[1], Zohreh Sharifi[1], Mohammad Ali Mansournia[2], Mahtab Maghsudlu[1]

*1. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran*
*2. Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Hepatitis C virus (HCV) viremia is described as persistent HCV RNA among HCV exposed individuals. HCV viremiac rate is defined as the proportion of HCV Ab positive and HCV RNA positive individuals to total HCV Ab positive individuals and provides the likelihood of HCV viremiac infection in a given population. Understanding the HCV viremiac rate is important to increase knowledge about HCV epidemiology. The aim of this study was to evaluate the HCV viremiac rate and demographic parameters and risk factors correlates among Iranian first time blood donors.
**Methods:** In this cross sectional study, serologically confirmed HCV positive blood donors who came back to the Iranian blood transfusion centers over the country for counseling from November 2015 to May 2017 were included and interviewed for HCV risk factors. HCV RNA RT-PCR was carried out for subjects by an in-house qualitative assay according to manufacturer's instruction. Logistic regression was performed for data analysis and STATA software version 13 was used for statistical analysis.
**Results:** Out of 239 subjects, 226 (94.5%) were first time donors and HCV viremia was detected in 155 (68.6%, 95% CI 62.4% - 74.8%) of first time donors. No statistically difference was found in demographic parameters and risk factors between HCV Ab positive/ HCV RNA positive and HCV Ab positive/ HCV RNA negative first time blood donors.
**Conclusion:** The results of this study show the likelihood of occurrence of active HCV infection among first time Iranian blood donors. The majority of first time Iranian blood donors are in active phase of HCV infection. The viremic status was not associated with demographic parameters and risk factors. More effective donor selection is required to improve blood safety and follow-up studies on viremic first time blood donors are recommended to clarify impact of factors on the occurrence of HCV viremia.

**Keywords**: Blood donors, Hepatitis C, Viremia

ICIA2018\Immunology of Infectious Diseases Viruses\poster\8212
**8212**

## Evaluation of coverage and HBsAb titer in the serum of Dental students

Abbas Azadmehr[1], Parviz Zoulghadri[2], Niloufar Sadat Miri[1], Aboulfazl Rahmani[1], Mohmmad Sadegh Falahatkar[2], Fatemeh Hajmanochehri[2]

1. *Department of Immunology, Babol University of Medical Sciences, Babol, Iran*
2. *Department of Periodontics, Dental School, Qazvin University of Medical Sciences, Qazvin, Iran*

**Background**: Hepatitis B is an infection disease that affects the liver and caused by the hepatitis B virus. Health workers such as dental students are at the higher risks of blood-transmitted infections. Up to now, the hepatitis B virus (HBV) vaccination was the useful and effective way to prevent HBV infection which requires antibody titers estimation following the vaccination to be sure about its efficiency.
**Methods:** The study included 33 clinical and postgraduate students (18 male and 15 female). The serum samples were collected and tested hepatitis B surface antigen (HBsAg) by enzyme-linked immunosorbent assay (ELISA) .The B hepatitis vaccination coverage and antibody titer were reported. The data were subjected to chi-square and Mann-whitney U tests.
**Results**: 92% of all studies received the three doses of the vaccine. Of all 33 participants, 29 individuals (87.9%) vaccinates against hepatitis B infection. 28 students (84.8%) showed positive antibody titer and 5 ones (15.2%) had negative antibody titer. No significant differences were noted among males and females regarding antibody titer (mean 410IU/Ml and 362.2 IU/mL for males and females).
**Conclusion:** Hepatitis vaccination coverage and positive antibody titer among the clinical and post graduate dental students were appropriate, however; regarding higher risks of blood-transmitted disease among them; it should be tried to increase the vaccination coverage more.

**Keywords**: B Hepatitis, Vaccination, Antibody titer, ELISA

ICIA2018\Immunology of Infectious Diseases Viruses\poster\8235
**8235**

# Hepatitis B Virus DNA is absent in Lebanese Blood Donors Who Are Positive for Both Anti-Hbc and Anti-Hbs Antibodies

Ghada Kchour, [1] Mohammad Fayyad Kazan, [2],* Antoine Haddad, [3] Bassam Badran, [1] , Mahdi Tarhini[4,*]

*1. Laboratory of Cancer Biology and Molecular Immunology, Faculty of Sciences I, Lebanese University, Hadath, Lebanon*
*2. Institute de Biology et de Medicine Moleculaires, Universite Libre de Bruxelles, 6041 Gosselies, Belgium*
*3. Department of Clinical Pathology and Blood Banking, Sacrecoeur Hospital, Lebanese University, Lebanon*
*4. Faculty of Health Sciences V, Lebanese University, and director of Clinical Pathology and Blood Banking Department ,Raee Hospital, Saida , Lebanon*

**Background**: A major risk during blood transfusion is the transmission of infectious agents such as hepatitis B virus (HBV). Screening for anti-HBc antibodies has long been used to test HBV infection and thus, blood safety. However, the development of advanced tools enabling HBV-DNA detection rendered the diagnostic benefit of anti-HBc test uncertain.
**Methods**: Sera of 7200 blood donors were first screened for HBs antigen (HBsAg) and anti-HBc antibody. Samples that were found to be HBsAg-negative but anti-HBc-positive were further tested for anti-HBs and HBV-DNA.
**Results**: Of the 7200 tested samples, 7143 (99.2%) were HBsAg-negative, while the remaining 57 (0.8%) samples were HBsAg-positive. Among the HBsAg-negative samples, 490 (6.8%) were anti-HBc-positive. Of the anti-HBc positive samples, 397 (81%) were anti-HBs positive, while the remaining 93 (19%) samples were anti-HBs-negative. Interestingly, all of the anti-HBc positive samples, which also were positive for anti-HBs, exhibited negative HBV-DNA results. On the other hand, the anti-HBc positive samples, which were negative for anti-HBs, showed both negative and positive HBV-DNA results.
**Conclusions**: Blood samples which are positive for both anti-HBc and anti-HBs are characterized by negative HBV load.

**Keywords:** Hepatitis B Virus (HBV), Anti-HBc, Anti-HBs, Blood Safety

ICIA2018\Immunology of Infectious Diseases Viruses\poster\8378
**8378**

# Hepatitis B Core Antibody Immunoglobulin M in Blood Donors with a History of Hepatitis B Virus Infection

Helia Azimi[1, 2], Maryam Vaezjalali[1, 3]

1. *Infectious Disease Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
2. *Department of Microbiology, Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran*
3. *Department of Microbiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background**: Hepatitis B is still an issue after blood transfusion. A reason could be the window period of hepatitis B infection in blood donors. In countries such as Iran, enzyme-linked immunosorbent assay (ELISA) is the only test to detect hepatitis B surface antigen in blood donors. This procedure may miss the window period of hepatitis B infected donors. The current study aimed to look for hepatitis B core antibody immunoglobulin M in blood samples of Iranian donors with a history of hepatitis B virus infection to detect infection window period.
**Methods**: Eighty serum samples with hepatitis B core antibody were collected from 1000 healthy blood donors, forty of them had been positive for hepatitis B virus DNA in authors' previous study and were diagnosed as occult hepatitis B infection. All 80 samples were tested for hepatitis B core immunoglobulin M.
**Results**: One thousand blood samples were collected from 64 (6.4%) female and 936 (93.6%) male subjects. None of the blood samples contained hepatitis B core immunoglobulin M. The study found no significant differences between male and female subjects in term of HBcAb positivity.
**Conclusions**: Hepatitis B core antibody immunoglobulin M positivity is different in healthy blood donors of different countries according to the prevalence of chronic hepatitis B and its vaccination. Based on the current study findings, all positive samples of hepatitis B core antibody in Iranian blood banks should be considered as candidates for occult hepatitis B not just the window period infected samples.

**Keywords**: Hepatitis B Virus, Blood Donors, Viral DNA, Immunoglobulin M

ICIA2018\Immunology of Infectious Diseases Viruses\poster\8410
**8410**

**Deceased serum samples have inhibitor components that distract viral RNA extraction procedure**

H. Jafarinejad[1], R. Yarmohamadi[1], A. Heravian[1], A. Radmanesh[1*], S. Bayanolhagh[1, 2*]

*1. The Legal Medicine Research Center, Legal Medicine Organization, Iran, Tehran*
*2. Iranian Research Center for HIV/AIDS, Iranian Institute for Reduction of high risk Behaviour, Tehran University of Medical sciences, Tehran, Iran*

**Background:** The blood viral RNA minikit is intended for molecular biology applications. This procedure intend for the diagnosis, treatment or research of a disease. Many of corpse's blood samples are hemolysis and have many protein that release in blood when dead and may have inhibitory role in RNA extraction procedure and decrease the efficiency of RNA production. The main of this study is how we can extract viral RNA from this cases?
**Methods:** During the procedure for purification of RNA from serum samples, first samples diluted in 1:10 concentration, then the solution are lysed using highly denaturing conditions that immediately inactivate RNases, allowing the isolation of intact RNA. The negative (not diluted) and positive (viral sample) control used in this study. After homogenization of the lysate by a brief centrifugation through spin column, ethanol is added to adjust binding conditions and the sample is applied to the spin column. RNA is bound to the silica membrane during a brief centrifugation step. Contaminants are washed away and total RNA is eluted in 60µl or more of RNase-free water for direct use in any downstream application.
**Results:** The samples that diluted in 1:10 concentration had viral RNA as positive control and detected in Real Time PCR method ($P<0.05$) and in compared with samples that not diluted, had no viral RNA ($P>0.05$).
**Conclusion:** It seems the corpse serum samples have inhibitor component that cover the spin column filter and decrease the yield of RNA product.

**Key word:** Deceased donors, Inhibitor components, Viral

ICIA2018\Immunology of Infectious Diseases Viruses\poster\8489
**8489**

# Investigation of serum level of IL-35 in HIV patients and its association with co-infection with human Pegi virus (GBV-C) and Hepatitis-C Virus

Alireza Najafi[1], Mohammad Jafari[2], Mostafa Haji Mollahoseini[3], Farah Bokharaei Salim[4,5], Maryam keykhaee[6]

1. *Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran*
2. *Cellular and Molecular Research Center, Gerash University of Medical Sciences, Gerash, Iran*
3. *Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
4. *HIV Laboratory of National Center, Deputy of Health, Iran University of Medical Sciences, Tehran, Iran*
5. *Department of Virology, Iran University of Medical Sciences, Tehran, Iran*
6. *Department of pharmaceutical biomaterials, faculty of pharmacy, Tehran university of Medical sciences, Tehran , Iran*

**Background**: Interleukin-35 (IL-35), a newly identified member of IL-12 family, functions as a crucial immunosuppressive factor in immune-mediated diseases, and the predominant mechanism of suppression is its ability to suppress T cell proliferation and effector functions. Although several previous studies investigated the role of IL-35 in infectious diseases such as hepatitis B virus and Influenza, however there are limited data available regarding the role of IL-35 in the immunopathogenesis of HIV. The aim of this study was to determinate the immune activation status in HIV patients with GBV-C and/or HCV coinfection through measuring IL-35 plasma levels.
**Methods:** A case-control study was carried out on 50 HIV infected patients (10 HIV mono-infected, 22 HIV/HCV co-infected, 7 HIV/GBV-C co-infected, and 11 HIV/HCV/GBV-C co-infected) and 30 matched healthy control. Plasmas were analyzed for HCV using serologic test and GBV-C by reverse transcriptase polymerase chain reaction (RT-PCR) and the level of IL-35 was measured by ELISA assay.
**Results:** The level of plasma IL-35 in all patients with HIV infection was more than healthy control (P: 0.011). In addition, HIV/HCV co-infected subgroup had an elevated IL-35 level in comparison to healthy controls (P: 0.008). Furthermore, the concentration of IL-35 was significantly higher in HIV/HCV/GBV-C co-infected patients compared with healthy controls (P: 0.0229).
**Conclusion:** These findings suggest that IL-35 may be a role in immunopathogenesis of HIV/HCV and HIV/HCV/GBV-C co-infection. Our results provide a basis for further investigation in the role of IL-35 in HIV infection.

**Keywords:** Interleukin (IL)-35, HIV, GBV-C AND HCV, co-infection

ICIA2018\Immunology of Infectious Diseases Viruses\poster\8591
**8591**

**The Frequency of Parainfluenza virus infection in Acute Respiratory infections**

Morteza Sagharjoghi Farahani [1], Arash Kalantari [2], Babak Khodadadi *[3], setare Mamishi [4]

*1. Young researchers and Elite Club, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran*
*2. Assistant professor of Tehran University of medical sciences. Imam Khomeini hospital complex vali-e-asr hospital Department of Pediatrics, Tehran, Iran*
*3. Young researchers and Elite Club, Khorramabad Branch, Islamic Azad University, Khorramabad, Iran*
*4. Department of Pediatrics, Division of Infectious Disease.Markaz Tebbi Hospital Tehran University of Medicine Sciences*

**Background:** Acute respiratory infections are one of the most common causes of morbidity in children. Parainfluenza viruses are important viral pathogens causing upper and lower respiratory infections in children. There are four type of Parainfluenza. The most common cause of laryngotracheobronchitis is parainfluenza type 1. The parainfluenza virus is transmitted via arousal and direct contact between children. The aim of this study was to determine the prevalence of parainfluenza virus infection among children aged less than 5 years referred to Markaz Tebbi Hospital.
**Methods:** Our diagnostic method for evaluating an outbreak of parainfluenza virus infection is to investigate the nasopharyngeal secretion of patients with immunofluorescence antibody. Also, the most common age group involved and various clinical symptoms are recorded.
**Results:** In our study, the frequency of respiratory tract infection due to parainfluenza virus was 26%. The age group of 25 to 36 months was the most commonly involved age group, the most common symptom was rhinorrhea(96%.) and the most common clinical sign was fever (68%).
**Conclusions:** This study demonstrate that the prevalence of parainfluenza virus and the most commonly occurring age is similar to other studies. Therefore, in this age group of children, the clinical symptoms of rhinorrhea and fever should lead us to parainfluenza virus infection and effective prevention strategies for respiratory virus infection should be done.

**Keywords:** parainfluenza virus**,** immunofluorescence antibody, respiratory infections.

ICIA2018\Immunology of Infectious Diseases Viruses\poster\8609
**8609**

# Association between Interleukin-18 gene Polymorphism (rs5744249 A/C) and Susceptibility to Chronic Hepatitis B Virus Infection

Haleh Sarrafnia[1], Seyed Reza Mohebbi[1], Shabnam Kazemian[1], Mahsa Saeedi Niasar[1], Afsaneh Sharifian[1], Hamid Asadzadeh Aghdaei[2], Mohammad Reza Zali[1]

*1. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and*
*2. Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research In*
*4. stitute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Infection with hepatitis B is one of the most notable causes of chronic liver disease. The outcome of hepatitis B virus (HBV) infection is mainly influenced by host genetic background such as single nucleotide polymorphisms (SNPs). Recent studies have indicated that gene polymorphisms in cytokines are associated with the development of chronic HBV infection and progression of the infection. Interleukin-18 is a proinflammatory cytokine that plays a significant role in immune responses against viruses. In this study, we aimed to explore whether interleukin-18 (IL-18) gene single nucleotide polymorphism (rs5744249) is associated with the outcome of hepatitis B virus (HBV) chronic infection or not.
**Methods:** In this case-control study, blood samples were collected from 100 chronic HBV infected patients and 100 healthy controls. Selection criteria for patient group were anti-HBc antibody (anti-HBcAb) and HBsAg positivity for more than 6 months and the control group consisted of anti-HBcAb and HBsAg negative individuals. After genomic DNA extraction, the SNP was genotyped by the PCR and restriction fragment length polymorphism (RFLP) method and then we used SPSS for statistical analysis.
**Results:** The results showed rs5744294 genotype frequencies of AA=81.7%, AC=15.8% and CC=2.5% in chronic patients and AA=75.8%, AC=22.5% and CC=1.7% in the control group. Results revealed that no difference was observed in the frequencies of neither genotype (P value=0.396) nor allele (P value=0.888) frequencies between two groups.
**Conclusion:** No correlation was detected between interleukin 18 gene Polymorphism rs5744249 A/C with hepatitis B virus infection or disease progression and that it could not be regarded as a host genetic factor associated with the hepatitis B virus infection. Other IL-18 SNPs are possibly more notable than the one studied.

**Keywords**: Interleukin-18, Hepatitis B, Single nucleotide polymorphism, rs5744249

ICIA2018\Immunology of Infectious Diseases Viruses\poster\8648
**8648**

**Seroepidemiology of Varicella Zoster Virus among nursery students in Langroud Nursing Faculty**

Reza Jafari-Shakib[1], Ashkan Rasouli[2], Mojtaba Farahbakhsh[3], Mahtab Gholamzadeh[2], Zahra Mohtasham Amiri[4]

1. *Department of Immunology, Faculty of Medicine, Guilan University of Medical Sciences, Rasht*
2. *Faculty of Paramedicine, Guilan University of Medical Sciences, Rasht*
3. *Department of Medical Biotechnology, Faculty of Paramedicine, Guilan University of Medical Sciences, Rasht*
4. *Department of Community and Preventive Medicine, Guilan University of Medical Sciences, Rasht*

**Background:** This study was done to investigate anti-VZV (varicella zoster virus) antibody among nursery students as a group of health care workers who can transmit the infection to coworkers or patients.
**Methods:** The anti-VZV IgG was measured in all nursery students in Langroud Nursing Faculty who wanted to participate in this cross-sectional study. The antibody was measured in 101 students by ELISA method.
**Results:** Seventy-three (72.3%) students had anti-VZV IgG, 27 students (26.7%) were seronegative and one students have borderline IgG.
**Conclusion:** There is a significant percentage of nursery student seronegative and they are susceptible to VZV infection. They can get infection from other staffs and patients in hospital and also transmit infection to others especially immunocompromised patients. Therefore, it is recommended to screen anti-VZV antibody in nursery students and vaccination seronegative ones.

**Keywords:** Varicella zoster virus, Nursery students, Seroepidemiology, Langroud

ICIA2018\Immunology of Infectious Diseases Viruses\poster\8649
**8649**

**Beclin1 inhibition promotes autophagy and decreases daclatasvir-induced apoptosis in Huh7 cells**

Somaieh Nasereslami [1*], Taravat Bamdad [1], Asghar Abdoli [2]

1. *Department of Virology, School of Medical Sciences, University of Tarbiat Modares, Tehran, Iran*
2. *Department of Virology, Pasture Institute, Tehran, Iran*

**Background:** Beclin1 is a well-known key regulator of autophagy, which is also a haploinsufficient tumor suppressor. Current studies revealed that down-regulation or monoallelic deletions of beclin1 were frequently found in various cancers. The purpose of this study was to investigate the effects of beclin1 inhibition on autophagy and daclatasvir-induced apoptosis of pancreatic cancer cells.
**Methods:** Beclin1 expression was inhibited by siRNA transduction and gene expression was determined by Real-time PCR and Western blot. The effects of beclin1 inhibition on autophagy and daclatasvir-induced apoptosis of Huh7 cells were analyzed through LC3 expression, cell viability, cell cycle and apoptosis using Western blot.
**Results**: We observed that beclin1 silence promoted microtubule-associated protein 1 light chain 3-II (LC3-II) protein formation and increased punctate fluorescent signals in Huh7 cells transfected with green fluorescent protein (GFP)-tagged LC3. Beclin1 inhibition showed a greater suppressive effect on daclatasvir-induced apoptosis of Huh7 cells.
**Conclusion:** Our data suggest that beclin1 silence not only up-adjusted autophagy process, but also played an important role in the regulation of apoptosis. Beclin1 inhibition could inhibit apoptosis signaling induced by daclatasvir in Huh7 cells.

**Keywords:** Daclatasvir, Huh7, Beclin1, LC3, Apoptosis

ICIA2018\Immunology of Infectious Diseases Viruses\poster\8654
**8654**

**Evaluation of anti-CMV antibody level in women of reproductive age**

Shayesteh Z[1], Yazdani Neyshabouri Sh[2], Beikzadeh L[3]*, Vazeh H[4], Farahmand M[5]

1. *Msc of medical Immunology, school of allied medical sciences, karaj, iran*
2. *phD of virology Islamic Azad University-Tehran Medical Branch Department of Microbiology*
3. *Msc of medical virology, school of allied medical sciences, karaj, iran (correspond)*
4. *Msc of medical virology, Golestan University of Medical Sciences, Department of Microbiology*
5. *Department of virology, school of public health, Tehran university of medical sciences, Tehran, Iran*

**Background:** One of the herpes family with the potency to life-extensive latency within the host is cytomegalovirus (CMV) that have double-stranded DNA genome. Alike all herpes viruses, HCMV undertake latency and reactivation within the host and has been appeared to pollute a broad cells in vivo. CMV inborn contaminations might be symptomless or may prompt hearing debilitation, mental hindrance, cerebral paralysis, and neurodevelopmental disabilities. The frequency of inborn CMV contamination is around 1%-7% of births. Anticipating the risk of CMV vertical transmission and planning preventive measures are facilitated by knowing these characteristics.
**Methods:** Among of 360 women in age of fertility attending health center chosen randomly from all women attending for test during the study period. Peripheral blood (۵ ml) was aspirated from them and CMV serology test was done using a standard ELISA kit to determine CMV IgM and IgG antibodies.
**Results:** The total number of participants in this study was 360. The evaluation of IgG concentration is indicated. According to the cut-off determined by the kit, which is 16.5, 280 specimens are positive and 80 cases are IgG negative. A Fisher's comparison test was used to compare IgG between two age groups. The results showed that the observed difference between the two age groups was not statistically significant ($p = 0.79$).
**Conclusion:** According to the findings, it can be seen that, there is not anyone in participants who is in the acute phase of diseas. Also, comparing IgG positive cases in two age groups >30 and <30 years old, there was no significant difference in age variable. Overall, 77% of cases were IgG positive, indicating a chronic phase of the disease in participants.

**Key words**: Reproductive age, antibody level, CMV

ICIA2018\Immunology of Infectious Diseases Viruses\poster\8697
**8697**

# Hepatitis C virus associated mixed cryoglobulinemia

Ramin Farajpour[1], Shiva Farajpour[2]

1. *Department of Neuroscience Research Center, Faculty of Medicine, Tabriz University of Medical Sciences*
2. *Department of Ophthalmology, Faculty of Medicine, Tabriz University of Medical Sciences*

**Background**: Mixed cryoglobulinemia (MC) is common in patients with chronic hepatitis C virus (HCV) infection. However, clinically significant vasculitis occurs only in a minority of these patients.
**Methods & results**: We hereby report a driver man with chronic HCV infection and MC presenting with polyarthralgia and skin purpura of the lower limbs. He later developed clinical remission and anti-viral therapy resulted in good clinical response.
**Conclusion**: The classical presentation of MC is a triad of cutaneous vasculitis, peripheral neuropathy and arthralgia. Anti-viral therapy is the standard treatment and good response depends on viral clearance.

**Keywords**: Hepatitis C, Mixed Cryoglobulinemia, Anti-viral therapy

ICIA2018\Immunology of Infectious Diseases Viruses\poster\9757
**9757**

**Polyclonal antibody preparation against bovine ephemeral fever virus**

Shokoofeh Almasi[1], Dr. Mehran Bakhshesh *[1], Dr. Mohammad Mehdi Ranjbar[1]

*1. Department of viral animal disease research and diagnosis, Razi vaccine and serum research institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran*

**Background:** Considering the numerous applications of polyclonal antibodies in research and clinical fields, the development of methods for the preparation of polyclonal antibodies against pathogens is very important. In this research, a method for producing polyclonal antibodies against the whole viral particle of the *Rhabdovirus*, bovine ephemeral fever virus (BEFV), is introduced.
**Methods:** Six rabbits of Dutch breed were selected and divided into experimental and control groups. Each rabbit from the experimental group received at least $10^6$ $TCID_{50}$ of the whole viral particles intravenously without adjuvant while the control group was injected with the cell culture supernatant free from the virus. The injections were administered at 0, 1 and 4 weeks intervals, and two weeks after the last injection the rabbits were bled and their sera were separated by centrifugation. The sera were tested by virus neutralization test (VNT), calculating the titer of antibodies to the live virus.
**Results:** This schedule resulted in a high titer of polyclonal antibodies to BEFV particles. Based on Karber formula, the rabbits from experimental group produced 288 to 290 titer of neutralizing antibodies as no titer of antibodies was detected in control group.
**Conclusion:** Bovine ephemeral virus has recently been isolated in Iran and studies are now being undertaken on characterization, epidemiology and particularly manufacturing a potent vaccine for prophylaxis against the virus. Therefore, the hyperimmune serum as a biological product of this research will definitely be exploited in the experiments required for these studies as well as possible commercial applications in the future.

**Keywords:** Bovine ephemeral fever, hyperimmune serum, rabbit

ICIA2018\Immunology of Infectious Diseases Viruses\poster\9803
**9803**

# Interferon-inducible protein interferes with HCV replication through the autolysosomal degradation of NS5A

Somaieh Nasereslami [1*], Taravat Bamdad [1], Asghar Abdoli [2]

1. *Department of Virology, School of Medical Sciences, University of Tarbiat Modares, Tehran, Iran*
2. *Department of Virology, Pasture Institute, Tehran, Iran*

**Background**: SCOTIN overexpression inhibits HCV replication and infectious virion production in cells infected with cell culture-derived HCV. HCV nonstructural 5A (NS5A) protein, which is a critical factor for HCV RNA replication, interacts with the IFN-b-inducible protein SCOTIN, which transports NS5A to autophagosomes for degradation. Furthermore, the suppressive effect of SCOTIN on HCV replication is impaired in both ATG7-silenced cells and cells treated with autophagy or lysosomal inhibitors. SCOTIN does not affect the overall flow of autophagy, however, it is a substrate for autophagic degradation. The physical association between the transmembrane/proline-rich domain (TMPRD) of SCOTIN and Domain-II of NS5A is essential for autophagosomal trafficking and NS5A degradation. Altogether, our findings suggest that IFN-b-induced SCOTIN recruits the HCV NS5A protein to autophagosomes for degradation, thereby restricting HCV replication.
**Methods**: In the present study, we propose that SCOTIN is one of the ISGs that interferes with HCV replication. IFNs type I exhibit most of their antiviral activities via hundreds of ISGs, which involve various proteins, including enzymes, transcription factors, heat-shock proteins and apoptotic proteins. SCOTIN expression was induced in Huh-neo cells treated with IFN-β, and the suppressive effect of IFN-β on HCV replication was reduced when SCOTIN expression was silenced. These results indicate that SCOTIN acts as one of the ISGs that contribute to the antiviral activity of interferon-β that is directed against HCV.
**Results:** IFN-β-inducible protein SCOTIN restricts HCV replication and SCOTIN promotes autophagosomal degradation of NS5A.
**Conclusion:** In this report, we demonstrate that an IFN-β-inducible host factor, SCOTIN, contributes to suppressing HCV replication via the autolysosomal degradation of the HCV NS5A protein. SCOTIN is an ER transmembrane protein that physically associates with NS5A and is also a substrate for autophagy-mediated degradation. Similar to the role of p62 autophagy receptor in the autophagy-mediated clearance of intracellular viruses and bacteria, SCOTIN recruits NS5A to LC3-II-containing autophagosomal compartments through physical interactions. These physical interactions and the colocalization of SCOTIN and NS5A in autophagosomes are critical for controlling the autophagy-mediated proteolytic degradation of the NS5A viral protein and HCV replication.

**Keywords:** IFN-b, HCV, NS5A, LC3, SCOTIN.

ICIA2018\Immunology of Infectious Diseases Viruses\poster\9809
**9809**

# HTLV-1 infected asymptomatic carriers overexpress the cell-mediated cytotoxicity genes

Asadollah Mohammadi[1], Bahare Fazeli[2], Reza Boostani[3], Houshang Rafatpanah[2]

*1. Cellular & Molecular Research Center, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran*
*2. Inflammation and Inflammatory Diseases Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*3. Department of Neurology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a chronic viral neuroinflammatory disease. Apoptosis plays an important role in the control of viral infection, this process occasionally makes it possible to distinguish asymptomatic carriers (ACs) from HAM/TSP patients.
**Methods:** The mRNA expression of Fas, FasL, TRAIL, perforin, granzyme A, granzyme B, and granulysin in the peripheral blood mononuclear cells (PBMCs) of 21 HAM/TSP patients, 21 ACs, and 21 healthy controls (HCs) were quantified. The Fas, FasL, TRAIL, granulysin serum levels, and the granulysin secretion level were evaluated and their interaction with the HTLV-1 proviral load (PVL), Tax and HBZ mRNA expression were also analysed.
**Results:** Fas, FasL, TRAIL, perforin, and granzyme B were significantly overexpressed in ACs whereas granulysin secretion significantly decreased in ACs and HAM/TSP patients. HAM/TSP patients have a higher PVL, Tax, and HBZ expression than ACs. Also, negative correlations between the mRNA expression of the Fas, FasL, TRAIL, perforin, granzyme B, and granulysin with HBZ levels were observed.
**Conclusions:** We found that ACs overexpress the genes which are involves in cell-mediated cytotoxicity. We conclude that successful suppression of the HTLV-1 PVL, Tax and HBZ expression, and control of disease progression is associated with the strong cytotoxic activity in the PBMCs of ACs.

**Keywords:** HTLV-1, Neuroinflammatory disease, HAM/TSP, Apoptosis.

ICIA2018\Immunology of Infectious Diseases Viruses\poster\9821
**9821**

**Frequency of influenza-A-H1N1 in patients with Acute Pneumonia admitted to Loghman Hakim Hospital**

Helya Sadat Mortazavi [1]; Abdolkarim Pajoumand [2]; Seyed Hamidreza Monavari [2]; Davood Javanmard [2]; Latif Gachkar [3]; Meinoosh Shabani [3*]

*1. Department of Medical Virology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*
*2. Department of Clinical Toxicology, Loghman Hakim Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Infectious Disease and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences and Health Services, Tehran, Iran*

**Background:** Here we scrutinized the incidence of Influenza-A-H1N1-related pneumonia in community-acquired pneumonia (CAP) and hospital-acquired pneumonia (HAP) at Loghman Hakim hospital, Tehran, Iran.
**Methods:** In this prospective study from November 22, 2016, to June 21, 2017, patients suspected of seasonal Influenza and confirmed CAP-HAP included. Samples tested using rapid antigen tests and quantitative Real-time PCR assay. P-value < 0.05 was considered significant.
**Results:** A total of 29 suspected cases of seasonal influenza had obtained either CAP or HAP. Of whom 51.7% were male, and the mean age was 60.07 ± 20.94 years. The most common findings were fever and chills, cough and phlegm (15 cases, 29.41%). The highest number of admission occurred in February (48.3%). Mean days of hospital stay was 11.31 ± 17.59 days and the average days of treatment with antibiotics were 10.78 ±17.13 days. 6.9% were positive in QReal-Time PCR assay (> 30 copies/ml). The highest prescribed antibiotic combination was ceftriaxone + Azithromycin, and only 31.3% of patients received empiric oseltamivir therapy. Also, 27.6% of patients were drug users. Consolidation (44.8%) was the most predominant radiological finding. We found a significant correlation in patient's systolic blood pressure, viral load, white blood cell count, BUN, CURB65 and radiological findings.
**Conclusion:** This study highlights the importance of the empiric influenza treatment before receiving the results of molecular tests in suspected cases. Moreover, precautions, including influenza and pneumococcal vaccination, should be emphasized by health care workers.

**Keywords:** Influenza A Virus, H1N1 Subtype, Quantitative Real-Time Polymerase Chain Reaction, Pneumonia

ICIA2018\Immunology of Infectious Diseases Viruses\poster\9917
**9917**

# Association between Chronic Hepatitis B Virus Infection and Polymorphisms of rs9277535 in HLA-DP in the Southern Khorasan population (East of Iran)

Hossein Javdani [1, 2], Abdolghader Taene [1, 2], Ali Farahi[1, 2]

1. *Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran*
2. *Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran*

**Background:** Chronic hepatitis B virus (HBV) infection is a major global health concern. It is assessed that two billion individuals worldwide are tainted with hepatitis B infection and approximately 350 to 400 million people are chronic carriers of HBV. The clinical results of HBV disease extend from asymptomatic disease to constant hepatitis, liver cirrhosis and hepatocellular carcinoma. The genes encoding HLA are the most polymorphic in the human genome, probably as a way to be able to respond to all capacity overseas antigens. In the present study, we aim to explore the association of the HLA-DP polymorphisms rs9277535 with HBV susceptibility in the Southern Khorasan population.
**Methods:** In our study, we used 60 patients with chronic HBV infections as a group of patients (HBsAg positive test results for at least 6 months) and 120 patients who have previously recovered from HBV infection, spontaneously (HBc-positive antibody / HBsAg negative) as a control group. The amplified PCR products were purified using PCR Purification Kit. To determined SNPs of HLA-DP (rs9277535), the purified PCR products were sequenced using the Applied Biosystems 3730/3730XL DNA analyzer sequencing.
**Results:** For the rs9277535 polymorphism, the patients carrying A allele or AT genotype had a higher risk of chronic HBV infection than the other patients carrying G allele or GT genotype. (P<0/005).
**Conclusion:** In conclusion, this study demonstrated that HLA-DP rs9277535 polymorphisms are strongly associated with HBV susceptibility in the Southern Khorasan population.

**Keywords:** Chronic hepatitis B, Immune response, Polymorphism, Human leukocyte antigens DP (HLA-DP)

ICIA2018\Immunology of Infectious Diseases Viruses\poster\9921
**9921**

# Relation between IFN-γ polymorphism and the outcome of hepatitis B infection in an Iranian population

Ali farahi[1,2] , Abdolghader Taene Taene[1,2] , Hossein Javdani Javdani[1,2]

1. *Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran*
2. *Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran*

**Background:** The chronic HBV infection is considered as a multifactorial issue, which can prompt to a vast variety of clinical outcomes. The host's immune system is the most crucial factor in viral persistence. IFN-γ is one of the immune factors that produce helpful substances essential for the protection against viral infections. The current study aimed to investigate the relation between a SNPs (rs2430561) of the IFN-γ first intron and the outcome of hepatitis B infection in an Iranian population.
**Methods:** Blood samples of 60 chronically HBV infected patients (cases) and 60 healthy subjects with the history of HBV infection (controls) were collected. Genomic DNA was extracted by salting-out method and DNA analysis genotype identification was performed by ARMs-PCR.
**Results**: The results indicated that the SNP frequency had a significant difference between the case and control groups. As the frequency of allele A was 73.3% and 46.7% in cases and controls, respectively.
**Conclusion:** This study demonstrate a significant relationship between the IFN-y (+874T/A, rs2430561) polymorphism and chronic HBV infection susceptibility in a sample of Iranian population. The result of current study suggests that carriers of mutant allele A at position +874 are more susceptible to chronic HBV infection and carriers of wild-type allele T at position +874 are predisposed to recover from HBV infection.

**Keywords:** IFN-γ polymorphism, hepatitis B infection, Iranian population

ICIA2018\Immunology of Infectious Diseases Viruses\poster\9972
**9972**

# Evaluation of HIV Viral Load and CD4 count distribution among HIV infected patients in co-infection with HCV and Human pegivirus (GBV-C)

Mohammad Jafari[1], Alireza Najafi[2], Mostafa Haji Mollahoseini[3], Farah Bokharaei Salim[4,5], Maryam keykhaee[6], Roghayeh babaei[4]

*1. Cellular and Molecular Research Center, Gerash University of Medical Sciences, Gerash, Iran Immunology Research Center, Immunology and Infectious Disease Institute, IRAN University of Medical Sciences*
*2. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. HIV Laboratory of National Center, Deputy of Health, Iran University of Medical Sciences, Tehran, Iran*
*4. Department of Virology, Iran University of Medical Sciences, Tehran, Iran*
*5. Department of pharmaceutical biomaterials, faculty of pharmacy, Tehran University of Medical sciences, Tehran, Iran*

**Background**: Systemic immune activation is critical to the pathogenesis of HIV disease, and is accentuated in HIV/HCV co-infected patients. On the other hand, harmful or beneficial effect of GBV-C on HIV progression is controversial. In this study, we examine immune pathogenesis of HIV co-infection through HIV viral load detection and CD4 percentage measurement in different infectious profile.
**Methods:** A case-control study was carried out on 50 HIV infected patients (10 HIV mono-infected, 22 HIV/HCV co-infected, 7 HIV/GBV-C co-infected, and 11 HIV/HCV/GBV-C co-infected). Plasmas were analyzed for HCV using serologic test and GBV-C by reverse transcriptase polymerase chain reaction (RT-PCR). The determination of the level of HIV-1 viral load was tested by an Artus HIV-1 RG RT-PCR Kit in accordance with the manufacturer's instructions. Whole blood analyzed for the percentage of CD4+ and CD8+ T-cells by CD3/CD4+ and CD3/CD8+ double staining method and flowcytometric analysis on lymphocyte population gate.
**Results:** HIV viral load in 36% of HIV/HCV/GBV-C sub group was detectable. In addition, patients with HIV/HCV co-infection had 13% HIV viremia. In monoinfected patients 10% of them had detectable viraemia. Surprisingly, The HIV virus in sample of HIV/GBV-C co-infected patients was undetectable. Furthermore, the average of CD4 percentage in HIV/HCV/GBV-C, HIV/HCV, HIV mono-infected, and HIV/GBV-C co-infected was 16.5%, 18%, 23, 4%, and 18% respectively.
**Conclusion:** These findings suggest that co-infection complicates the clinical progress of HIV in infected patients, and may also harmfully affect on treatment of HIV infection.

**Keywords:** HIV co-infection, HIV viral load, CD4 count.

ICIA2018\Immunology of Infectious Diseases Viruses\poster\11073
**11073**

# Rotavirus infection induces nitric oxide production by Caco-2 cells

Behnoush Baradaran[1], Sara Soudi[1], Hoorieh Soleimanjahi[1]

*1. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

**Background:** Intestinal epithelial cells are the first barrier against intestinal pathogens. Rotavirus is the leading cause of severe gastroenteritis in infants and young children that infects villus epithelial cells of small intestinal. After virus entry to the cells, signaling pathways are activated, resulting in the induction of proinflammatory cytokine production and increase in oxidative stress. In this research the effect of rotavirus on nitric oxide production by Caco-2 cells were measured.
**Methods:** To this purpose, Caco-2 cells were cultured at $3\times10^{5}$/ml in each well of 12 well plats. Rotaviruses were added at $10^4$, $10^3$, $10^2$ virus/ml to the cells in triplicate. No virus treated group were considered as control. 24 and 48 hours post virus treatment, supernatant of the cells were collected and amount of the NO production were measured by griess method.
**Results:** According to obtained results, $10^4$, $10^3$, $10^2$ and no virus treated groups produced the mean amount of 20.47, 18.5, 18.5 and 12.6 nM of nitric oxide after 24 hours of infection, respectively. This amount were increased significantly to 30.0 and 28.2 nM in $10^4$and $10^3$ virus treated groups, but no significant differences were seen in both $10^2$ virus treated and no treated groups.
**Conclusion:** Nitric oxide production by epithelial cells may insert both protective effect against infection and negative effect on host cells by induction of excessive oxidative stress. The result showed that nitric oxide production by Caco-2 cells are dependent to both primary infection dose and duration of pathogen-host interaction.

**Keywords:** Rotavirus, Caco-2 cells, nitric oxide

# Immunology of Mycobacterium Tuberculosis

## Poster Presentation

ICIA2018\ Immunology of Mycobacterium Tuberculosis\poster\7969
**7969**

# Evaluation of Host Interaction and *Mycobacterium Tuberculosis* in Outbreak of Tuberculosis Disease with Evaluation of MMP-3 and MMP-9 Genes Expression in Host and ESAT-6 in Microbe in Patient of TB

Shadi Abbasnia[1], Kiarash Ghazvini[2], Saeed Amel Jamehdar[2], Narges Valizadeh[1] , S.A.R Rezaee[1]

*1. Inflammation and Inflammatory Disease Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*2. Department of Microbiology & Virology, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** Tuberculosis (TB) caused by infection of *Mycobacterium tuberculosis (Mtb)*. Different manifestations of TB result from interactions between the bacteria and host defense. A family of host molecules which may complicate in TB manifestation by extracellular matrix degradation is matrix metalloproteinases (MMPs). In this study, the interaction of host and *Mtb* was evaluated by assessing MMP-9 and MMP-3 gene expression in host and ESAT-6 in *Mtb*.
**Method:** Lung lavage fluid from 30 patients with clinical symptoms of tuberculosis but negative direct and culture *Mtb* tests, was taken by a pulmonologist. Out of 30 patients, 14 were $TB^+$ and 16 $TB^-$. After extracting the RNA and synthesis of cDNA, expression of MMP-3 and MMP-9 in host and ESAT-6 in *Mtb* were evaluated by TaqMan Real Time PCR.
**Results:** The findings show that MMP-9 gene expression in $TB^+$ and $TB^-$ were (2.56±0.68) and (1.13±0.35), respectively, which the higher expression (2.26 times) in TB+ was significant (p=0.05). Gene expression of MMP-3 in TB+ was (0.22±0.09) and in TB- was (0.64±0.23) which the difference was not statistically significant.
ESAT-6 gene expression in patients with TB+ was (0.28±0.091). In addition, although, there was not any correlation between MMP-3 or 9 with ESAT-6, there were significant correlations between MMP-9 and WBC (R=+0.61, P=0.02), BMI (R=-0.59, P=0.02) and albumin (R=+0.68, P=0.007), and also between MMP-3 and INOS (R=+0.94, P≤0.001), *Mtb*-Ag8 (R=+0.92, P≤0.001) and T-bet (R=+0.84, P=0.002), WBC (R=-0.60, P=0.04), PMN (R=-0.86, P=0.001) and lymphocytes (R=+0.80, P=0.003).
**Conclusion:** The results showed that increased expression of MMP-9 in TB+ could be complicated in lung injuries in TB, which is a good target for therapy. Moreover, ESAT-6 seems to have no effect on lung damage.

**Keywords:** *Mycobacterium tuberculosis*, ESAT-6, MMP-3, MMP-9, Taqman Real-Time PCR

# Immunology of Rheumatic Diseases

# Poster Presentation

ICIA2018\Immunology of Rheumatic Diseases\poster\5326
**5326**

# The correlation between vitamin D plasma levels with Foxp3 gene expression in Rheumatoid arthritis patients

Parisa Zafari[1], Nasrin Iranshai[2], Mahdi Taghadosi[2]

1. *Department of immunology, school of medicine, Mazandaran University of Medical Sciences*
2. *Department of immunology, school of medicine, Kermanshah University of Medical Science*

**Background**: Rheumatoid arthritis (RA) is an autoimmune disease with an inflammatory nature that affects the small joints of the hands and feet. It seems that the function of Regulatory T cells (Tregs), the main cells in controlling and inhibiting of inflammation, have been compromised in RA. Foxp3 expression as major transcription factor of these cells depends on variety of environmental and internal factors. Vitamin D is one of the environmental factors that in addition to its regulatory role in the immune system, it is also important to induce Foxp3 expression. Therefore, the aim of this study was to investigate the effect of plasma levels of vitamin D on the expression of Foxp3 gene in patients with RA.
**Methods:** 20 untreated RA patients and 40 healthy subjects participated in this study. Plasma levels of vitamin D were measured by competitive ELISA method. Foxp3 transcription factor gene expression was also measured using real-time PCR technique.
**Results:** As expected, Foxp3 expression was lower in RA patients than healthy subjects but there was not a significant correlation between vitamin D plasma levels and Foxp3 gene expression.
**Conclusion:** Vitamin D deficiency cannot be considered as an environmental factor affecting RA pathogenesis unlike undeniable effect of this vitamin in pathogenesis of other autoimmune disease such as multiple sclerosis.

**Keywords**: Foxp3, rheumatoid arthritis, vitamin D

ICIA2018\Immunology of Rheumatic Diseases\poster\6313
**6313**

# Analysis of the gene expression of Helios and the methylation status of Foxp3 TSDR sequence in the newly diagnosed Rheumatoid Arthritis patients

Mahdi Taghadosi[1], Parisa Zafari[2], Nasrin Iranshahi[1]

1. *Department of immunology, school of medicine, Kermanshah University of Medical Science*
2. *Department of immunology, school of medicine, Mazandaran University of Medical Sciences*

**Background**: The control of auto-reactive cells may be compromised in Rheumatoid Arthritis (RA). Regulatory T cells (Tregs), key controller of immune system, may have impaired function in RA disease. Foxp3 is a master regulator of Treg cells which its expression is under tight control of epigenetic mechanisms. By analysis of epigenetic modulation of Foxp3 Treg-specific demethylated region (TSDR) and Helios gene expression, the present study aims to evaluate Treg cells alterations in RA disease.
**Methods**: Tewnty newly diagnosed RA patients and 41 healthy subjects participated in this study. Foxp3 and Helios expression was measured using real-time PCR technique. Methylation level of TSDR was analyzed by bisulfite treatment and Quantative methylation specific PCR (Q-MSP) method.
**Results**: The gene expression of Foxp3 and demethylation rate of Foxp3 TSDR sequence was decreased in RA patients compared with healthy subjects ($P < 0.001$ and $P = 0.006$ respectively). The Helios expression was significantly higher in RA patients compared to healthy controls ($P = 0.048$). Positive correlation between Foxp3 expression with Helios expression and demethylation rate of TSDR was detected ($P=0.016$ and $P= 0.010$ respectively).
**Conclusion**: Our results suggest that epigenetic alterations may have an important role in the pathogenesis of RA.

**Keywords**: Rheumatoid arthritis, Foxp3, Helios, epigenetic, TSDR

ICIA2018\Immunology of Rheumatic Diseases\poster\7351
**7351**

# Determination of IL-17A & IL-17F gene polymorphisms and serum levels of Anti-CCP in patients with Rhematoid arthritis

M.Khojasteh[1], R.Mansori[1], A.Dehghan[1], M.Mahmoodi[2], M.Vakili[1], A.Shahmohammadi[1], Mazarzadeh[1]

1. *Department of Immunology, International campus of Yazd, Iran*
2. *Department of Rheumatology, Medical university of Tehran, Iran*

**Background:** Rheumatoid arthritis (RA) is a chronic, systemic inflammatory autoimmune disorder that is symmetric in small and sometimes large joints. This disease affects about 1% of the world's population. Studies have shown RA is a multifactorial disease that is the result of the interaction of genetic and environmental factors. Genetic factors have a special role in severity disease. On the other hand the disease process triggers a cascade of inflammatory cytokines leading to activation of autoreactive inflammatory cells, which ultimately leads to the destruction of bone and cartilage. IL17 is the main pro-inflammatory cytokine that secrets from Th17 and expresses in synovium of joint fluid in RA patients. IL17A and IL17F (two main family members of IL17) induce expression of cytokines and other inflammatory processes in bone and involved in rheumatoid arthritis. Therefore, in this study, single nucleotide polymorphism rs2275913 of gene IL17A and single nucleotide polymorphism rs763780 of IL17F gene in patients and controls were examined and compared.
**Methods:** In this study, 100 patients with RA and 92 healthy controls were selected for IL17A and 116 patients with RA and 107 healthy individuals were selected for IL17F. DNA was extracted from peripheral blood mononuclear cells by DNA extraction kit. Primers were designed by PRIMER 3 software and then the appropriate restriction enzymes were selected. Allele frequencies and genotypes of these polymorphisms were analyzed using PCR-RFLP technique. After statistical analysis, a significant association between rs2275913 single nucleotide polymorphism with the risk of disease was observed (P- Value:0.0001,OR:2.97,CI:95%). But there was no significant association between rs763780 and risk of disease.( P-Value: 0.201)
**Results:** Also in this study, the relationship between the genotypes mentioned above and Anti-ccp antibodies was investigated. Anti-ccp is a specific and offensive diagnostic marker for rheumatoid arthritis that can predicts disease, there was no significant correlation between the genotypes and positive Anti-ccp.
**Conclusion**: Based on this study, rs2275913 polymorphism of IL17A gene may be related to the risk of rheumatoid arthritis in Iranian patients.

**Keywords:** rheumatoid arthritis, IL 17, single nucleotide polymorphism

ICIA2018\Immunology of Rheumatic Diseases\poster\7487
**7487**

# T helper subsets and regulatory T cell in different groups of Rheumatoid Arthritis patients

Saeid Taghiloo[1], Abolghasem Ajami [1,2]*, Mohsen Tehrani[1,2], Arezou Abbasi[1], Reza Alizadeh Navaei[2], Mohsen Akhiani[3], Alireza salami[4]

1. *Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
2. *Molecular and Cell-Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran*
3. *Alborz Hospital, Alborz University of Medical Sciences, Karaj, Iran*
4. *Department of Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Rheumatoid arthritis (RA) is one of the most common autoimmune diseases in the world. Recent studies have indicated the imbalance of Th subsets and Treg activity in development, progressing and remission of disease. In this study, we investigate the expression gene of four major transcription factors T-bet (Th1), GATA (Th2), RORc (Th17) and Foxp3 (T reg) in peripheral blood of different group of RA patients.
**Methods:** In a case-control study relative gene expression of transcription factor of Th subsets and T reg were investigated in patients and controls. Tewnty newly diagnosed patients, 20 patients under treatment, 20 patients in remission state, 20 patients with osteoarthritis and 20 age and sex matched healthy controls were selected. In this study diagnosis and classification of patients was done according to American college of Rheumatology criteria. two ml of peripheral blood collected from all patients, RNA was extracted and cDNA was synthesized. The expression of transcription factors were evaluated by Real-time PCR, GAPDH gene was considered as housekeeping gene.
**Results:** The relative expression of T-bet in RA patients were significantly higher than healthy controls ($P = 0.002$). Also, the relative expression of Foxp3 in RA patients was significantly lower than healthy controls ($P < 0.0001$). There was no significant difference in the relative expression of GATA3 and RORc in the RA patients, osteoarthritis and healthy controls ($P = 0.3$, $P = 0.2$, respectively).
**Conclusion:** The results of this study indicated that Th1 and Treg cells are important in RA, nevertheless the role of Th17 cells seem to be of little importance in these patients. It appears that Th2 cells do not interfere with the development of RA.

**Keywords:** Rheumatoid Arthritis, Osteoarthritis, T-bet, GATA3, FOXP3, RORc

ICIA2018\Immunology of Rheumatic Diseases\poster\7516
**7516**

# Changes in Th17 cells frequency and function after Nanocurcumin therapy in ankylosing spondylitis patients

Atiye Mellati[1, 2], Sanam Dolati[1, 2], Mehdi Yousefi[1, 2]

1. *Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran.*
2. *Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background**: Ankylosing spondylitis (AS) is a type of inflammatory disease that commonly contains spine and sacroiliac joint. It was described by chronic inflammation, bone reformation and back pain. Though there is no certain cure for AS some anti-inflammatory drugs can improve clinical symptoms and slow down the progression. Th17 play a key role in pathogenesis of inflammatory diseases. For this reason we tried to investigate anti-inflammatory effects of nanocurcumin on Th17 in AS patients.
**Methods:** Tewnty four AS patients were enrolled and divided into two groups of 12 patients in which one group received nanocurcumin and another received placebo as control group. After 16 week treatment, PBMCs were isolated from all 24 patients' blood. Then mRNA expression of Th17 associated cytokines (IL17 and IL23) and transcription factor (RORγt) in pre and post-treatment patients were assessed using Real-time PCR and CYBR green method. Secretion of Th17 associated cytokines was assessed using ELISA method and finally Th17 frequency was evaluated by flow cytometry technique.
**Results:** Nanocurcumin resulted in significant decrease in RORγt mRNA expression (P value=0.0001). IL17 mRNA expression decreased (P value=0.011) while IL23 mRNA didn't change compared with placebo group. IL17 secretion showed significant decrease compared with placebo and post treatment (P value=0.029) but no significant decrease in IL23 was observed. Furthermore results of flowcytometry technique showed that there is no decrease in Th17 frequency in nano-curcumin receiving group.
**Conclusion**: The results demonstrated that nanocurcumin may provide a promising treatment of AS and development of inflammatory cytokines suppression.

**Keyword:** Ankylosing spondylitis, Nanocurcumin, T helper17, Inflammation

ICIA2018\Immunology of Rheumatic Diseases\poster\7859
**7859**

**Genepolymorphisms and serum levels of IL-10 in behcet's disease**

Zohreh Babaloo[1], Babak Afkari [2], Shahriar Alipour[3], Ebrahim Sakhinia [4]

*1. Immunology Department, Medicine Faculty, Tabriz University of Medical Sciences*
*2. Immunology Department, Medicine Faculty, Tabriz University of Medical Sciences*
*3. Genetic Department, Medicine Faculty, Tabriz University of Medical Sciences*
*4. Genetic Department, Medicine Faculty, Tabriz University of Medical Sciences*

**Background**: Interleukin 10 (IL-10) is a cytokine with potent anti-inflammatory properties that plays a fundamental role in restricting host immune response to pathogens, by means of that is a crucial importance for chronic inflammatory disease studies. Therefore, the goal of this study was to measure the correlation of the IL-10 gene polymorphisms with the susceptibility of Behçet's disease compared with the control group in the Azeri population and to determine the expression of this gene in the two groups. Also, real-time PCR was performed for evaluate the IL-10 mRNA expression of the associated polymorphisms.
**Methods**: In this study, blood samples from 47 (1 missed) patients and 58 (3 missed) healthy control were taken and then mononuclear cells isolated with ficoll protocol. The DNA and RNA were subsequently extracted and were then examined for −592A/C (rs1800872) of IL-10 gene single nucleotide polymorphism (SNP) using RFLP-PCR. Allele and genotype distributions were evaluated among groups using chi-square or Fisher's test. Then, the extracted RNA was converted to cDNA using the RT-PCR method, afterward the expression of IL-10 was evaluated by Real-time PCR. Serum levels of IL-10 were measured using Enzyme-linked immunosorbent assay (ELISA).
**Results:** Rate of the rs1800872 A allele was statistically lower in the control group compared with BD patients ($p = 0.0315$ and OR = 1.90 (1.05–3.42)). Also, as we expected, the expression level of the IL-10 gene was significantly decreased in the patient group compared to the control.
**Conclusions**: Our study showed that the rs1800872 A allele of the IL-10 gene may contribute to the genetic susceptibility of BD by regulating the expression of IL-10. Also as we expected, the expression level of this gene was significantly decreased in the patient group compared to the control group.

**Keywords**: rs1800872 SNP, Behçet's disease, IL-10

ICIA2018\Immunology of Rheumatic Diseases\poster\7984
**7984**

# The effect of progesterone on MMP7 & MMP13 expression in a model of experimental systemic sclerosis

Elahe Izadi[1], Fatemeh Vafashoar[1], Hadi Poormoghim[2], Mohamad Ali Assarehzadegan[1] , Jalil Kuhpayehzadeh[3] Nazanin Mojtabavi[1*]

1. *Immunology department, Iran University of medical sciences, Tehran/ Iran*
2. *Scleroderma Study group, Firuzgar Hospital, Iran University of medical sciences, Tehran/Iran*
3. *Community Medicine and Public health research center, Iran University of medical sciences, Tehran/Iran*

**Background:** Gender medicine is a new era of science which focuses on the impact of sex hormones and gender on normal physiology, pathobiology and clinical features of diseases. In our previous study we showed that supra physiological dose of progesterone exacerbate the lung fibrosis in a mouse model of scleroderma. Matrix metalloproteinases are a group of enzymes which play a role in tissue remodeling and fibrosis. Whereas the abnormal expression of MMP2 and MMP9 are indicated in the pathogenesis of systemic sclerosis, fewer studies are done on MMP13 and MMP7 which are expressed by epithelial cells, fibroblasts and macrophages of the lungs. They are involved in the pathogenesis of COPD, IPF and different lung diseases therefore we aimed to investigate the effect of progesterone on the expression of these two enzymes in lungs of mouse model of scleroderma.
**Method**: Female mice received progesterone for 28 and 21 days in addition to 28 days bleomycin. On day 29 mice were sacrificed and the expressions of these two enzymes in lungs were analyzed by real time PCR.
**Result:** We found that bleomycin significantly downregulated the expression of MMP7 and MMP13 and co-administration of progesterone and bleomycin declined the expression of these enzymes but not in a significant manner.
**Conclusion:** While progesterone cannot reduce the expression of MMP7 and MMP13 in a mouse model of lung fibrosis further investigations are required to find modulators of MMP-7 and MMP-13 in this disorder.

**Keywords:** Systemic sclerosis, Progesterone, Fibrosis, Bleomycin, MMP7, MMP13

ICIA2018\Immunology of Rheumatic Diseases\poster\8090
**8090**

# The correlation between rheumatoid factor (RF) and anti cyclic citrullinated peptides (Anti-CCP) with gene expression of FoxP3 in rheumatoid artheritis patients

Nasrin Iranshahi[1], Seyed Mojtaba Amiri[2], Parisa Zafari[3,4], Mahdi Taghaddosi[1]*

*1. Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah,Iran*
*2. Department of Epidemiology and Biostatistics, School of Public Health, Kermanshah University of Medical Sciences, Kermanshah,Iran*
*3. Departmen of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
*Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran*
*4. Assistant professor in Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah,Iran*

**Background:** Rheumatoid arthritis (RA) is a chronic autoimmune disorder that affects 1-2% of people worldwide. Inflammation is an important factor in the pathogenesis of RA. Anti- Cyclic Citrullinated Peptid (Anti-CCP) antibody and Rheumatoid Factor (RF) are autoantibodies that promotes inflammatory reactions and have a crucial role in RA pathogenesis. Treg cells are necessary for the maintenance of immune homeostasis and prevention of autoimmunity. FoxP3 is essential transcription factor for development of these regulatory cells. In this study, we surveyed the effects of FoxP3 gene expression in peripheral blood on plasma levels of Anti-CCP and RF.
**Methods**: Peripheral blood samples were collected from 47 patients and 44 healthy subjects. Then plasma levels of Anti-CCP were evaluated using ELISA method. Also RF was detected with latex agglutination test and gene expression of FoxP3 analyzed by real time PCR.
**Results**: The levels of Anti-CCP ($P<0.001$) and RF ($P< 0.001$) were significantly higher in the patients in comparison with healthy subjects. Also significant reverse correlation between RF and Anti-CCP with gene expression of FoxP3 have been shown in our study (r: -0.630, r: -0.584) respectivly. The sensitivity and specificity of Anti-CCP and RF was (89.1%, 86.95%) and (91.3%, 91.1%), respectively for the diagnosis of RA.
**Conclusion**: Our data illustrated that FoxP3 gene expression have reverse significant correlation with plasma concentration of anti-CCP and RF.

**Keywords**: Rheumatoid arthritis, FoxP3, RF, Anti-CCP

ICIA2018\Immunology of Rheumatic Diseases\poster\8091
**8091**

# Importance of immunological biomarkers in assessment of disease activity and progression in rheumatoid arthritis

Nasrin Iranshahi[1], shirin Assar[2], Parisa Zafari[3,4], Seyed Mojtaba Amiri[5], Adel Fekri[1], Mahdi Taghaddosi[*]

*1. MSc student in department of Immunology, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*2. Assistance professor of Rheumatology in Clinical Research Development Center, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*3. Departmen of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran*
*4. MSc student in department of Biostatistics and Epidemiology, school of health, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*5*. Assistance professor in department of Immunology, Kermanshah University of Medical Sciences, Kermanshah, Iran*

**Background:** Rheumatoid arthritis (RA) is a chronic autoimmune disorder that affect 1-2% of people worldwide. Disease Activity Score (DAS-28) is a clinical parameter of RA progression. This factor is measured according to joints tenderness and swelling. Many elements could cause inflammation in synovial microenvironment including pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) and other mediators produced during immune response. Neoptrin is one of the metabolites that is produced following catabolism of guanosine triphosphate (GTP). The high concentration of neopterin is indicator of a more severe inflammation. Other disease mediators including Anti-CCP and RF are autoantibodies which are indispensable in the diagnosis of RA.
**Methods:** Peripheral blood samples were collected from 47 patients and 44 healthy subjects. Then plasma levels of neopterin and Anti-CCP were evaluated using ELISA method. Also RF positive patients were detected with latex agglutination test and ESR levels were obtained from patient's records.
**Results:** The levels of neopterin ($P<0.038$), Anti-CCP($P<0.001$) and RF($P<0.001$) were significantly higher in the patients in comparison with healthy subjects. Also a significant correlation between RF and DAS-28 ($P=0.001$) were shown in our study. The sensitivity and specificity of anti-CCP and RF were (89.1%, 86.95%) and (91.3%, 91.1%) respectively for the diagnosis of RA.
**Conclusion:** Our data showed that neither neopterin nor Anti-CCP has any significant correlation with DAS-28 while the correlation between RF and DAS-28 was statistically meaningful.

**Keywords:** Rheumatoid arthritis, DAS28-ESR, Neopterin, RF, Anti-CCP

ICIA2018\Immunology of Rheumatic Diseases\poster\8425
**8425**

# Frequency of different autoantigens among ANA seropositive patients referred to Shafa medical laboratory of Birjand City, Iran

Arghavan Asghari[1], Neda Mahavar[1], Elham Atabati[2], Mohammad Fereidouni[1]

*1. Asthma, Allergy & Immunology Research Center, School of Medicine, Birjand University of Medical Sciences, Birjand, Iran*
*2. Department of Rheumatology, Vali-Asr Hospital, Birjand University of Medical Sciences, Birjand, Iran*

**Background**: Prevalence of autoimmune disorders has increased dramatically during the last decades particularly in developing countries. Autoantibodies are responsible for autoimmune inflammation and are considered as the helpful markers for accurate diagnosis. It is shown that specific autoantibodies can lead to a specific disease, so it is important to figure out which autoantibodies are more prevalent in our population to have a proper diagnosis, management and follow up. The aim of this study was to evaluate the frequency of different autoantibodies in patients with positive anti-nuclear antibodies referred to the Shafa laboratory of Birjand city of Iran 2017.
**Methods**: Serum samples of patients with symptoms of autoimmune diseases were collected and presence of anti-nuclear antibody (ANA) as well as the profile of autoantibodies against at least 16 auto antigens were evaluated by immunofluorescence and immunoblotting test respectively.
**Results**: one hundred and fifty five (mean age: 43.58±14.50, range 13-83 years, M/F ratio: 0.11) subjects with positive ANA test participated in this study. The most observed autoantibodies were anti SSA and anti-RO52 (42.22% ؛42.22%). autoantibodies against gp120, mi2b, mi-2 and pm-scl 75 had the least frequency (0.6%;0.64%;0.64%), Respectively.
**Conclusion**: The results of current study showed that 90% of patients with positive ANA test were female and autoantibodies are the most frequent autoantibodies.

**Keywords**: Anti-nuclear antibody, Autoimmune disorders

ICIA2018\Immunology of Rheumatic Diseases\poster\8483
**8483**

## Association of polymorphisms in ERAP1 and risk of ankylosing spondylitis in an Iranian population

Hamed Mohammadi[1,2], Maryam Hemmatzade[1,2], Farhad Babaie[1,2], Alireza Rezaiemanesh[3], Jafar Majidi[1,2], Behzad Baradaran[1,2]

*1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
*2. Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
*3. Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*

**Background:** Endoplasmic reticulum aminopeptidase 1 (ERAP1) gene variants reduce the risk of progression ankylosing spondylitis (AS) through a decrease in ERAP1 activity. We determined the role that ERAP1 gene plays in Iranian patients with ankylosing spondylitis (AS) in terms of disease susceptibility and disease severity.
**Methods:** We evaluated two variants (rs27044 and rs17482078) of ERAP1 gene in HLA-B27 positive individuals (160 ankylosing spondylitis and 160 controls). The genotypes of involved polymorphisms (rs27044 and rs17482078) in ERAP1 were identified by SSP-PCR.
**Results:** There was statistically association between ERAP1 rs27044 polymorphism and risk of AS and the carriers with rs27044 CG genotype have an enhanced risk for AS (CG versus GG, OR = 1.60, 95% CI = 1.10–2.1, P = 0.006). However, we found no evidence for the association of 17482078 polymorphism in ERAP1 with AS risk.
**Conclusion:** Our results demonstrated that ERAP1 rs27044 polymorphism associates with the susceptibility of AS.

**Keywords:** Ankylosing spondylitis, ERAP1, Polymorphism, HLA-B27

ICIA2018\Immunology of Rheumatic Diseases\poster\8509
**8509**

# Gene expression profile of pro-inflammatory and anti-inflammatory cytokines in Peripheral Blood Mononuclear Cells of Patients with Ankylosing Spondylitis

Farhad Babaie[1,2] , Hamed Mohammadi[1,2], Sorayya Salimi[1,2], Alireza Rezaiemanesh[3], Behzad Baradaran[1,2], Zohreh Babaloo[1,2]

1. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
3. *Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*

**Background:** Ankylosing spondylitis (AS) belongs to a typical group of arthritides called seronegative spondyloarthritides. AS targets fundamentally the spine and sacroiliac joints and is characterized by entheseal inflammation, dactylitis and uveitis. The role of pro-inflammatory and anti-inflammatory cytokines in ankylosing spondylitis is still unrevealed. Our study aims to determine gene expression profile of pro-inflammatory and anti- inflammatory cytokines and their correlations with AS clinical manifestation patterns, including the age of symptom onset, Bath AS Disease Activity Index (BASDAI), Bath AS Functional Index (BASFI) and Bath AS Metrology Index (BASMI).
**Methods:** Peripheral blood mononuclear cells (PBMCs) were isolated from 40 patients with AS and 30 healthy controls, then total RNA of PBMCs was extracted, followed by cDNA synthesis from the mRNA of PBMCs. Quantitative Real time PCR was performed to evaluate mRNA expression of pro-inflammatory (INF-γ, IL-17F, IL-17A, TNF-α, IL-33, and IL-6) and anti-inflammatory cytokines (IL-10, IL-37 and TGF-β).
**Results:** A significant up-expression of pro-inflammatory (IL-17A, IL-17F, TNF-α, IL-33 and IL-6) and anti-inflammatory cytokines (IL-10, IL-37, and TGF-β) was observed in AS patients compared to the control group but, there was no significant difference in INF-γ expression between groups. There was no significant correlation between gene expression and AS clinical manifestation patterns.
**Conclusion:** These results demonstrated the critical role of pro-inflammatory and anti-inflammatory cytokines in the immunopathogenesis of AS. On the other hand, these cytokines may be responsible for inflammatory-related outcomes of AS.

ICIA2018\Immunology of Rheumatic Diseases\poster\8575
**8575**

# Study of retinal damage following cyclosporine-A eating using electroretinogram technique

Samaneh Gholami[1], Agheel Tabarmolahasan[1], Mohammad Masoud Shoushtarian[1], Seyed Hamid Aghajanzadeh[1], Safdar Mehdizadeh Shermeh[1]

*1. Islamic Azad University of Babol.*

**Background:** Cyclosporine A is prescribed as an immunosuppressive agent in many diseases. One of the side effects of cyclosporine is visual disturbances and retinal damage. Different methods are available to investigate the damage of retina, the best of which is the electroretinogram technique. In this study, the effects of cyclosporine on the retina of patients with rheumatoid arthritis were studied.
**Methods:** In this observational study, which was a cross-sectional descriptive-analytic study, 55 patients receiving cyclosporine due to rheumatoid arthritis between 2012-2014 were randomly selected and subjected to electroretinography and voltage then delay phase was determined and compared with the normal range. The results were analyzed by means of statistical analysis of variance. Significant level was set at 0.05.
**Results:** The average recorded voltage in ERG was 74.7 with a standard deviation of 29.79, which showed a statistically significant difference from normal value of 125 (P=0.0001). The mean recorded latency was 33.9 with a standard deviation of 3.58, which means no significant difference from normal value of 32.39 (P=0.545).
**Conclusion:** Based on the results, it is concluded that the use of cyclosporine significantly reduces the voltage recorded in the electroretinogram, but does not affect the latency phase. Therefore, patients taking this medicine should continue their treatment according to the ophthalmologist

**Keywords:** Electrotonography, Cyclosporine, Retina

ICIA2018\Immunology of Rheumatic Diseases\poster\8685
**8685**

**TNF-α -induced protein 3 gene polymorphisms in Azari patients with Behcet's Disease**

Saeede Ghasemi[1], Yasamin Pahlavan[2], Alireza Khabbazi[3]

*1. Department of Biology, Faculty of Sciences, Sciences and Researches of East Azarbijan Branch, Islamic Azad University*
*2. Department of Molecular Medicine, School of Advanced Medical Sciences, University of Tabriz Medical Sciences*
*3. Department of Romathology, School of Medicine, University of Tabriz Medical Sciences*

**Background:** Behçet's Disease is an inflammatory and chronic autoimmune disease with an unknown cause, which is associated with oral ulcers, skin and genital lesions. The aim of our study was the investigating the association of polymorphisms of TNF-α -induced protein 3 (TNFAIP3) gene with susceptibility to Behcet's Disease (BD) in Azari population of Iran.
**Methods:** In this cross sectional study we considered the single-nucleotide polymorphisms rs9494885 and rs7753873 of TNFAIP3 in 50 Iranian Azary patients with BD and 50 healthy controls by polymerase chain reaction-restriction fragment length polymorphism.
**Results:** A significant difference was found for the rs7753873 polymorphism between the BD and control groups. The frequency was 6.6% in BD patients compared to 20% of controls (p=0.04). We found no significant differences between the BD and control groups regarding the distribution of the polymorphism frequencies.
**Conclusion:** The rs7753873 polymorphism of TNFAIP3 has negative association with BD in Iranian Azari population.

**Keywords:** TNF-α, polymorphisms, Behcet's Disease

ICIA2018\Immunology of Rheumatic Diseases\poster\9945
**9945**

# Evaluation of CD4+/CD25+/high/CD127low/- Regulatory T Cells in Rheumatoid Arthritis Patients

Samaneh Delavari[1], Ata Ghadiri[2], Mehri Ghafourian[3], Elham Rajaei[4], Afshin Amari[5]

1. *Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
2. *Cellular and Molecular Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
3. *Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
4. *Department of Internal Medicine, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
5. *Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

**Background**: Rheumatoid arthritis (RA) is the most common inflammatory rheumatic disease with an unknown etiology which is characterized by destruction of articular cartilage and bone loss. The hallmark of disease is defect in immune tolerance. Regulatory T cells (Treg) play a critical role in protection of peripheral tolerance. We investigated the percentage of $CD4^{+}/CD25^{+/high}/CD127^{low/-}$ Treg cells in RA patients in comparison to healthy individuals.
**Methods:** The number of Treg $CD4^{+}/CD25^{+/high}/CD127^{low/-}$ cells was assessed by multi-color flow cytometry. The clinical disease activity of RA patients was determined by disease activity score 28 (DAS-28). The correlation of DAS-28 and ESR with Treg cells in peripheral blood of RA patients was evaluated.
**Results**: The percentage of $CD4^{+}/CD25^{+/high}/CD127^{low/-}$ Treg cells in RA patients was significantly decreased compared with healthy individuals. The percentage of $CD4^{+}/CD25^{+/high}/CD127^{low/-}$ Treg cells negatively correlated with DAS-28 and ESR.
**Conclusion:** In our study, we conclude that defect of Treg cells play a key role in the pathogenesis of this disease. Further studies are necessary to clarify the role of Treg cells in clinical course of rheumatoid arthritis.

**Keywords:** Rheumatoid arthritis, Regulatory T cells, Flow cytometry

ICIA2018\Immunology of Rheumatic Diseases\poster\9984
**9984**

**MicroRNAs as potential and novel biomarkers in adult Iranian patients with systemic lupus erythematosus**

Majid Khoshmirsafa[1], Farhad Seif[1], Reza Falak[1], Mehdi Shkarabi*[1]

*1. Department of Immunology School of Medicine University of IRAN*

**Background:** MicroRNAs (miRNAs) have emerged as effective immune-modulators of gene expression in immune responses. Previous miRNA studies have been showed some unique miRNA signatures that are associated with systemic lupus erythematosus (SLE), but their role as diagnostic and prognostic biomarkers of Lupus Nephritis (LN) has not been well surveyed.
**Methods:** In this ongoing study, we assessed miRNA expressions (miR-200a, b and c) in peripheral blood mononuclear cells (PBMCs) obtained from SLE patients with different kidney involvements using quantitative PCR (q-PCR).
**Results:** MiR-200c displayed significant increment in SLE patients (LN) in comparison with healthy controls (P value < 0.05).
**Conclusion:** The role of miRNAs in immunopathogenesis of rheumatic diseases was determined, it is possible that certain miRNAs may serve as therapeutic and diagnostic biomarkers for SLE and LN.

**Keywords:** Systemic lupus erythematosus, Lupus Nephritis, MicroRNA, q-PCR

# Immunomodulation and Immunoregulation

# Poster Presentation

ICIA2018\Immunomodulation and Immunoregulation\Poster\8515
**8515**

**Evaluation of Immunomodulatory Effect of Monolaurin**

Elham Rahimi[1], Maryam Ekhtelat[1], Mojgan Naghitorabi[1], Ata Ghadiri[2]

*1. Cellular and Molecular Research Center, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
*2. Department of Immunology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

**Background:** Fatty acids, monoglycerides and some esters of fatty acids show antitumor and antimicrobial effect. In this study, we evaluated the monolaurin (1-glycerol-monolaurate; the esterified form of lauric acid) effect on macrophage activation.
**Methods:** For this purpose, monolaurin effect on peritoneal macrophages of mice (BALB/c) and LPS stimulated macrophages were examined in triplicate. MTT assay with different concentrations (10, 50, 100, 200, 400 and 800 µg/ml) of monolaurin was done for evaluation of macrophages viability and the level of nitric oxide in supernatant of culture was measured by nitric oxide (**NO**) assay kite. Data analysis was performed by the SPSS software using ANOVA.
**Results:** MTT assay showed that monolaurin in amount of 100 µg/ml and greater significantly reduced viability of macrophages (stimulation index). Also, the amount of NO production in stimulated macrophages in concentrations of more than 100 µg/ml of monolaurin, significantly was lower compared to the LPS control ($p<0.05$).
**Conclusion:** Regarding immune-modulatory effects of monolaurin, it can be used to help cure disorders of immunity and inflammation. Of course, in vivo evaluation are necessary to confirm it.

**Keywords:** Immunomodulatory, Monolaurin, Macrophage, MTT assay, Nitric oxide

ICIA2018\Immunomodulation and Immunoregulation\Poster\8536
**8536**

# Effect of low molecular weight garlic proteins on the T regulatory cell induction in co-culture of PBMCs and colorectal cancer cell lines

Mona Amani[1], Elahe Safari[1], Samaneh khorami[1]

*1. Immunology Department, School of Medicine, Iran University of Medical Sciences*

**Background:** Cancer is one of the non-infectious diseases with high mortality all over the world. In this regard, colorectal cancer is the third most common cancer worldwide. There is different therapeutic methods such as chemotherapy, radiotherapy and surgery using to treat the cancer. Between this methods perhaps we can bringing to account the herbal medicine to use as supplement in prevention and/or control the cancer. Among various types of herbal medicine it can mention to garlic as the eldest plant with medicinal properties that its medicinal usage was very common from ancient years. Garlic active ingredients have different biological effect. They can improve immune system to defend against different microorganism and also cancer. It can also use as treatment of cardiovascular disease. Thus in this study we were decided to extract the low molecular weight proteins of garlic and then treat the co-culture of PBMCs and colorectal cancer cell lines SW48 and SW837 with the garlic protein extract to examine the immunomodulatory effect of this protein in the tumor microenviroment as the most important factor that affect tumor growth and expansion.
**Methods:** After extraction from garlic cloves, protein fractions were separated with G-75 gel filtration chromatography. To check the purity of separation, Sodium Dodecyl Sulfate Poly Acrylamide Gel Electrophoresis(SDS-PAGE) were done. To define the protein identity, MALDI-TOF spectrometry were done. In the cell culture phase, PBMCs and cell lines alone and in co-culture were treated with desire protein and PBMCs proliferation was assayed by using fluorescent dye, CFSE. Also these cell culture supernatants were collected to evaluate the secretion of inflammatory and inhibitory cytokines in this milieu. Finally, to measure the inhibition rate of inhibitory cell induction like Treg and MDSC in co-culture medium in the presence of desire protein, PBMCs were cultured with cell lines and then stained with specific antibodies against Treg and MDSC surface antigens and the results were assayed with FACS.
**Results:** We identify that this protein extraction was a lectin binding protein with molecular weight of 11-16 kDa that exhibit immunomodulatory effect. In proliferation assay, this protein was able to stimulate PBMCs alone and in co-culture with tumor cell lines. In cytokine assay we observed that PBMC treatment with protein extraction caused reduction in TGF-β and Galectin-3 secretion; in opposite IL-6 and IFN-γ secretion level was upregulated and it has no significant effect on IL-10 secretion in comparison with negative control which had no treatment. Also we investigated that it could inhibit immune suppressor cell, Treg and MDSC induction in the co-culture milieu.
**Conclusion:** By stimulating PBMCs proliferation, inhibiting suppressor cell induction and upregulating inflammatory cytokine and reversely reducing inhibiting cytokines; garlic protein extract may use as immunomodulatory supplement in cancer treatment. Also in vivo study should be done.

**Keywords:** Immunomodulation , Tumor , garlic.

ICIA2018\Immunomodulation and Immunoregulation\Poster\8718
**8718**

## Comparison of cytokine profile between patients with end-stage renal diseases and healthy individuals

Farshid Fathi[1], Hossien Moteddayyen[2], Noushin Lotfi[1], Shohreh Mohammadi[3], Hossien Hafezi[4], Erfan Sadeghi[5]

1. *Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
2. *Department of Laboratory Medicine, Kashan University of Medical Sciences, Kashan, Iran*
3. *Khorshid Hospital, Isfahan University of Medical Sciences, Isfahan, Iran*
4. *Department of Dermatology, Isfahan University of Medical Sciences, Isfahan, Iran*
5. *Department of Biostatistics and Epidemiology, School of Public Health, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background:** Cytokines have a pivotal role in regulating the function of the cells from both the innate and adaptive immune system. Imbalance and dysregulation in cytokine profile produced by different immune cells can influence the risk of developing autoimmune and allergic disorders. We, therefore, focused to determine whether there is a difference in cytokine profile between renal patients who suffered from some autoimmune disorders and healthy subjects.
**Methods:** Heparinized whole blood (5 ml) was obtained from patients with end-stage renal failure and healthy subjects. Plasma was isolated from whole blood and the level of interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), interleukin-4 (IL-4), interleukin-17A (IL-17A) and interleukin-10 (IL-10) was measured by an Enzyme-linked immunosorbent assay (ELISA) kit.
**Results:** Our results indicated that the level of IL-17A and TNF-α was significantly increased in renal patients with autoimmune disorders compared to healthy individuals ($P<0.05$). However, there was no significant difference in the level of IFN-γ and IL-10 between renal patients suffered from autoimmune disorders and healthy subjects. Moreover, a significant reduction in IL-4 level was observed in renal patients compared to healthy subjects ($P<0.001$).
**Conclusion:** Regarding the fact that cytokines play an important role in regulating the immune responses which lead to the development of autoimmune disorders, dysregulation of cytokine production from different immune cells in patients with autoimmune diseases may be contributed to the immunopathogenesis of renal failures.

**Keywords:** Renal patients, Autoimmune diseases, cytokines

# Immunoparasitology

# Poster Presentation

ICIA2018\Immunoparasitology\Poster\8336
**8336**

# The influence of fetal calf serum and hemin in growth and infectivity of *Leishmania major* in BALB/c mice

Sima Habibzadeh [1,2], Sima Rafati[1]

1. *Immunotherapy and Leishmania Vaccine Research Dept., Pasteur Institute of Iran, Tehran, Iran*
2. *Islamic Azad University, Pharmaceutical Sciences Branch, Tehran, Iran*

**Background**: Leishmaniasis is a common disease between humans and animals in tropical and subtropical regions. The causative agent is *Leishmania* which existes in two forms of promastigote and amastigote. Like other cells require nutrients to grow. Various culture media especially in liquid form designed for parasite cultivation. Most of these media contain either Fetal Calf serum (FCS) or blood as essential ingredient which is highly problematic. We have designed liquid media without FCS. Although is not expensive but the addition of hemin is obligatory for *in vivo* infectivity of *L. major* in BALB/c mice. This molecule as the prostatic group of hemoproteins, has important roles in regulating vital cell functions and transformation of promastigote to amastigote.
**Methods**: In the present study, *L. major* parasites were cultured in two different media, LBM (LB-BHI-M199) and LBM with hemin (LBM H+) in comparison with M199 FCS 5% (M199 5%) as control positive group. After several passages, stationary-phase promastigotes were injected to the left footpad of mice. *In vivo* infectivity of parasites were evaluated at six weeks post infection, using footpad swelling and quantitative real-time PCR.
**Results**: There is no swelling in the footpad of mice infected with parasite growing within LBM without hemin. In addition, the level of swelling was similar in animals infected with parasite growing in LBM H+ media and M199 5%. Furthermore, by real-time PCR, the lowest parasite was detected in group infected by parasite in LBM without hemin in compare with LBM+ hemin and M199 with 5%FCS.
**Conclusion:** *Leishmania* like other trypanosomatids needs an external source of heme such as hemoglobin, blood, serum, hemin and hematin for their growth. This is due to lack of two essential enzymes for heme biosynthesis pathway. In this study we examined the effect of hemin on the infectivity of *L. major* parasite in BALB/c mice and prove it as an essential substance.

**Keywords**: *L. major*, Heme, Infectivity, BALB/c

ICIA2018\Immunoparasitology\Poster\8600
**8600**

# Prevalence Cutaneous Leishmaniasis in the Western Cities Areas of Kermanshah Province, West Iran, During 2010- 2015

Mehrdad Pooyanmehr[1], Mohammad Reza Khedri[2], Erfan Bahrami[2], Amir Amiri Paryan[3*]

*1. Department of Basic Sciences and Pathobiology, Microbiology section, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran*
*2. Veterinary Student, Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran*
*3. Veterinary Student, Pathobiology & Basic Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran*

**Background:** Leishmaniosis is one of the most important tropical diseases and zoonotic infection. Natural transmission of Leishmania parasites is carried out by biting of female sandflies genus Phlebotomus from reservoir hosts to human (caused by obligate intracellular protozoa of the genus Leishmania). There are three forms of leishmaniosis: Cutaneous leishmaniasis (CL, rural and urban), visceral leishmaniosis (VL) and muco cutaneous leishmaniosis (MCL). Cutaneous leishmaniosis only causes ulcers on skin. In past ten years, prevalence and incidence of leishmaniosis remarkably increased in many regions of Iran.
**Methods:** The data were taken from internal reports of Health Centers western cities areas of kermanshah province (Ravansar, Javanrood, Pavehe and Salasebabajany) in the period six years (2010-2015). Thirty-five patients with CL were included in this study. The diagnosis of CL was based on clinical, Para Clinical findings and microbiologic procedures on chronic (Non-Healing) skin lesions. Laboratories in Health Centers four city confirmed of the diagnosis for detecting *Leishmania* parasites in infected tissue (through light-microscopic examination of stained specimens, culture techniques and molecular methods).
**Results:** Results indicated that All Thirty-five patients' referent were who diagnosed as CL. The clinical presentation of CL was typical in 31 patients, Ravansar 13 (0.013%), Javanrood 8 (0.008%), Pavehe 1 (0.001%) and Salasebabajany 9 (0.009%) per100.000 persons, by clinical para clinical findings and pathologic documentation in 4 cities medical centers registries patients at six years period respectively.
**Conclusion:** CL is a very contagious and important infectious disease worldwide. The diagnosis of CL may be difficult, especially in atypical presentations and Non-endemic areas. Thus, CL should be kept in mind, especially in many regions of Iran.

**Keywords**: Zoonotic, cutaneous, leishmaniosis, western cities areas, Kermanshah

ICIA2018\Immunoparasitology\Poster\9850
**9850**

# The Effect of *Leishmania Major* Lipophosphoglycan 3 on Human T Lymphocytes In Vitro

Maryam Hosseini[1], Mostafa Haji-Fatahaliha[2], Sanaz Rasolzadeh[1], Mehdi Yousefi[1]

1. *Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Leishmaniasis is a vector-borne disease threatens 350 million people in 98 countries over the world. No effective drug or vaccination strategy has been designed against leishmaniasis so far. Lipophosphoglycan 3 (LPG3) as one of the class II LPG genes from HSP90 family involves in assembly and synthesis of lipophosphoglycan (LPG) and other phosphoglycan residues implicate in parasite virulence. The aim of this study was to investigate the ability of recombinant LPG3 (rLPG3) to induce human T-cell lineages in vitro.
**Methods:** Peripheral blood mononuclear cells were obtained from heparinized blood samples of 10 healthy volunteers. Then $CD4^+$ naïve T-cells were separated using magnetic-activated cell sorting technique and their purity was evaluated by flow cytometry. Isolated T-cells were treated with different concentrations of recombinant *Leishmania major* LPG3. After 48 h incubation, the activity of T-cells was investigated by assessing hallmark cytokines of T helper (Th) 1, 2 and 17 lineages using real-time PCR and ELISA techniques.
**Results:** The purity of isolated $CD4^+$ T-cells was about 90%. rLPG3 could induce IFN-γ mRNA expression in moderate and high doses but no significantly effect on IL-4 and IL-17A expression was shown in treated T cells. Moreover, similar observations were achieved in cytokine secretion and showed stimulatory effect of rLPG3 in IFN-γ secretion especially in high concentration ($p<0.05$).
**Conclusion:** Our results demonstrated that LPG3 could stimulate Th1 responses effectively but further researches are needed to investigate its anticipated adjuvant usage in *Leishmania* vaccination.

**Keywords:** Leishmaniasis, LPG3, T cell

# Immunopharmacology and Medicinal Plants

# Poster Presentation

ICIA2018\Immunopharmacology and Medicinal Plants\poster\5301
**5301**

**The hydro alcoholic extract of *Hypericum perforatum* attenuates the inflammatory reactions in acetic acid induced ulcerative colitis**

Siavash Mashhouri [1], Seyyed Meysam Abtahi Froushani [2]

*1. Graduate of Veterinary Medicine, Department of Microbiology, Faculty of Veterinary Medicine, University of Urmia*
*2. Department of Microbiology, Faculty of Veterinary Medicine, University of Urmia*

**Background**: This study was done to investigate the beneficial effects of the hydro alcoholic extract of *Hypericum perforatum* to control the inflammation in animal models of ulcerative colitis.
**Methods**: Luminal instillation of acetic acid was used to induce ulcerative colitis in the male Wistar rats. Rats in the treatment groups received herbal extract (150 mg/kg PO) or Prednisolone (4 mg/kg PO) daily for 10 consecutive days. When the animals were sacrificed, the disease activity index, the levels of malondialdehyde, myeloperoxidase, nitric oxide, total protein and the concentration of TNFα IL1ß and IL6 were determined in the colonic homogenized tissues specimens.
**Results**: Both therapeutic regimes with herbal extract and Prednisolone could ameliorate the clinical scores and the mortality rate of ulcerative colitis in a comparable manner. The levels of TNFα, IL1ß and myeloperoxidase activity were regressed in the gut tissues of Prednisolone treated rats more than ulcerative colitis rats treated with herbal extract. Nevertheless, *H. perforatum* could suppress the levels of IL6, nitric oxide and malondialdehyde more significantly than Prednisolone.
**Conclusion**: It seems that the hydro alcoholic extract of *Hypericum perforatum* may be used as a natural source to control the inflammation in the ulcerative colitis.

**Keyword**: *Hypericum perforatum*, ulcerative colitis, Acetic acid, inflammation.

ICIA2018\Immunopharmacology and Medicinal Plants\poster\6363
**6363**

# Effects of Viola tricolor flower hydroethanolic extract on lung inflammation in a mouse model of chronic asthma

Elham Harati, Alireza Razavi[2]., Maryam Bahrami[1], Mohammad Kamalinejad[3]., Maryam Mohammadian[1]., Hamid Reza Sadeghipour[1].

*1. Department of Physiology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
*2. Department of Pathobiology, Division of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
*3. Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Asthma is a chronic inflammatory disease of the lungs driven by T cell activation. *Viola tricolor* L. as a traditional medical herb could suppress activated T lymphocytes and has been used empirically for asthma remedy. In the present study, we investigated the anti-inflammatory effect and underlying mechanism of *Viola tricolor* on asthma characteristics induced by ovalbumin (OVA) in mice.
**Methods:** Sixty BALB/c mice were randomly divided into six groups: normal control, OVA control, OVA mice treated with *Viola tricolor* (50, 100 and 200 mg/kg) and dexamethasone (3 mg/kg). All mice except normal controls were sensitized and challenged with OVA. Asthmatic mice were treated orally in the last 7 days of OVA challenge. The total and differential leukocyte counts, Interleukin (IL)-4 and interferon (IFN)-γ levels in bronchoalveolar lavage fluid (BALF) were determined. H&E staining for lung inflammation was performed.
**Results:** *Viola tricolor* treatment at 200 mg/kg significantly decreased IL-4 level but did not considerably affect the IFN-γ level. Therefore, it effectively reduced asthma characteristics including infiltration of leukocytes particularly eosinophil and peribronchial inflammation as compared to dexamethasone. However, *Viola tricolor* at 100 mg/kg had the most prominent inhibitory effect on the IL-4 level and also markedly increased IFN-γ level. As result, it prevented further reduction of inflammatory parameters in this group compared to the *Viola tricolor*-treated group at 200 mg/kg. It seems that a significant increase in IFN-γ level at 100 mg/kg could prevent the beneficial effects of reducing IL-4 in the asthma treatment.
**Conclusion:** Our study demonstrated that *Viola tricolor* has anti-inflammatory effects via inhibition of Th2 cytokine production and validated its empirical usage in traditional medicine.

**Keywords:** Asthma; Inflammation; Murine model; *Viola tricolor* L.

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7338
**7338**

# Improving Doxorubicin Chemotherapy Efficacy by Using Citrus Unshiu Extract That Reduces Inflammatory Cytokines-Induced Cachexia in a Murine Colon Cancer Model

Sahar Tahaghoghi Hajghorbani [1]*, Alireza Rafiei [1], Mohamad Ali Ibrahim Zade [2], Javad Akhtari[3]

*1. Department of Immunology, Medical School, Mazandaran University of Medical Sciences, Sari, Iran*
*2. Pharmaceutical Sciences Research Center, School of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran*
*3. Mazandaran university of Medical Sciences, Nanobiomedicine, Sari, Iran*

**Background:** Several medical trials on the treatment of cancer cachexia have been performed using synthetic drugs that can stimulate appetite and suppress systemic inflammation. But the long-term use of drugs induces adverse effects. Therefore, finding novel agents that efficiently prevent or treat cancer-induced cachexia with minimal adverse effects is very important to improve the cancer chemotherapy efficacy and survival time. In the present study, Citrus Unshiu (a seedless and easily peeled citrus fruit) Peel (CUP) extract was evaluated as a safe and potent agent along with chemotherapy.
**Methods:** Water CUP extract was prepared. The phytochemical profile of CUP was analyzed using the Hitachi HPLC system. Prophylactic and therapeutic extract was fed to male BALB/c mice alone or together with doxorubicin chemotherapy. Inflammatory cytokines (IL-1β, TNF-α and IL-6) and NO production were evaluated using enzyme-linked immunosorbent assay (ELISA) and Griess reagent, respectively. Body weight, tumor size, food intake and survival rate were assessed in mice
**Results:** HPLC analyses revealed the high levels of Naringin and Hesperidin content in the CUP extract. We found that daily oral administration of CUP to male BALB/c mice bearing C26 reduced the losses in body weight and serum inflammatory cytokines level that eventually result in cancer-induced cachexia symptoms. Also the tumors in mice who received extract grew more slowly and the survival rates were greater than mice in the other groups
**Conclusion:** These findings collectively suggest that WCUP reduces systemic inflammation in tumor-bearing mice and suppresses the production of pro-cachectic cytokines followed by the prevention of weight loss and tumor growth.

**Keywords:** Immunotherapy, Cachexia, Colon Cancer, Unshiu Extract

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7384
**7384**

**Effect of D-panthenol on the adjuvant induced rheumatoid arthritis (RA) in Wistar rats.**

Navid Mijani, Norouz DelireZh, Seyyed Meysam Abtahi Froushani

*Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** The anti-inflammatory benefits of D-panthenol have been documented earlier. This study was done to evaluate the effect of D-panthenol on the rat model of rheumatoid arthritis (RA).
**Methods:** RA was induced by injection of complete Freund's adjuvant into the footpad of Wisatr rats. Then, rats were allocated in 3 groups: treated with D-panthenol (500 mg/kg-orally), treated with Prednisolone (10 mg/kg-orally) and un-treated group. All treatments were initiated at day 5 after induction. The change in the dorso-plantar diameter of hands and legs of each rat were recorded every other day until 23 days after induction.
**Results:** The edema and swelling of the soles of the feet of RA rats received D-panthenol or prednisolone were significantly decreased in a similar manner. The serum levels of nitric oxide and myeloperoxidase concurrent with proliferation of spleen lymphocytes were significantly lessened in treatment groups compared to control rats. The level of decrease in the level of serum nitric oxide was higher in the D-panthenol group compared to the prednisolone group.
**Conclusion:** D-panthenol may be considered as a useful drug to control RA disease.

**Keywords:** D-panthenol, Rheumatoid arthritis, Immunity responses

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7386
**7386**

**Dexpanthenol has beneficial effects on the acetic-acid induced ulcerative colitis**

Seyedeh Mehrnaz Mostatabzadeh, Seyyed Meysam Abtahi Froushani

*Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** The anti-inflammatory benefits of Dexpanthenol have been documented earlier. Here, we investigated the protective potential of Dexpanthenol in ameliorating animal model ulcerative colitis (UC). **Methods:** UC was induced in the male Wistar rats by luminal instillation of acetic acid. Rats in the treatment groups treated with Dexpanthenol (100 mg/kg PO) or Prednisolone (2 mg/kg PO) daily for 10 consecutive days. At the end, the animals were sacrificed and the disease activity index, the levels of myeloperoxidase, malondialdehyde, nitric oxide and total protein were evaluated in the colonic homogenized tissue specimens. **Results:** The data indicated that both therapies with Dexpanthenol and Prednisolone could suppress the clinical scores and the mortality rate of ulcerative colitis in a comparable manner. The levels myeloperoxidase activity was lessened in the guts of Prednisolone treated rats more than Dexpanthenol groups. Nonetheless, Dexpanthenol downregulated the levels of nitric oxide and malondialdehyde more significantly than Prednisolone.
**Conclusion:** These data suggest that the Dexpanthenol may be used as an appropriate medication to ameliorate the signs of ulcerative colitis.

**Keywords:** Dexpanthenol, Ulcerative colitis, Anti-inflammatory.

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7405
**7405**

# Comparison of Immunomodulatory Effects of Some *Allium* species from Iran on Cytokine Generation

Hosseinpur Z[1], Radjabian T[1,2], Ghazanfari T[1], Naghizadeh MM[1]

1. *Immunoregulation Research Center, Shahed University, Tehran, Iran*
2. *Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran*

**Background:** The literature review has demonstrated that extract of *Allium sativum* (garlic) modulates the cytokines release patterns in lymphocytes. But there are unveiled data about immunomodulatory potentials of the other *Allium* species. So, in the present research, the effects of extracts from some wild grown *Allium* species of Iran on IFN-γ, IL-4 and IL-17 production were evaluated.
Methods: The bulbs of some *Allium* species were collected from their natural localities and the aqueous extracts of them were prepared. Lymphocytes were isolated from BALB/c mice and were incubated with various concentrations of the bulb extracts. Finally, the levels of IFN-γ, IL-4 and IL-17 were measured.
**Results:** Bulb extracts of *A. sativum* had stimulatory effects on production of IFN-γ and IL-17, so the extracts could probably switch immune responses towards Th1 and Th17 cells pattern. Bulb extracts of *A. asarense* increased secretion values of IL-4 from lymphocytes. Whilst, bulb extracts of *A. sativum* decreased the amounts of IL-4, especially in the higher concentrations. Therefore, *A. asarense* and *A. sativum* may be significantly acted as a stimulator and inhibitor of Th2 cells, respectively, in some concentrations. On the other hand, bulb extract of *A. jesdianum* significantly decreased the amounts of IL-4 and IL-17, especially in the higher concentrations. Hence it seems that this species reduced the response of Th2 and Th17 cells.
Conclusion: In general, bulb extracts of the *Allium* species had immunomodulatory effects on the lymphocyte cytokines levels, which are involved in immune related-diseases, and these species may be considered for treatment studies.

Keywords: *Allium* species, Bulb extract, Cytokines, IFN-γ, IL-4, IL-17

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7407
**7407**

**Assessment of the Immunomodulatory Effects of Some *Allium* species on TNF-α and NO Production**

Hosseinpur Z[1], Radjabian T[1,2], Ghazanfari T[1], Nasiri Z[1]

1. *Immunoregulation Research Center, Shahed University, Tehran, Iran*
2. *Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran*

**Background:** The effects of *Allium sativum* (garlic) extract, as a medicinal plant, have been demonstrated on variety of immune cells and their secretory mediators associated with various immune-related diseases. According to the efficiency of garlic bulb extracts in animal studies and unveiled data about the other *Allium* species on immune responder cells, the effects of aqueous extracts from the bulbs of some wild grown *Allium* species from Iran on TNF-α and NO generation in BALB/c mice macrophages were evaluated.
Methods: The bulbs of some *Allium* species were collected from their natural localities and the aqueous extracts of them were prepared. Peritoneal macrophages were isolated from BALB/c mice and were incubated with different concentrations of the bulb extracts. Then, the amounts of TNF-α and NO production in macrophages were measured.
Results: In non-pathogenic conditions of this research, NO generation in macrophages was increased after incubation with bulb extracts of *A. elburzense* and *A. Asarense,* but their stimulatory effects was not significant. The effects of different concentrations of the bulb extracts of the examined *Allium* species were not significant on TNF-α generation, except for 0.05 mg/ml ($P<0.001$) bulb extract of *A. sativum*. In spite of the fact that the immunomodulatory effects of the bulb extracts on TNF-α and NO production were not significant, but the identical patterns were observed for the stimulatory effects of the bulb extracts of *A. elburzense* and *A. Asarense* on the production of these factors in the pretreated macrophages. Also, similar patterns were obtained in reduction of NO production by macrophages after treatment with the bulb extracts of *A. sativum* and *A. Iranicum.*
Conclusion: In general, bulb extracts of the *Allium* species had modulatory effects on the studied mediator factors, which are involved in immune related-diseases, and these species may be considered for treatment studies.

Keywords: *Allium* species, Bulb extract, Macrophage, NO, TNF-α

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7451
**7451**

**Synergistic benefits of thymol and nicotine in alleviating rat model of rheumatoid arthritis (RA)**

Sara Golbahari, Seyyed Meysam Abtahi Froushani

*Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** One logical approach to enhancing rheumatoid arthritis (RA) treatment result is the combination of available medications to provide more favorable outcomes. Immunomodulatory benefits of thymol and nicotine were documented. Here, we investigated synergistic effects of combination therapy by suboptimal doses of atorvastatin and ATRA in experimental autoimmune encephalomyelitis (EAE), an animal model of MS.
**Methods:** RA was induced by injection of complete Freund's adjuvant into the footpad of Wisatr rats. Then, animals were allocated in 4 groups: treated with nicotine (2.5 mg/kg-orally), treated with thymol (100mg/kg-orally), combined therapy (one half doses of each medication) and untreated group. All therapies were initiated at day 5 after induction and continued throughout the study until the day 23 when animals were sacrificed.
**Results:** Combined in EAE mice with half doses of thymol and nicotine lead to the more favorable clinical outcome than treatment with optimal doses of either medication alone. All medications caused a significant decrease in the serum levels of CRP, myeloperoxidase, splenocytes proliferation index and IL-17 and IFN-γ secretion after ex vivo stimulation of splenocytes. Without any advantage in anti-proliferative effect, combined therapy regressed secretion of pro-inflammatory IL-17 cytokine and nitric oxide in splenocytes more than either medication alone.
**Conclusion:** Combined thymol and nicotine have synergistic benefits and this approach may be a useful strategy to control RA.

**Keywords:** Rheumatoid arthritis, thymol, Nicotine, Combination therapy.

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7463
**7463**

# Hydroalcoholic extract of Cinnamon has anti-inflammatory benefits on the acetic-acid induced ulcerative colitis

Mojtaba Salamatian[1], Vahid Mohammadi[1], Seyyed Meysam Abtahi Froushani [2]

*1. Department of internal medicine and clinical pathology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*
*2. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Recent evidence has proposed the anti-inflammatory benefits of Cinnamon. Here, we evaluated the protective potential of hydroalcoholic extract of Cinnamon in alleviating ulcerative colitis (UC) induced in rats by acetic acid.
**Methods:** Luminal instillation of acetic acid was applied to induce UC in the male Wistar rats. Rats in the treatment groups received hydroalcoholic extract of Cinnamon (400 mg/kg PO) or Prednisolone (2 mg/kg PO) daily for 10 consecutive days. At the end, the rats were sacrificed and the disease activity index, the levels of myeloperoxidase, nitric oxide and total protein were evaluated in the colonic homogenized tissue specimens.
**Results:** The results exhibited that both therapies with hydroalcoholic extract of Cinnamon and Prednisolone could suppress the clinical scores and the mortality rate of ulcerative colitis in a comparable manner. The levels myeloperoxidase activity was decreased in the colonic homogenate of Prednisolone treated rats more than Cinnamon group. Nevertheless, hydroalcoholic extract of Cinnamon regressed the levels of nitric oxide in gut tissues more prominent than Prednisolone.
**Conclusion:** Hydroalcoholic extract of Cinnamon may be used as an appropriate medication to reduce the inflammatory mediators in UC.

**Keywords:** Hydroalcoholic extract of Cinnamon, Ulcerative colitis, Anti-inflammatory.

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7497
**7497**

# Immunostimulatory effects of hydroalcoholic extract of Mallow in NMRI mice immunized by Sheep Red Blood Cells.

Zahra Zeinaly, Seyyed Meysam Abtahi Froushani, Hadi Esmaili Govarchin Galee

*Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Mallow (*Malva sylvestris*) is a medicinal herb used in folklore medicine for treatment of some infection and inflammation. Here, we investigated the effects of hydroalcoholic extract of Mallow on the immune responses of in NMRI mice Immunized by Sheep Red Blood Cells (sRBC).
**Methods:** 14 male NMRI mice were divided into two groups and were immunized with the sRBC antigen, twice, with one-week interval. Treated mice received the hydroalcoholic extract of Mallow (200 mg/Kg-orally, every day) for 3 weeks from the beginning of the survey. Finally, the levels of anti-sRBC antibody and the specific cellular immune responses were assumed by microhemagglutination test and footpad thickness, respectively. Furthermore, neutrophils were checked for phagocytosis and respiratory burst.
**Results:** Hydroalcoholic extract of Mallow significantly potentiated specific cellular immunity and concurrently potentiated specific humoral immunity in mice after challenge with sRBCs. Furthermore, hydroalcoholic extract of Mallow significantly increased the respiratory burst in neutrophil population. Nonetheless, neutral red uptake by neutrophils didn't show any significant change between groups.
**Conclusion:** It seems that Hydroalcoholic extract of Mallow can be applied as the immunostimulatory agent.

**Keywords:** Mallow (*Malva sylvestris*), Immunostimulator, NMRI mice.

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7510
**7510**

**Synergistic antitumor effect of NVP-BEZ235 and CAPE on MDA-MB-231 breast cancer cells**

Samira Torki[1], Amin soltani[1], Hedayatollah Shirzad[2], Nafiseh Esmaeil [3], Mahdi Ghatrehsamani[1]

1. *Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran*
2. *Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran*
3. *Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background:** Triple negative breast cancer (TNBC) is the most lethal and aggressive kind of breast cancer. Studies with TNBC cells suggest that tumor environmental cytokines such as tumor growth factor β1 (TGF-β1) have important roles in tumors fate.
**Methods:** In the present study, we aimed to investigate the effect of phosphatidyl inositol 3-kinase/AKT/ mammalian target of rapamycin (PI3K/Akt/mTOR) signaling pathway dual inhibitor, NVP- BEZ235 and Caffeic acid phenyl ester (CAPE) on TNBC cell lines (MDA-MB-231), stimulated with TGF-β1 for 14 days in vitro.
**Results**: We found that TGF-β1 as a local tumor environmental cytokine plays important role in progression and invasiveness of TNBC cells. NVP-BEZ235 inhibited the enhanced cell viability and CXCR4 expression induced by TGF-b1. In addition, the combined treatment of TNBC cell lines with CAPE and NVP-BEZ235 synergistically inhibited cell growth and reduced CXCR4 expression. Also, treatment of MDA-MB-231 cells with CAPE and NVP-BEZ235 led to decreasing the expression levels of p-FOXO3a in a time-dependent manner.
**Conclusion**: Overall, these results suggest that, tumor metastasis and progression in TNBC cells can be effectively reduced through the concurrent use of NVP-BEZ235 and CAPE. This could be of particular interest in assessing the effects of this therapy in the reduction of tumor metastasis and progression in other tumor types.

**Keywords:** Caffeic acid phenyl ester; NVP-BEZ235; Triple negative breast cancers; TGFβ1

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7570
**7570**

# Effect of cinnamon and turmeric aqueous extracts on serum interleukin-17F level of high-fructose fed rats

Maryam Sohrabi[1], Mahdi Alahgholi-Hajibehzad[2,3], Zahra Gholami Mahmoodian[1], Sayed Ali Hosseini Siyar[4], Alireza Zamani [2,3*]

1. *Department of Anatomy, School of Medicine, Hamadan University of Medical Sciences. Hamadan, Iran*
2. *Department of Immunology, School of Medicine, Hamadan University of Medical Sciences. Hamadan, Iran*
3. *Molecular Immunology Research Group, Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
4. *Department of Animal Science, Agriculture Faculty, Bu-Ali Sina University, Hamadan, Iran*

**Background:** Studies indicate overweight and obesity induce a chronic low-grade inflammation. Inflammation predisposes obese people to some chronic diseases and cancers. This study was conducted to evaluate the effect of two weight lowering and anti-inflammatory agents, cinnamon and turmeric, on the serum level of interleukin-17 (IL-17) as a pro-inflammatory cytokine.
**Methods:** Sixty-four rats were designated in eight groups. All groups except control, cinnamon and turmeric groups received fructose solution. The control group received normal diet. The other groups fed with normal diet plus: cinnamon, turmeric, fructose with cinnamon and fructose with turmeric. The serum level of IL-17F was detected by enzyme-linked immunosorbent assay (ELISA).
**Results:** High fructose consumption led to increase the body weight gain and IL-17. While, feeding with cinnamon and turmeric caused to decline body weight gain but surprisingly increased IL-17F.
**Conclusion**; Although, some studies showed cinnamon and turmeric supplementation decrease IL-17 under the standard diet but in the presence of high fructose diet and overweighting their effects were reversed.

**Keywords:** Interleukin-17, Cinnamon, Turmeric, Fructose, Inflammation, Overweight

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7583
**7583**

# Citrus Unshiu Extract Decreases Inflammatory Cytokines and MDA Production In LPS-Stimulated Macrophage Cell line and C-26 Tumor-Bearing Mice

Sahar Tahaghoghi Hajghorbani [1]*, Alireza Rafiei [1], Mohamad Ali Ibrahim zade [2], Zahra Hoseini Khah[3]

*1. Department of Immunology, Medical School, Mazandaran University of Medical Sciences, Sari, Iran*
*2. Pharmaceutical Sciences Research Center, School of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran*
*3. Department of Molecular Cell-Biology, Research Center, Faculty of Medicin, Mazandaran University of Medical Sciences, Sari, Iran*

**Background:** Citrus unshiu (Marcov) is an easy-peeling citrus fruit, which has been used as a traditional Chinese medicine to treat common cold, relieve exhaustion, and cancer. Hesperidin, naringin and nobiletin are representative components of citrus unshiu peel. However, its effects on inflammation remain unknown. In this study, we investigated the in vitro and in vivo effects of citrus unshiu peel (CUP) on the production of pro-inflammatory mediators. In addition, the effects of daily oral administration of CUP on tumor growth in mice bearing tumor was evaluated.
**Methods:** Water extract of citrus unshiu peel (WCUP) was prepared. Cytotoxicity was analyzed using MTT assay to find out the effective dose of extract. The phytochemical profile of WCUP was analyzed using the Hitachi HPLC system. Murine macrophage RAW 264.7 cell lines were pretreated with CUP and stimulated with LPS. Inflammatory cytokines (IL-1β, TNF-α and IL-6) and NO production was evaluated using enzyme-linked immunosorbent assay (ELISA) and Griess reagent, respectively. Body weight, tumor size, food intake and survival rate were assessed in male BALB/c mice.
**Results:** HPLC analyses revealed that the naringin content in the CUP extract was 22% (2, tR: 13.87 min). We found that CUP represses LPS-induced MDA, as well as TNF-α, IL-1β and IL-6 production. In addition we observed that daily oral administration of CUP to male BALB/c mice bearing C26 reduced the losses in body weight and serum inflammatory cytokines levels compared with saline treatment. Tumor growth was inhibited in 45% of the mice in this group.
**Conclusion:** WCUP has the in vitro and in vivo anti-inflammatory effects and it can be used as a nutritional supplement for the management of cancer patients with severe weight loss.

**Keywords:** Immunotherapy, TNF-α, Colon Cancer, Unshiu Extract

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7597
**7597**

# Modulatory effect tretinoin on histopathology of pancreas of streptozotocin-induced diabetes in C57BL/6 mice

Farin Malekifard[1], Nowruz Delirezh[2], Rahim Hobbenaghi[3], Hassan Malekinejad[4]

1. *Department of Microbiology, Facutly of Veterinary Medicine, Urmia University, Urmia, Iran*
2. *Department of Microbiology, Facutly of Veterinary Medicine, Urmia University, Urmia, Iran*
3. *Department of Pathobiology, Facutly of Veterinary Medicine, Urmia University, Urmia, Iran*
4. *Department of Pharmacology, Urmia University of Medical Sciences, Urmia, Iran*

**Background:** It has been shown that some drugs such as tretinoin have immunomodulatory and anti-inflammatory activity, which might represent a potential preventive therapy for autoimmune diseases. The present study was undertaken to examine the protective effect of tretinoin on histopathology of pancreas in diabetic mice.
**Methods:** Diabetes was induced by multiple low-dose of streptozotocin (MLDS) injection (40 mg/kg/day for 5 consecutive days) in male C57BL/6 mice. Mice were considered diabetic when their fasting blood glucose levels were >250 mg/dl. Subsequently, the mice were allocated to three therapeutic groups (n=7 per group) (normal control group, MLDS group and treatment group). In Treatment group, mice were treated with tretinoin (20 mg/kg/day i.p.) for 21 days. Animals were euthanized on day 22 and pancreases were isolated and stained with hematoxylin &eosin (H&E) and Gomeri aldehyde fuchsin (GAF) for histological analyses (the number of islets and β cells, diameter of islets) of pancreas.
**Results:** The number of islets and β cells and diameter of islets were decreased in diabetic rats compared to control animals ($P<0.05$). Tretinoin treatment in streptozotocin-induced diabetic mice increased the mean diameter of islets and the number of islets and beta cells compared with the diabetic group ($p<0.05$).
**Conclusion:** Tretinoin improved pancreas tissue during destruction of the pancreatic beta-cells in streptozotocin-induced type 1 diabetes in mice.

**Keywords** Type 1 diabetes, Tretinoin, Streptozotocin, Pancreas

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7625
**7625**

**Immunomodulatory effect *of Cinnamomum aromaticum* on dendritic cell**

Abbas Azadmehr[1,2], Niloufar Sadat Miri[1], Mojtaba Shahnazi[3], Robabe Latifi[3], Sahar Mosalla[3], Reza Hajiaghaee[4]

1. *Department of Immunology, Babol University of Medical Sciences, Babol, Iran*
2. *Department of Immunology ,Qazvin University of Medical sciences .Qazvin, Iran*
3. *Department of Parasitology, Qazvin University of Medical Sciences, Qazvin, Iran*
4. *Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran*

**Background:** Extracts from medicinal plants have been reported to possess good immunomodulation. This study was conducted to assess the immunomodulatory effect of *Cinnamomum aromaticum* extract on dendritic cells (DC).
**Methods:** For this study, DCs were isolated from the spleen of Balb/c mice by using monoclonal antibody and magnetic bids (MACS method). $10^5$ cell/well were cultured with 10, 50 and 100 µg/ml concentration of C*innamomum aromaticum* extract. The expression of maturity markers of DCs (CD40, CD86, MHCII) were measured by flow cytometry method.
**Result**: In this present study, the result indicated that the extract of *Cinnamomum aromaticum* significantly ($P < 0.05$) decreased all maturity markers dose-dependently (10, 50 and 100 µg/ml) in DC cells via the immunomodulatory effect.
**Conclusion:** These results suggest that *Cinnamomum aromaticum* extract has potential immunomodulatory effect by inhibition of maturity markers on APCs. So, this medicinal plant can be used as an immunomodulator agent in inflammatory disease. However future studies are necessary.

**Keywords:** *Cinnamomum aromaticum,* Antigen presenting cells, Immunomodulation

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7626
**7626**

# Immunosuppression properties of *Cinnamomum camphora* extract on dendritic cells maturation markers

Niloufar Sadat Miri[1], Abbas Azadmehr[1,2], Mojtaba Shahnazi[3], Sahar Mosalla[3], Robabe Latifi[3], Reza Hajiaghaee[4]

1. *Department of Immunology, Babol University of Medical Sciences, Babol, Iran*
2. *Department of Immunology ,Qazvin University of Medical sciences, Qazvin, Iran*
3. *Department of Parasitology, Qazvin University of Medical Sciences, Qazvin, Iran*
4. *Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran*

**Background:** In these studies we examined the impact of *Cinnamomum camphora* extract on dendritic cells. Here, we investigated the effect of *Cinnamomum camphora* extract on maturation and function of DCs.
**Methods:** DCs were collected from Balb/c mice spleen undergoing MACS method. Then, the cells were cultured and treated with 10, 50 and 100 µg/ml concentration of *Cinnamomum camphora* extract. The expression of CD40, CD86 and MHCII were evaluated by flow cytometry method.
**Result:** The result of our study demonstrated that this medicinal plant have immunomodulatory effect by significantly inhibition of CD40 and MHCII of DCs markers dose-dependently ($P<0.05$) in all concentration (10-100 µg/ml). In addition, the extract of *Cinnamomum camphora* only in the concentration of 100 µg/ml significantly ($P<0.05$) decreased the expression of CD86 marker.
**Conclusion:** Our findings showed that *Cinnamomum camphora* extract has immunosuppression property. However, further investigations to identify effective compounds in these extract and their effect on immune response are necessary.

**Keywords:** *Cinnamomum camphora*, Antigen presenting cells, Immunosuppression

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7650
**7650**

**Apoptosis and necrosis of a human colon cancer cell line after treatment by the extract of *Scrophularia megalantha***

Narges Amirjamshidi[1], Hesam Babaei Khameneh[1], Azin Aghamajidi[1], Abbas Azadmehr[1], Ebrahim Zabihi Neyshabouri[2], Reza Hajiaghaee[3], Amrollah Mostafazadeh[2]

1. *Department of Immunology, Babol University of medical sciences, Babol, Iran*
2. *Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran*
3. *Medicinal Plants* Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran

**Background:** Medicinal plants are often used as cost-effective and safe anticancer agents. In this line, there is some evidence in literature indicating that *Scrophularia* species are used in traditional medicines to cure many diseases such as eczema, ulcers, cancer, etc. The objective of the present study was to investigate the apoptosis-inducing potential of the *S.megalantha* extract on a human colon cancer cell line; HCT–116.
**Methods:** HCT–116 cells were cultured with various concentrations of *S. megalantha* extract for 48 h. MTT assay was done to determine cytotoxicity and GI50 index. The cell death pattern was determined by staining with Annexin V-FITC (fluorescein isothiocyanate) and PI (propidium iodide).
**Results:** The GI50 of the *S. megalantha* extract was found for the colon cancer cells. Flow cytometry analysis showed that the dominant pattern of cell death induced by the *S. megalantha* extract was necrosis, whereas apoptosis was detected with higher doses of this extract.
**Conclusion:** Our experiments indicated that the extract of *S.megalantha* induced both apoptosis and necrosis to inhibit the proliferation of colon cancer cells. This extract would be a useful agent for improving the treatment of colon cancer. However, further experiments are necessary.

**Keywords:** Colon cancer; *Scrophularia megalantha*; Apoptosis; Herbal extract

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7733
**7733**

# Effect of dimethyl fumarate on *in vitro* differentiation of naive CD4+ T cells to regulatory T cells

Haideh Namdari[1], Maryam Izad[2], Zahra Amirghofran[1, 3*]

1. *Department of Immunology, Medical School, Shiraz University of Medical Sciences, Shiraz*
2. *Department of Immunology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran*
3. *Autoimmune Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background**: Over-activated Th17 cells have been associated with the pathogenesis of autoimmune disorders. Regulatory T cells (Tregs) by controlling these cells can dampen autoimmunity and protect body against tissue injury. We investigated the effects of dimethyl fumarate (DMF), a fumaric acid ester isolated from *fumaria officinalis*, on cell proliferation and differentiation of Tregs and the Th17 response.
**Methods:** The effect of DMF on cell proliferation and the viability was determined by BrdU and propidium iodide (PI) assay. CD4+ naïve T cells were isolated from mouse spleen and after stimulation with anti-CD3/anti-CD28 antibodies, cultured in the presence of Treg-inducing cytokines and DMF. We performed flow cytometry for assessment of Tregs development and real-time PCR for gene expressions of specific transcription factors and cytokines.
**Results:** Cell proliferation analysis showed the inhibitory effects of DMF at various concentrations. Non-cytotoxic concentrations determined by PI assay were used for the next experiments. In differentiation study, naïve $CD4^+$ T cells were treated with TGF-β and IL-2 cytokines to differentiate toward Treg cells. The rate of Treg development was approximately 47%. Treatment of cells with DMF reduced the rate of naïve $CD4^+$ T cells differentiation to Tregs to less than 18%. Real-time PCR showed that treatment of mice splenocytes with DMF significantly decreased Foxp3 (0.32±0.11 RFC, $p<0.01$), IL-10 (0.3±0.15 RFC, $p<0.01$) and IL-17 (<0.2 RFC, $p<0.001$) gene expressions. The expressions of TGF-β and RORγt also decreased but the levels compared to the control were not significant.
Conclusion: Decreased Tregs generation and IL-10 expression as well as reduced IL-17 cytokine by DMF, suggest the suppressive effect of this compound on Tregs and Th17 cells. Further studies for determination of the effect of DMF on the Treg/Th17 balance at cellular level are recommended.

**Key words**: DMF; immunomodulation; $CD4^+$ T cells; regulatory T cells

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7860
**7860**

**Anti-inflammatory effects of Lavandula angustifolia in rat model of myocardial infarction**

Nahid aboutaleb[1], Faramarz Souri[2], Yaser Azizi[1]

1. *Physiology Research Center, Iran University of Medical Sciences, Tehran, Iran*
2. *Physiology Department, Faculty of Medicine,* Ahvaz Jundishapur University of Medical Sciences, *Ahwaz, Iran*

**Background:** Studies have demonstrated that acute inflammatory response after myocardial infarction confers irreversible loss of a large number of cardiomyocytes, myocardial necrosis and subsequent heart failure. In the present study, we focused on the anti-inflammatory effects of Lavender in myocardial ischemia/reperfusion injury and its potential mechanisms.
**Methods:** Male Wistar rat weighing 250-300 g were randomly assigned into three major groups: Sham (n=10), control IR-vehicle (n=10), two cohorts treated with different doses of Lavender oil from Lavandula angustifolia +IR (L1/100mg/kg and L2/200mg/kg, n=10 per group). Lavender was administrated interaperitoneally in rats instantly after left anterior descending coronary artery (LAD) ligation for 20 min. At 24 h after reperfusion, rats were sacrificed under deep anesthesia and the heart was stored at -80 ˚C prior to inflammation assessment. The expression of pro-inflammatory and anti-inflammatory cytokines was evaluated by the immunohistochemical assay.
**Results:** LAD ligation remarkably increased the expression of pro-inflammatory cytokines (TNFα and IL1β) and decreased the expression of anti-inflammatory cytokines (IL10) 24 h after reperfusion. After administration of different doses of Lavender in myocardial ischemia/reperfusion injury rats, the expression of TNFα and IL1β were significantly decreased compared with control IR-vehicle in a dose dependent manner. Moreover, Lavender post-treatment markedly increased the expression of IL10 relative to control IR-vehicle cohort in a dose dependent mechanism.
**Conclusion:** In summary, our result showed that Lavender post-treatment could ameliorate myocardial injury after MI by targeting inflammatory cytokines. These findings establish a fundamental foundation for future drug design to treat MI.

**Keywords:** Lavender, Inflammatory Cytokines, Ischemia reperfusion

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7869
**7869**

# Evaluation of the anticancer effects of aqueous extract of persimmon seed on K562 cell Line (Chronic Myeloid Leukemia)

Mohammad Khojasteh[1], Elham Ahmadi[2], Rozhin Jamali[1]

*School of Veterinary Medicine, Islamic Azad University, Sanandaj Branch, Sanandaj, Iran*
*Department of Pathobiology, School of Veterinary Medicine, Islamic Azad University, Sanandaj Branch, Sanandaj, Iran*

**Background:** Chronic myeloid leukemia (CML) is a myeloproliferative disease originating from a constitutively active tyrosine kinase termed Bcr-Abl, which is expressed by an oncogene resulting from a reciprocal translocation between chromosome 9 and chromosome 2. Plant-derived compounds and their semisynthetic serve as a major source of pharmaceuticals for human diseases. It is estimated that approximately 25% of prescriptions handled contain a plant-derived natural product and 74% of the 119 most important drugs currently contain ingredients from plants used in traditional medicine. Persimmon is used in traditional medicine against several human ailments, including cancer. The aim of this study was the investigation of the effect of aqueous extract of persimmon seed on K562 cell line.
**Methods:** First, the chronic myeloid leukemia K562 cell line was cultured. Concentrations of 25, 50, 100, 200, 400 and 800 µg/ml of aqueous extract of persimmon seed were prepared and the cells were treated for 24, 48 and 72 hours. Next, the inhibitory effects of this extract on K562 cells proliferation was measured by MTT assay. Hoechst (33342) staining and DNA electrophoresis was used to check apoptosis. Data analyses were performed using SPSS 23 software.
**Results:** In this study, anticancer effect of aqueous extract of persimmon seed on K562 leukemia cell line was confirmed. The results also showed that the aqueous extract of persimmon seed, compared with the control group, had cytotoxic activity that is highest in 800 µg/ml at 72 hours. Statistical analysis indicated that this extract inhibited the growth of K562 cells ($P<0.05$).
**Conclusion:** The findings of the present study showed that the aqueous extract of persimmon seed possessed inhibitory effect on K562 cell line. In order to find the underlying mechanism of this activity, further research should be carried out.

**Keywords:** Leukemia, Apoptosis, Persimmon seed

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7897
**7897**

**Dopamine can instruct M2 anti-inflammatory macrophages**

Hanieh Nikkhah, Seyyed Meysam Abtahi Froushani

*Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Previous documents indicated that dopamine possesses a spectrum of the direct and indirect role in modulating the immune system. This survey was designed to evaluate the effects of dopamine on the polarization of macrophages.
**Methods:** The resident macrophages were collected from the peritoneal cavity of Wistar rats by injecting 20 ml of ice-cold PBS and treated with dopamine for 24h in the concentration of $5\times10^{-7}$ M (maximum physiologic concentration).
**Results:** The flow cytometry results demonstrated that the dopamine treatment significantly increased the levels of CD68+/CD206+ cells (M2 macrophage marker), when macrophages were cultured with dopamine, compared to macrophages alone (46.9%±2.1 vs. 23.7%±2.98, $p<0.001$). The mRNA level of NF-κB p65 in macrophages pulsed with dopamine was down-regulated compared macrophages without treatments. Albeit, MTT reduction assay indicated that dopamine treatment couldn't any significant changes in the vitality of macrophages.
**Conclusion:** Collectively, dopamine treatment of macrophages instructs M2 anti-inflammatory macrophages. These findings might offer new insight into the potential mechanisms that underlie the immunomodulatory effects of dopamine.

**Keywords:** Macrophages, Dopamine, anti-inflammatory.

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7907
**7907**

# Zerumbone may diminish the expression of TGF-β produced by inflammatory fibroblasts

Zahra Radaee[1], Alireza Zamani[1,2], Razieh Amini[2,3]

1. *Immunology Department, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
2. *Research Center of Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
3. *Student Research Committee, Hamadan University of Medical Sciences, Hamadan, Iran*

**Background:** Inflammatory cytokines, TGF-β, play a pivotal role in controlling tumor progression. TGF-β belongs to the family of regulatory cytokines that interfere with the dual use of a wide range of cells that contribute to physiological and pathological processes such as fetal development, tumorigenicity, immune response, inflammation, and cancer. Fibroblasts play an important role in progression of human cancers. Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine that act as a major role in regulation of the inflammatory process in tumor growth. Zerumbone, the first sesquiterpene isolated from ginger, has been observed to show antitumor activities in multiple cancer cells. The current study was done to explore the role of TNF-α and zerumbone on TGF-β expression.
**Methods:** The cytotoxicity of zerumbone in fibroblast was determined using MTT assays. The influence of TNF-α on expression of TGF-β was measured using the SYBR green based QPCR and enzyme-linked immunosorbent assays. We also examined the effects of zerumbone on expression of TGF-β.
**Results**: We could show a significant increase of the expression of TGF-β in fibroblast cells after stimulation by TNF-α. On the other hand, we found that treatment of TNF-α stimulated fibroblasts by zerumbone decreased TGF-β expression.
**Conclusion:** Our study had suggested that the expression of TGF-β produced by inflammatory fibroblasts can be regulated by zerumbone and as a results, It can be suggested that treatment with the Zerumbone may be an ideal choice for immunological treatment of cancer.

**Keywords:** TGF-β, TNF-α, Fibroblast, Zerumbone.

ICIA2018\Immunopharmacology and Medicinal Plants\poster\7942
**7942**

# Effects of *Achillea millefolium* Extract on Pancreas Tissue in Diabetic Rats

Mahsa Safari[1] , Mohammad Shahbazzadeh[2]

*Department of biochemistry, Faculty of management, Payam-e-Noor University, Tehran, Tehran, Iran*

**Background:** Diabetes is the third leading cause of death in word. Hyperglycemia and oxidative stress have the main factors involved in pathogenesis of diabetes. On the other hand, the antioxidant system is the first defense mechanism of body against oxidative stress. *Achillea millefolium* possesses hypoglycemic and antioxidant effects. This study surveyed the effects of different doses of *Achillea millefolium* Hydro- alcoholic extract (50,100,150 mg/kg) on histological changes of Langerhans islets and serum insulin, nitric oxide and glucose levels.
**Methods**: A total of 45 male Wistar rats were divided into 8 groups (control, diabetic with STZ, treatment with *Achillea millefolium* (50,100,150 mg/kg) and diabetic treated with *Achillea millefolium*) (50,100,150 mg/kg). Data were analyzed by one-way ANOVA, and P value <0.05 was considered significant.
**Results**: *Achillea millefolium* extract (100 and 150 mg/kg) significantly decreased serum glucose level ($p<0.01$) and improved the diameter of islets ($p<0.05$) in diabetic rats treated with *Achillea millefolium* extract, compared with the diabetic group. Moreover, at dose of 150 mg/kg, the extract improved serum insulin ($p<0.01$), decreased nitric oxide ($p<0.01$) and increased the weight ($p<0.01$) and number of islets of diabetic rats ($p<0.05$). Histopathological studies also confirmed these changes.
**Conclusion**: *Achillea millefolium* can improve insulin secretion and serum glucose levels in an animal model of STZ induced diabetes, possibly by reducing nitric oxide production and preventing pancreatic tissue oxidative damage.

**Keywords**: *Achillea millefolium*, Diabetes, Pancreas, Hyperglycemias, Rats

ICIA2018\Immunopharmacology and Medicinal Plants\poster\8097
**8097**

# Anti-angiogenic activity of β-D-mannuronic acid (M2000) as a novel NSAID with immunosuppressive properties under *in vitro* and *in vivo* models

Mohsen Rastegari Pouyani[1,2], Ali Mostafaie[3], Kamran Mansouri[3], Shahabeddin Mortazavi Jahromi[1, 4], Hamid Reza Mohammadi Motlagh[3], Abbas Mirshafiey[1]

1. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
2. *Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
3. *Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran*
4. *Department of Cellular and Molecular Biology, Kish International Campus, University of Tehran, Kish, Iran*

**Background:** Angiogenesis, the process by which new capillaries are formed from pre-existing ones, plays a pivotal role in the pathogenesis of several disorders including cancer and chronic inflammatory disorders. The β-D-mannuronic acid (M2000), patented (DEU;102016113018.4), is a novel NSAID with immunosuppressive effects and is a matrix metalloproteinase (MMP) inhibitor. Since MMPs play an important role during angiogenesis and due to the existence of various molecular links between angiogenesis and inflammation, we hypothesized that M2000 might also have anti-angiogenic properties.
**Methods:** Cytotoxic and anti-proliferative effects of M2000 on human umbilical vein endothelial cells (HUVECs) were examined by trypan blue and MTT assays, respectively. For in vitro angiogenesis assay we designed a three-dimensional (collagen-cytodex) model. Finally, we used the chick chorioallantoic membrane (CAM) model for in vivo assessment of M2000 effects on angiogenesis.
**Results:** Based on our cytotoxicity assay, endothelial cells showed a high tolerability towards increasing amounts of M2000. According to the anti-proliferative assay M2000 had little or no anti-proliferative effect on HUVECs. Our in vitro anti-angiogenesis model was indicative of weak anti-angiogenic effects of M2000. In the in vivo model, however, M2000 showed a much more potent anti-angiogenic activity in a dose-dependent manner.
**Conclusion:** M2000 could be considered as an anti-angiogenic molecule which more likely exerts its activity mainly via indirect effects on endothelial cells and its anti-inflammatory effects may partly be attributable to its anti-angiogenic activity. Therefore, it might be recommended as a candidate for prevention and treatment of cancer, chronic inflammatory diseases and other angiogenesis-related disorders.

**Key words:** Angiogenesis, M2000, Cancer, Collagen-cytodex, Inflammation, CAM

ICIA2018\Immunopharmacology and Medicinal Plants\poster\8132
**8132**

# Hydroalcoholic extract of *Lavandula officinalis* (lavender) suppresses the phagocytosis and respiratory burst of neutrophils

Negin Mohammadpoor[1], Seyyed Meysam Abtahi Froushani[1], Bahman Mansouri Miotlagh[1], Sahar Etemadi[1]

*1. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** *Lavandula officinalis* (lavender) is a kind of medicinal plants. It is under clinical research for its anti-inflammatory benefits. This study was performed to investigate the effects of lavender on the neutrophils isolated from orally treated mice with hydroalcoholic extract of Lavender.
**Methods:** 20 male NMRI mice were allocated into two equal groups: Treatment and Control groups. The animal in treatment groups orally received with hydroalcoholic extract of lavender for 4 constitutive weeks (400 mg/Kg-Daily). Control mice received PBS at the same volume. At the end, peripheral blood neutrophils were isolated by dextran and used for *ex vivo* assays.
**Results:** Neutral red uptake and MTT reduction assay test showed that Lavender couldn't change the vitality of neutrophils. Lavender significantly decreased the respiratory burst and phagocytosis potential in neutrophil population after challenge with opsonized yeast.
**Conclusion:** It seems that Lavender can be used as an anti-inflammatory agent in immunopathologic conditions induced by neutrophils.

**Keywords:** Neutrophils, Lavender, Inflammation.

ICIA2018\Immunopharmacology and Medicinal Plants\poster\8231
**8231**

# Isolation of Ellagic acid from pomegranate peel and study of it's anti-inflammatory effect in mouse

Elham Abbasi

*Kermanshah University of Medical Sciences School of Medicine*

**Background:** Inflammation is a protective response to eliminate the primary stimuli, causing cell injury or necrosis and tissue damages from the tissues of reference. Previous studies have shown that there is a correlation between inflammation and heart disease, cancer and chronic inflammatory diseases. On the other hand, pomegranate has been proved to encompass various notable polyphenols in its peel, including punicalagin, hydrolysable tannin which could be hydrolyzed into ellagic acid. Ellagic acid has long been considered a phenolic compound with anti-inflammatory and anti-cancer effects compound to be considered. So, the present study aimed to purify ellagic acid from pomegranate husk and analyze the anti-inflammatory effects of ellagic acid on inflammation model induced by LPS in mouse.
**Methods:** After grounding pomegranate peel into powder, dried powder was extracted with aqueous-alcoholic solution. In order to purify ellagic acid, recrystallization by methanol was undertaken. The purified product was then assayed and confirmed using FTIR and C-NMR. To assess the anti-inflammatory activity of purified ellagic acid mouse samples were classified into test and control groups before induction by LPS. Test groups were treated with ellagic acid in doses of 10, 50 and 100 milligrams per kilogram of mouse body weight. Finally, the level of TNF-α cytokine in serum and peritoneal fluid in control and test groups was determined by Eliza kit.
**Results:** The results from purification of ellagic acid showed high purity and yield of 2% from pomegranate peel. Test groups treated with three doses of purified ellagic acid exhibited a significantly decreased TNF-α level in serum and peritoneal fluid comparing to control groups.
**Conclusion:** Purified ellagic acid with high purity and efficiency reduced the level of TNF-α cytokine. In this study it was shown that a significant impact on the TNF level is observed. The results presented here could be investigated by further studies and various other methods.

**Key words**: inflammation, Ellagic acid, tumor necrosis factor, lipopolysaccharide

ICIA2018\Immunopharmacology and Medicinal Plants\poster\8318
**8318**

# The effect of Iranian propolis on pro- inflammatory cytokines IL-1B, IL-6 and TNF- α

Mina Motallebnejad[1], Hamid Reza Nouri[2], Fariba Asgharpour[3]

*1. Department of Oral and Maxillofacial, Oral Health Center, Health Research Institute, Babol University of Medical Sciences, Babol, I.R. Iran*
*2. Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, I.R. Iran*
*3. Dental Materials Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, I.R. Iran*

**Background:** Propolis is a resinous substance collected by bees from different plant sources and its compounds are divers by location and their characteristics depend on the type of plants in each region. Propolis is rich in flavonoids and antioxidants and indicate a broad spectrum of biological activities, such as antioxidant, antimicrobial, anticancer and anti-inflammatory properties. In this study investigated the pro-inflammatory effects of Iranian propolis on cytokine production (IL-6, IL-1β and TNF- α) in murine macrophage cell line (RAW 264.7).
**Methods:** Propolis was collected in the beehive located in the Polur of Mazandaran province (PEEP) in the spring of 2016. RAW264.7 cells were treated for 12 h with or without various concentrations of PEEP and LPS (1 pg/ml). After that, total RNA was isolated cDNA synthesized and, then cytokines expression was evaluated by quantitative real-time PCR.
**Results:** IL-1β significantly was decreased in presence of LPS plus PEEP in concentrations of 0.15 µg/mL and 1.5 µg/mL and PEEP (15 µg/mL) significantly inhibited IL-6 mRNA expression, but PEEP was not effective in of TNF-α production.
**Conclusion:** These results suggest that PEEP has inhibitory effects on macrophage activity by reducing expression of IL-1β and IL-6, and may have further-reaching implications for the pharmaceutical industry.

**Keywords:** Propolis, Pro-inflammatory, Cytokines, Murine macrophage

ICIA2018\Immunopharmacology and Medicinal Plants\poster\8497
**8497**

# The Effect of Methanol Extract of Echinops Lasiolepis on TNF-α Production in LPS-activated J774 A.1 Mouse Macrophages

Fateme Sadat Dashti, Hossein Hadinedoushan, Maryam Asadi

*Department of Immunology, Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

**Background:** Plants as medicines have always played a vital role in human life. Tumor necrosis factor alpha (TNF)-α is one of the macrophage-derived inflammatory cytokine with pleotropic effects in the inflammation process. Some studies have been demonstrated that some of the Echinops species have anti-inflammatory activity. In fact, Echinops lasilepis is introduced as one of the native plants of Yazd. Thus, the present study intended to assess the inflammatory activity of Echinops lasiolepis on TNF-α secretion in J774 A.1 mouse macrophages.
**Methods:** At first, methanol extract was prepared by maceration. 105 cells/ well were seeded in 96-well plate in triplicate and were treated with different concentrations of extract and 100 ng/ml Lipopolysaccharides. MTT cytotoxicity assay was used to determine the cell viability. Concentrations of extract with cell viability of more than 90% were used to evaluate the level of TNF-α in the macrophage culture using enzyme-linked immunosorbent assay.
**Results:** Viability of cells at different extract concentrations of 0.1, 1, 10, 50, 100 and 200 µg/ml were 91.68, 95.27, 94.2, 90.8, 85.38 and 71.38, respectively. Therefore, cells treated with 50 µg/ml and lower concentrates of extracts showed more than 90% of viability and their supernatants were used for TNF-α assay. The study results revealed that all concentrations of extract reduced the production of TNF-α.
**Conclusion:** Our findings showed that methanol extract of Echinops lasiolepis may have anti-inflammatory activity via reducing TNF-α production.

**Keywords :** Tumor necrosis factor , J774A.1 cell line , Echinops Lasiolepis

ICIA2018\Immunopharmacology and Medicinal Plants\poster\8578
**8578**

**Influence of Acemannan and Glucomannan as an immunomodulator on E7d vaccine**

Mahboubeh Nikookalam[1], Zeinab Salehian[1], Sahar Mirzaee[1], Mehdi Mahdavi[2], Mohammad Hossein Yazdi[3,4]

*1. Department of Biotechnology, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran (IAUPS)*
*2. Department of Immunology, Pasteur Institute of Iran, Tehran, Iran*
*3. Department of Pharmaceutical Biotechnology and Biotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
*4. Evidence-Based Medicine Group, Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran*

**Background :** Acemannan and glucomannan are mannose containing polysaccharides in Aloe Vera mucilage with immune enhancement capabilities were studied for its impact on the immunization profile of E7d Vaccine. These polysaccharides absorbed after oral feeding of mice and remained unchanged in the blood stream. Subsequently it can act as an immunomodulator which is induced both Th1 and Th2 immune response.
**Materials and methods**: 60C57BL/6 mice were divided into six groups as follow: Group 1, E7d-Alum-Gel; Group 2, E7d-Alum; Group 3, E7d-Montanid -Gel; Group 4, E7d-Montanid; Group 5 and Group 6,Control-Gel and PBS respectively. The oral feeding of Aloe vera gel with 0.05% polysaccharide was done for a period of 30 days. The mice were immunized with E7d vaccine three times on day 0, 14 and 28th. Then tumor was implanted subcutaneously and 2 weeks later, the serum samples of the mice were collected to evaluate the cytokines and antibodies levels by ELISA assay.
**Result:** analysis of humoral immune responses represented a noteworthy increase of total antibody level in E7d-MONTANID-Ge lgroup in comparisonto E7d-montanid group. Result of survival rate was best in E7d –ALUM-GEL and CONTROL GEL groups.
**Conclusion:** Aloevera polysaccharides as the potential of enhancing the immune system and also has antitumor activity and may have the capacity to use along with chemotherapy drugs. This effect was possibly derived from inducing IL-2, TNF-α, IFN–γ and other cytokines which are producedin the body and also improves the immunity response activity

**Keyword:** Acemannan, Glucomannan, E7d vaccine, Immunomodulator

ICIA2018\Immunopharmacology and Medicinal Plants\poster\8608
**8608**

# The Study of Immunomodulatory Effect of Hydroalcoholic Extract Hypericum Scabrum on Wound Healing in Kurdish Ethnomedicine

Amir Amiri Paryan[1*], Mehrdad Pooyanmehr[2], Mohammad Reza khedri[3], Erfan Bahrami[3]

*1. Veterinary Student, Pathobiology & Basic Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran*
*2. Department of Basic Sciences and Pathobiology, Microbiology section, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran*
*3. Veterinary Student, Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran*

**Background**: Several plants are used in traditional medicine for treatment of human diseases such as wound. Wound is the result of damages to the skin and other soft tissues. In traditional medicine, it has been reported that are species of Hypericum Scabrum (HS) has healing effect on peptic ulcer, depression and wound. The aim of this study is to appraise the effect of hydroalcoholic extract of HS on wound healing.
**Methods**: In this study 24 male wistar rats weighting about 200±25gr. Animals were anesthetized by mixture of ketamine and xylazine in ratio of 3 to 1. A wound was created on the back of rats by incision an area of 2×2 cm. The animals were divided into four equal groups, first group the wound treated and dressed with Phenytoin 1%, second group cream of hydroalcoholic extract of HS 10%, third group received Eucerin and fourth group was left untreated (control).14 days after incision, wound healing process was evaluated microscopically and macroscopically.
**Results:** The results showed wound healing process and re-epithelialization in group 2 were more quickly than other groups and compared to group 3and4 there was significant difference. Treatment with HS reduced lymphocytes, macrophages and total white cell count compared with other treatments. Probably, effective compounds in the HS extract such as flavonoids, in addition to stimulating macrophages, natural killer cells and fibroblastic factors, angiogenesis and the production of interferon ɣ, can repair damaged tissue.
**Conclusion:** In this study, cream of hydroalcoholic extract of HS is effective in wound healing and accelerated the treatment. HS may have reduced inflammation.

**Keywords:** Wound healing, Mmacrophage, Hypericum scabrum, Inflammation, Treatment

ICIA2018\Immunopharmacology and Medicinal Plants\poster\8612
**8612**

# Umbelliprenin cytotoxicity increased in combination with arsenic trioxode in MT-2 leukemia/lymphoma cells

Zahra Sadat Delbari[1], Houshang Rafatpanah [1], Fatemeh B. Rassouli[2], Mehrdad Iranshahi[3]

1. Inflammation and Inflammatory Diseases Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
3. Department of Pharmacognosy and Biotechnology, Biotechnology Research Center, Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

**Background:** Adult T-cell leukemia/lymphoma (ATLL) is a peripheral T-cell lymphoma caused by human T-cell leukemia/lymphoma virus type1 (HTLV-1). Iran, especially Khorasan province, is known as one of the endemic regions for HTLV-1. Despite advances in treatment of ATLL, the average survival rate of this malignancy is low. Umbelliprenin is a sesquiterpene coumarin with broad-spectrum anticancer and antiprolifrative activities. To introduce more effective anticancer strategies against ATLL, here we investigate whether Umbelliprenin could enhance the efficacy of arsenic trioxide, a chemical drug prescribed for ATLL.
**Methods:** In this regard, at first MT-2 cells, an ATLL cell line, were treated with increasing concentrations of Umbelliprenin (25, 30, 40 and 50 µg/ml) and arsenic trioxide (2, 4, 8 and 16 µM) for 24, 48 and 72 h, and the IC50 value of drugs was determined. Then, cells were treated with combination of Umbelliprenin (25 µg/ml) and arsenic trioxide (2 µM) for 72 h. Finally, viability of cells was measured by alamarBlue® test, and apoptosis was determined by PI staining and flow cytometry.
**Results:** Obtained results indicated that 25 µg/ml Umbelliprenin increased the toxicity of 2 µM arsenic trioxide, and therefore, had synergic effects with this anticancer drug.
**Conclusion:** In conclusion, current findings suggest that combinatorial use of Umbelliprenin and arsenic trioxide can be helpful in ATLL treatment.

**Keywords**: Umbelliprenin, Adult T-cell Leukemia/Lymphoma, Cytotoxicity, Arsenic trioxide.

ICIA2018\Immunopharmacology and Medicinal Plants\poster\8626
**8626**

**Effects of sesquiterpene lactone parthenolide on the expression of NF-κB in human lymphoma cells**

Hamide Kouhpeikar[1], Mohammad Hadi Sadeghian[1], Houshang Rafatpanah[2], Fatemeh B. Rassouli[3], Mehrdad Iranshahi[4]

1. Cancer Molecular Pathology Research Center, Department of Hematology and Blood Bank, Faculty of Medicine, Mashhad University of Medical Sciences, Iran
2. Inflammation and Inflammatory Diseases Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
4. Department of Pharmacognosy and Biotechnology, Biotechnology Research Center, Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

**Background**: Parthenolide (PTL), a sesquiterpene lactone (SL) originally purified from the shoots of feverfew (Tanacetum parthenium), has shown potent anticancer and anti-inflammatory activities. It is currently being tested in cancer clinical trials. Adult T-cell leukemia/lymphoma (ATLL) is an aggressive malignancy of mature activated T cells caused by human T-cell lymphotropic virus type I(HTLV-1). Iran, especially Khorasan province, is known as one of the endemic regions for HTLV-1. Despite advances in treatment of ATLL, the average survival rate of this malignancy is low. The virus carries the Tax oncogene which constitutively activates the NF-κB pathway.
**Methods:** In present study, we investigated parthenolide effects on the expression NF-κB (REL-A) in human lymphoma cells. In this regard, MT2 cells were treated with 1 µg/ml parthenolide for 72 h, while cells treated with 0.2% DMSO (used as parthenolide solvent) were considered as relevant control. Then, the total cellular RNA was extracted and treated with DNase I. In the following, cDNAs were synthesized and their fidelity was confirmed by PCR using GAPDH primers. Real-time RT-PCR was conducted using Taq man prob and specific primers for NF-κB (REL-A).
**Results:** Results of current study revealed that parthenolide (in non-toxic concentration) significantly ($p<0.05$) down regulated the expression of NF-κB (REL-A) in MT2 cells.
**Conclusion:** Accordingly, this natural sesquiterpene lactone could be considered as an effective agent to attenuate malignant properties of human lymphoma cells in future *in vivo* studies.

**Key words:** parthenolide, Adult T-cell leukemia/lymphoma, HTLV-1, NF-κB

ICIA2018\Immunopharmacology and Medicinal Plants\poster\9753
**9753**

# A Comparative Study on the Effects of *Allium asarense* and *Allium sativum* Extracts on Lymphocytes

Hatami H[1], Radjabian T[1,2], Hosseinpur Z[1], Ghazanfari T[1] , Naghizadeh MM[1]

1. *Immunoregulation Research Center, Shahed University, Tehran, Iran*
2. *Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran*

**Background**: Garlic (*Allium sativum*) is used as an effective herbal medicine for the treatment of the autoimmune diseases and cancers. Although there are many studies on two *Allium* species (*Allium sativum* and *Allium cepa*), there is no scientific report on the other *Allium* species. The aim of this study was to compare the *in vitro* effects of aqueous bulb extracts of wild *A. asarense* and cultivated *A. sativum* on lymphocytes.
**Methods:** Lymphocytes were isolated from BALB/c mice and were incubated with various concentrations of the aqueous bulb extracts of the studied *Allium* species. Finally, the cytokines concentration and lymphocyte viability were evaluated and compared.
**Results:** The results did not show significant increases in the viability of lymphocytes after incubation with *A. asarense* bulb extract, however significant increases were observed in the viability of lymphocytes after treatment with lower concentration (≤0.05mg/ml) of *A. sativum* extract. Although no significant changes were found in IL-17 and IL-4 concentration after treatment with two *Allium* extracts, IFN-γ concentration was decreased by *A.asarense* extract and was increased by *A.sativum* extract.
**Conclusion:** It suggests that similar to *A. sativum*, the extract of *A. asarense* may be regulate the IFN-γ levels.

Keywords: *Allium* species, IFN-γ, IL-4, IL-17

ICIA2018\Immunopharmacology and Medicinal Plants\poster\9754
**9754**

# Evaluation of *Allium asarense* and *Allium sativum* Aqueous Extracts on Macrophages

Hatami H[1], Hosseinpur Z[1], Radjabian T[1,2], Ghazanfari T[1]

1. *Immunoregulation Research Center, Shahed University, Tehran, Iran*
2. *Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran*

**Background**: It has been showed that some of *Allium* species have immunomodulatory effects on human immune system. Recent studies on *A. cepa* and *A. sativum* showed that their extract have therapeutic effects including anti-cancer, anti-diabetes, anti-oxidant activities. In this research we performed a comparative study about the effects of bulb extracts from wild *A. asarense* and cultivated *A. sativum* on macrophages responses. Methods: Peritoneal macrophages were isolated from BALB/c mice and were incubated with various concentrations of the aqueous bulb extracts of the studied *Allium* species.Then, the amounts of TNF-α and NO generation and MTT in macrophages were measured.
**Results:** Our results showed that the bulb extracts of both *Allium* species had positive effects on macrophages viability, but the significant stimulatory effects of *A. asarense* bulb extract were observed at the lower concentrations (≤0.01 mg/ml). Also significant increases were found in TNF-α concentration after treatment with 0.1, 0.05 and 0.001 mg/ml of the *A. asarense* bulb extract and 0.05 mg/ml *A. sativum* bulb extract. No significant changes were observed in nitric oxide levels after treatment with the extracts.
**Conclusion:** Generally, it seems that in addition to *A. sativum, A. asarense* could be used as an effective medicinal plant for immunoregulation.

Keywords: *Allium sativum*, *Allium asarense*, Nitric Oxide, TNF-α

ICIA2018\Immunopharmacology and Medicinal Plants\poster\9765
9765

**Parthenolide cytotoxicity increased in combination with Arsenic trioxode in MT2 lymphoma cells**

Hamide Kouhpeikar[1], Mohammad Hadi Sadeghian[1], Houshang Rafatpanah[2] , Fatemeh B. Rassouli[3], Mehrdad Iranshahi[4]

*1. Cancer Molecular Pathology Research Center, Department of Hematology and Blood Bank, Faculty of Medicine, Mashhad University of Medical Sciences, Iran*
*2. Inflammation and Inflammatory Diseases Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*3. Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran*
*4. Department of Pharmacognosy and Biotechnology, Biotechnology Research Center, Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background**: Adult T cell leukemia-lymphoma (ATLL) is an aggressive lymphoid neoplasm that occurs only in patients with human T-lymphotropic virus, type I (HTLV-1) viral infection. Iran, especially Khorasan province, is known as one of the endemic area for HTLV-1. Despite advances in treatment of ATLL, long term recovery of this malignancy has been failed. Parthenolide, a naturally occurring sesquiterpene lactone derived from fever few (Tanacetum parthenium), exhibits exceptional anti-cancer and anti-inflammatory properties, making it a prominent candidate for further studies and drug development. To introduce more effective anticancer strategies against ATLL, here we investigate whether parthenolide could enhance the efficacy of arsenic trioxide, a chemical drug prescribed for ATLL
**Methods:** In this regard, at first MT-2 cells, an ATLL cell line, were treated with increasing concentrations of parthenolide (1.25, 2.5 and 5 µg/ml) and Arsenic (1, 2, 4 and 8 µM) for 24, 48 and 72 h, and the IC50 value of drugs was determined. Then, cells were treated with combination of parthenolide (1 and 2 µg/ml) and Arsenic (1 and 2 µM) for 72 h. Finally, viability of cells was measured by alamar blue test
**Results:** Obtained results indicated that 1 µg/ml parthenolide increased the toxicity of Arsenic2 µM, and therefore, had synergic effects with this anticancer drug
**Conclusion:** In conclusion, Current findings suggest that combinatorial use of parthenolide and arsenic trioxide could be considered for designation of novel chemotherapy regimes

**Key words:** parthenolide, Arsenic trioxide , Adult T-cell leukemia/lymphoma, HTLV-1
.

ICIA2018\Immunopharmacology and Medicinal Plants\poster\9798
**9798**

# Anti- inflammatory effect of *Beta vulgaris* leaf aqueous extract in rat

Azad Bahrampour[1], Roonak Azizbeigi[2]

*1. Faculty of Veterinary Medicine, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran*
*2. Department of Physiology, Faculty of Veterinary Medicine, Sanandaj Branch, Islamic Adzad University, Sanandaj, Iran*

**Background:** Inflammation is a local response of tissue to injury or infection. It has been indicated that there is a protective response which is involving leukocytes, blood vessels, and inflammatory mediators. Inflammation which is unregulated mechanism can be result in excessive free radical activity and tissue destruction. *Beta vulgaris*, belonging to Chenopodiaceae, is used in traditional medicine to treat a wide variety of diseases. *B. vulgaris* leaves contain many possesses different potential included antioxidants, anti-rheumatic, and anti-cancer activity. Therefore, this study was conducted to evaluate the effect of *B. vulgaris* leaf aqueous extract on the inflammation induced by xylene.
**Methods:** A number of 25 adult male Wistar rats were divided randomly into 5 groups (n=5) Control (C), xylene (S), and different dose of (100, 200, and 300 mg/kg;ip) which is ,contain B1, B2, and B3 groups ,respectively. *B. vulgaris* leaf aqueous extract administrated 30 min before xylene. The volume injection of xylene was (0.03 ml/kg;sc). After treatment, the animal's leukogram and neutrophil/lymphocyte ratio (NLR) were calculated. Also, the inflammation was measured according to the weight difference of a plug with 7 mm of thickness in treated and untreated ears.
**Results:** The results indicated that the inflammation was significantly reduced in the B3 group compared to S group ($P<0.01$). The leukocytes count decreased significantly in extract-treated groups compared to the xylene group ($P<0.05$). NLR in the S group was significantly higher than B2 and B3 groups ($P<0.05$). There was no significant difference between B1 and S groups in the NLR ($P>0.05$).
**Conclusion:** Our finding suggested that the *B. vulgaris* leaf aqueous extract can control xylene induced inflammation. It seems that more researches are needed to investigate the role of this plant in the inflammatory processes.

**Keywords:** *Beta vulgaris*, Inflammation, Leukocytes, rat

ICIA2018\Immunopharmacology and Medicinal Plants\poster\9888
**9888**

# Medicinal plant Ganoderma Lucidum reduced l-arginine induced nitric oxide release by macrophages

Sajjad Ahmadi Renani[1], Kazem Ahmadi[2]

*1. Tehran University of Medical Science, Tehran, I.R.Iran*
*2. Department of Immunology, Faculty of Medicine, Baqiyatallah University of Medical Science, Tehran, I.R.Iran*

**Bacground**: Inflammation is an unpleasant process which occurs in response to a wide range of agents such as infection and immune disorder. In this regard, over production of some pro-inflammatory mediators such as Nitric Oxide (NO) could lead to chronic diseases of the inflammatory origin. Ganoderma lucidum (*G. lucidum)* is a medicinal plant that in healthy individual boosts the immune system. The aim of this study was looking on the effect of *G.Lucidum* on NO release as an immune mediator by activated macrophages treated with L-Arginine.
**Methods**: In the in vivo study, mice were *orally treated with G.Lucidum* ranging from 2.5µg to 80µg/day/mouse in drinking water for 30 days. Peritoneal Macrophages of *G.Lucidum* treated and non-treated mice were extracted, counted and the $1x10^6$ suspension cells were cultured in 24 wells plate in the presence or absence of 1 mM L-arginine and appropriate LPS in 2 ml volume phenol red and L-arginine free RPMI 1640 medium. Cells were incubated at $37^{0c}$ and 5% $CO_2$ in air for 24h. Supernatants were collected, centrifuged and the amount of $NO_2$ as an indicator of NO production was measured by green,s method.
**Results**: The results showed that L-Arginine treated macrophages were produced more NO comparing to control groups $p<0.001$. Meanwhile *G.Lucidum* reduced L-arginine induced NO release in a dose dependent manner.
**Conclusion:** Previous studies indicate that NO is partly involved in inflammatory disease, and the *G.Lucidum* works as an anti-inflammatory factor. Therefore, the reduction of NO release by *G.Lucidum* treated macrophages show the possibility of using *G.Lucidum* treatment as a safe option in inflammatory disease.

**Keywords**: *G.Lucidum,* Inflammation, Macrophages, Nitric Oxide.

ICIA2018\Immunopharmacology and Medicinal Plants\poster\9897
**9897**

# Anticancer activity of Nanoemulsion formulation of Medical leech protein extraction on human breast cancer cells MCF-7

Sara Mohamadi Saani[1], Amir Shakouri[2], Jalal Abdolalizadeh[3], Saeed Zeinali Heris[1]

1. *Faculty of Chemical and Petroleum Engineering of Tabriz University*
2. *Drug Applied Research Center of Tabriz medical science*
3. *Paramedical Faculty, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background**: Nanoemulsion serves as an attractive vehicle for the delivery of drugs. In medicine, Nanoemulsion has glowed a rapidly growing notice as it potentials to solve an amount of issues associated with conventional therapeutic agent. Over the past several decades, significant progress has been made in the improvement and application of engineered nanoparticles to treat cancer more effectively. For example, therapeutic agents have been combined with nanoparticles engineered with optimal sizes, shapes, and surface properties to increase their solubility, prolong their circulation half-life, develop their bio distribution, and reduce their immunogenicity. Nowadays nanoemulsion is using to increase penetrate tissue and release drug at the target cells with linking target antibody on surface of Nanoemulsion.
**Methods:** High energy techniques are used for the preparation of nanoemulsions. In high energy methods, great disruptive forces are provided by the usage of mechanical devices such as ultrasonicators, and high pressure homogenizers which yields small sized droplets. This method is widely used for the producing nanoemulsions. High pressure homogenizer is used to yield nanoemulsions. Sonication can be widely used to formulate nanoemulsions on small scale. In addition, we use dynamic light scattering (DLS) to monitor the formation of the nanoemulsions complexes. For demonstrate the anti-cancer effect of encapsulation of medical leech protein extract in nanoemulsions, human breast adenocarcinoma cell line (MCF-7) was obtained from the American Type Cell Collection (ATCC). Cells were cultured at an initial inoculum cell concentration of $10^4$ cells/cm2 in 15 ml Roswell Park Memorial Institute medium (RPMI) with 10% FBS (v/v) strep in Corning® 75 cm2 cell culture flask. The cultured cells were incubated at 37°C in 5 % CO2 humidified atmosphere. Nanoemulsions treatment on cell capability of MCF-7 cells were specified using a colorimetric technique, which is an LDH assay on a 96-well plate in RPMI with 10% of FBS per well at cell density 1 × 210 cell/wells incubated for 24 h, 48 h (37 °C, 5% CO2 and 95% humidity). DAPI staining is used for exhibit the apoptosis of the cells that treated with nanoemulsions.
**Results:** DLS showed that the size of nanoemulsions droplets is less than 100 nm and PdI is about 0.130. LDH assay demonstrated that MCF-7 Cancer Cells were sensitive to nanoemulsions that encapsulate with medical leech protein extract and it can be destroyed over 60% of cancer cells agent's normal cell line (HUVEC). In DAPI staining DNA of cancer cells that treated with nanoemulsions were degraded.
**Conclusion:** Nanoemulsions technology is useful way to treat cancer cells with targeting drug like this project with no side effect and Cytotoxicity in human body.

**Keywords**: Nanoemulsions; Breast Cancer; Medical leech**;** MCF-7 Cell line

ICIA2018\Immunopharmacology and Medicinal Plants\poster\9910
**9910**

# The effect of Ginger powder on IL2, TNFα and IL1β cytokines gene expression levels in Patients with Active Rheumatoid Arthritis

Aryaeian Naheed[1], Mahmoudi Mahdi[2], Shahram Farhad[2], Poursani Shiva[2], Jamshidi Fatemeh[2], Tavakoli Hajar[3]

*1. Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Tehran, Iran*
*2. Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran*
*3. Departments of Cellular and Molecular Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects the joints and consequently leads to the destruction of cartilage and bone lesions. Ginger is a native plant to India, which traditionally has been used in treatment of osteoarthritis, joint and muscle pain, neurological diseases, and inflammation of gums, tooth pain, asthma, stroke, diabetes, and constipation. This study aimed to determine the effect of ginger on some immunological and inflammatory factors in patients with rheumatoid arthritis.
**Methods:** In this study, 66 patients with active rheumatoid arthritis were enrolled. Patients were randomly divided into two groups that were treated using ginger and placebo (wheat flour) respectively. To determine the effect of confounding factors on the findings of the study, questionnaires for nutrient intake, physical activity, medication, and determination of body mass index were filled for each participant, at the beginning and end of the study. Serum CRP and mRNA levels of IL-1β, IL-2, and TNF-α were measured by ELISA and Quantitative Real-Time PCR respectively.
**Results:** Results of the study showed ginger supplementation caused a significant decrease in CRP ($P$= 0.05), and IL-1β ($P$= 0.02). TNFα mRNA level in ginger group compared to placebo group decreased, although the difference was not significant between the two groups ($P$= 0.09). Ginger had no effects on IL2 expression.
**Conclusions:** The study showed that ginger reduces inflammatory markers, CRP, and IL-1β in patients with active RA and it seems that ginger can improve the inflammation in the patients.

**Keywords:** Ginger, Active rheumatoid arthritis, Inflammatory markers

ICIA2018\Immunopharmacology and Medicinal Plants\poster\9911
**9911**

# The therapeutic effects evaluation of Boswellia serrata extract in experimental autoimmune encephalomyelitis in mice C57BL / 6

Abdolghader Taene [1], Gholamreza Anani Sarab [2]

1. *Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran*
2. *Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran*

**Background:** Multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), are inflammatory autoimmune diseases of the central nervous system. Boswellia serrata is widely used as an anti-inflammatory, anti-arthritic, anti-proliferative, anti-microbial and analgesic effect remedy in traditional and herbal medicine. In this study, we investigated the effect of methanolic extracts from Boswellia serrata on EAE and on the serum cytokine level and Total Antioxidant Capacity (TAC) in C57BL/6 mice.
**Methods:** EAE is induced in C57BL/6 mice by immunization with an emulsion of MOG35-55 in complete Freund's adjuvant (CFA), followed by administration of pertussis toxin (PTX) in PBS, first on the day of immunization and then again the following day. The mice were administered with PBS in the control groups and Boswellia serrata extract (200 mg/kg, daily) in the treatment group. Serum levels of IL17A and IL23 were measured using enzyme-linked immunosorbent assay (ELISA). Histopathologic assessments were performed by hematoxylin and eosin (H and E) and luxol fast blue (LFB) staining. TAC of samples were measured by using the determination of ferric reducing ability power (FRAP).
**Results:** Treatment of mice with daily oral 200 mg/kg Boswellia serrata significantly reduced the clinical symptoms and cerebral infiltration of inflammatory cells in C57BL/6 mice with EAE. Also treatment prevented weight loss and increased serum levels of IL17A, IL23 in immunized mice with MOG35-55. TAC production was significantly elevated in Boswellia serrata treated mice( $P<0/005$).
**Conclusion:** These results showed that treatment with Boswellia serrata may attenuate disease severity, inflammatory responses in EAE-induced mice and may be potentially useful for the treatment of Multiple Sclerosis (MS).

**Keywords:** experimental autoimmune encephalomyelitis, Boswellia serrata, Total Antioxidant Capacity, multiple sclerosis

ICIA2018\Immunopharmacology and Medicinal Plants\poster\9915
**9915**

**The anti-inflammatory effects of *Matricaria chamomilla* hydro-alcoholic and aqueous extracts**

Zahra Asadi[1], Mojtaba Bagheri[1], Tooba Ghazanfari[2]

1. *Department of Biochemistry, Institute of Biochemistry and Biophysics, University of Tehran*
2. *Immunoregulation research center, University of Shahed*

**Background:** *Matricaria chamomilla* (MC) is one of the most useful medicinal herbs in Iran, with anti-inflammatory, anti-oxidant and anti-bacterial properties in traditional medicine. Essential oil and phenolic compounds (e.g., coumarins, flavones, flavonols, and flavanones) are two major groups of the MC compounds. Although the anti-inflammatory activities of some of these compounds have been investigated, the effects of the MC extracts on the immune cells inflammatory parameters have not fully been investigated so far. We, therefore, planned to investigate the effects of the MC hydro-alcoholic (70%) and aqueous extracts on the cell viability and the amount of nitric oxide (NO) production in mouse macrophages *in vitro*.
**Methods:** The mouse peritoneal macrophages were separated and after preparation they were incubated with various concentration of the MC hydro-alcoholic and aqueous extracts (25, 50, 100, 200, 400, 800 and 1600 µg/ml) in the presence of lipopolysaccharide (LPS) (10 µg/ml) for 20 hrs. Extract without cells was taken as the negative control. Then, the macrophages cell viability was measured by using the MTT assay. The amount of the NO produced by the macrophages was measured by Griess test using the cell culture supernatant.
**Results:** Both hydro-alcoholic and aqueous extracts of the MC significantly reduced the amount of NO in all concentration except 1600 µg /ml. The MC hydro-alcoholic exposure significantly reduced the macrophages cell viability in the high concentration; i.e., 800 and 1600 µg/ml. The macrophage cell viability exposed to the MC aqueous extract increased for the concentration of 100, 400 and 800 µg/ml.
**Conclusion:** The MC hydro-alcoholic and aqueous extracts have strong anti-inflammatory property that can result in the reduction of the NO production in macrophages. Also, the MC hydro-alcoholic extract can kill activated macrophages. It seems that the MC aqueous extract exerted it anti-inflammatory effect by inducing the alternative macrophage activation.

**Keywords:** MC hydro-alcoholic extract, MC aqueous extract, Macrophage, Inflammation.

ICIA2018\Immunopharmacology and Medicinal Plants\poster\9959
**9959**

# Anti-inflammatory effects of aqueous extract of *Eryngium campestre* on Ethylene Glycol-Induced calcium oxalate kidney stone in rats

Saman Seyedabadi[1], Mohammad Sadegh Naghizadeh[2], Sajjad Esmaeili[2], Mehran Falahpour[2], Gholamreza Anani Sarab[3]

1. *Department of medical biotechnology, faculty of medicine, Birjand University of Medical Sciences, Birjand, Iran*
2. *Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran*
3. *Infectious Disease Research Center, Birjand University of Medical Sciences, Birjand, Iran*

**Background:** Kidney stone is one of the most prevalent diseases known to man and is the third most common disorder of the urinary tract. It is a common chronic disorder that affects 10-15% of the general population around the world. This study aims to evaluate the anti-inflammatory effect of E. campestre using the aqueous extracts obtained from aerial parts on Ethylene Glycol (EG)-induced calcium oxalate (CaOx) kidney stone in rats.
**Methods:** 64 male Wistar rats randomly divided into 8 groups including: negative control: standard diet and drinking water; received 1 ml of normal saline orally each day, kidney stone control: standard diet and 0.1 % of EG in drinking water and during the study; received 1 ml of normal saline orally each day, 3 prophylactic groups with100, 200, 400 doses: standard diet and 0.1 % of EG in drinking water during the study; received 100,200, 400 mg/kg extract daily, respectively and 3 treatment groups with100, 200, 400 doses: standard diet and 0.1 % of EG in drinking water during the study, and from the day 15 until the end of the study; received the extract at the doses of 100,200, 400 mg/kg daily, respectively. After the day 30, rats anaesthetized and killed. Blood collected and the kidneys excised immediately. Slides from each one's kidneys prepared and stained with Hematoxylin & Eosin method, also levels of IL-1β and IL-6 determined in rat's serum by rat competitive ELISA kit.
**Results:** E. campestre reduced both cytokines IL-1β and IL-6, showing a significant reduction in IL-1β and IL-6 in all prophylactic groups. Also, IL-1β and IL-6 reduced significantly in the treatment groups in 400 and 200 dosage, respectively (p-value <0.5).
**Conclusion:** This study suggests a potential therapeutic application of E. campestre extract for prophylaxis and treatment of the inflammation caused by CaOx kidney stone.

**Keywords:** kidney stone, cytokines, E. campestre, inflammation

ICIA2018\Immunopharmacology and Medicinal Plants\poster\10019
**10019**

# Cytotoxic effects of combined copper oxide nanoparticles produced by walnut shells using and hydroalcoholic extract of *Aloe vera* against K562 cell line

Ardeshir abbasi[1], Nasim Rahmani Kukia [2], Seyyed Meysam Abtahi Froushani [2]

1. *Department of Immunology, Tarbiat Modares University of Medical Sciences, Tehran, Iran*
2. *Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** In recent studies, anticancer effects of *Aloe vera* and copper oxide nanoparticles (CuO NPs) have been documented. Here, we tested whether combining of the hydroalcoholic extract of *A.vera* and green CuO NPs produced by using eco-friendly and non-toxic walnut shells could provide synergistic cytotoxicity against K562 cell lines and peripheral blood mononuclear cells (PBMC).
**Methods**: first, green CuO nanoparticles were synthetized and characterized. And, hydroalcoholic extract of *A. vera* was produced. The K562 cells or PBMCs (1×105 cells/100 µl/well) were incubated for 24h with serial dilution of *Aloe vera* extract (0, 20, 40, 60, 80, 160 and 320 mg/ml) or serial dilution of green CuO NPs (0, 50,100,200 and 400 µM). After incubation, the survivability of present cells was determined by MTT methods. In another experiment, The K562 or PBMC cells were treated with a combination of *Aloe vera* extract and Green CuO NPs at minimal cytotoxic concentrations and the inhibitory present was calculated.
**Results:** The hydroalcoholic extract of *A. vera* and green CuO NPs had cytotoxic effects against K562 cell-line in a dose-dependent manner. Unlike *A.vera*, the marginal safety of green CuO NPs is low because the IC50 value of the green CuO NPs against K562 cell line had no significant difference comparing the IC50 value of the Green CuO NPs against PBMCs. Moreover, combined treatment with minimal cytotoxic doses provided synergistic benefits and lead to more cytotoxic effect against K562 cell-line than their individual treatment of K562 without any additive cytotoxic effect against PBMCS.
**Conclusion**: This combination provides more favorable cytotoxicity against K562 cell-line without any additive cytotoxicity against PBMCs.

**Keywords:** K562, Green synthesis, walnut shells, Copper oxide nanoparticle, *Aleo vera.*

ICIA2018\Immunopharmacology and Medicinal Plants\poster\10036
**10036**

## Effects of Umbelliprenin on the expression of NF-κB in human leukemia/lymphoma cells

Zahra Sadat Delbari[1], Houshang Rafatpanah[1], Fatemeh B. Rassouli[2], Mehrdad Iranshahi[3]

*1. Inflammation and Inflammatory Diseases Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*2. Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran*
*3. Department of Pharmacognosy and Biotechnology, Biotechnology Research Center, Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** Adult T-cell leukemia/lymphoma (ATLL) is a peripheral T-cell lymphoma caused by human T-cell leukemia/lymphoma virus type1 (HTLV-1). The virus carries the Tax oncogene. Tax is a potent oncogene that deregulates cellular gene expression by persistently activating signaling pathways such as NF-κB. Iran, especially Khorasan province, is known as one of the endemic regions for HTLV-1. Despite advances in treatment of ATLL, the average survival rate of this malignancy is low. In this regard, introducing novel anticancer agents *in vitro* could help clinicians to design more effective therapeutic regimes for ATLL. Umbelliprenin (UMB) is a natural coumarin extracted from dried roots of *Ferula szwitsiana* with broad-spectrum anticancer effects.
**Methods:** In present study, we investigated Umbelliprenin effects on the expression NF-κB (REL-A) in human lymphoma cells. In this regard, MT-2 cells were treated with 25 µg/ml Umbelliprenin for 72 h, while cells treated with 0.2% DMSO were considered as relevant control. Then, the total cellular RNA was extracted and treated with DNase I. In the following, cDNAs were synthesized and their fidelity was confirmed by PCR using GAPDH primers. Real-time PCR was conducted using Taq man prob and specific primers for NF-κB (REL-A).
**Results:** Results of current study revealed that Umbelliprenin (in non-toxic concentration) significantly ($p$<0.0001) down regulated the expression of NF-κB (REL-A) in MT-2 cells.
**Conclusion:** Regarding the role of NF-κB in the proliferation of cancer cells and chemoresistance, the results of this study, introduces this natural coumarin as an effective factor in designing novel approaches against ATLL.

**Keywords:** Umbelliprenin, Adult T-cell leukemia/lymphoma, HTLV-1, NF-κB

# Innate Immunity and Inflammation

# Poster Presentation

ICIA2018\Innate Immunity and Inflammation\Poster\5260
**5260**

# Effects of acetyl salicylic acid (ASA) and its derivatives on inflammation in diabetes

Shabnam Ghazanfari Sarabi[1], Mehran Habibi Rezaei[1,2]

*1. School of Biology, College of Science, University of Tehran, Tehran, Iran*
*2. Nano-Biomedicine Center of Excellence, Nanoscience and Nanotechnology Research Center, University of Tehran, Tehran, Iran*

**Background:** Glycation- the leading cause of Diabetes- is a non-enzymatic interaction between carbohydrates and proteins forming advance glycated end products (AGEs). AGEs and RAGEs (receptor for AGEs) interaction cause the formation of pro-inflammatory molecules and free radical such as reactive oxygen species (ROS) which promote the pathology of diabetes. Gene expression by NF-κB and activator protein-1 (AP-1) transcription factors originate from ROS release. NF-κB not only directly responds to the oxidative stress but also induces several inflammatory cytokines, which associate with insulin resistance. Oxidative stress can give rise to ROS formation. Hemoglobin (Hb) is one of the main targets of carbohydrates attack. Hb glycation leads to its structural modification such as heme degradation, the release of iron and free radical generation. The anti-glycation agents thought to be useful as therapeutics for prevention of diabetes inflammation. Aspirin (ASA) is among the first identified glycation inhibitors. However, ASA has its own side effects. In this regard, discovering a new glycation inhibitor without side effects has grown in importance.
**Methods:** In this study, glycation of 15μM bovine serum methemoglobin (MetHb), with 30mM fructose during 20 days of incubation was done. One and half mM ASA and two other rationally related compounds, including benzoic acid (BA) and p-nitrobenzoic acid (NBA) were added in order to analyze the formation of AGEs by determining their absorbance at 340nm as reported previously.
**Results:** Consequently, absorbance increased with time for all preparations except the native Hb. BA showed the maximum amount of AGEs production, whereas the absorbance of NBA-treated sample was not above ASA-treated sample.
**Conclusion:** We reported the more preventive effect of NBA against ASA on AGEs formation consequently decreasing heme-loss and ROS production during Hb glycation. No clinically human side-effects have been reported for NBA.

**Keywords:** Diabetes Inflammation, Free radicals, NF-κB, Aspirin

ICIA2018\Innate Immunity and Inflammation\Poster\6365
**6365**

## Lack of Association Between rs17568 Polymorphism in OX40 Gene and Myocardial Infarction

Abdolreza Sotoodeh Jahromi[1], Saeideh Erfanian[1], Mehrnoosh Maalhagh[1], Mohammad Shojaei[1]

*1. Research Center for Non.communicable Diseases, Immunology Department, Jahrom University of Medical Sciences, Jahrom, Iran*

**Background:** Tumor necrosis factor (TNF) is one of the inflammatory cytokines which has an important role in inflammation and migration of other inflammatory cells to the atherosclerotic plaques. OX40 is a member of the TNF superfamily receptor protein. OX40 and OX40 ligand are co-stimulators for T-cells and can increase inflammatory response in atherosclerotic plaques. The aim of this study was to determine the association of rs17568 polymorphism in OX40 gene with premature myocardial infarction.
**Methods:** This case control study was done on 100 patients with premature acute myocardial infarction (AMI) and a similar number of sex, age and some other cardiovascular risk factor matched healthy people. The OX40 rs17568 polymorphism was genotyped, using PCR-RFLP method.
**Results:** Allele frequency of rs17568 SNP was lower non-significantly in Premature AMI, compared to healthy subjects (49% vs. 51%). The analysis of rs17568 (A/G) polymorphism showed an odds ratio of 1.127 (95% CI: 0.635-1.999; P= 0.686) for the GG genotype and 5.761 (95% CI: 1.200-27.655; P= 0.029) for the AG genotype, compared to the AA genotype.
**Conclusion:** The results of this study indicate that the rs17568 SNP of OX40 gene is not associated with premature AMI in the evaluated population.

**Keywords:** rs17568 Polymorphism, OX40 Gene, Myocardial Infarction

ICIA2018\Innate Immunity and Inflammation\Poster\7378
**7378**

# An Investigation into the Relation of High Sensitive C Reactive Protein (hs-CRP) with Cardiac Diastolic Function in Primary Hypertension

Reza Gheitasi[1], Samiramis Qavam[2], Mohammad Reza Hafezi Ahmadi[*]

*1. Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
*2. Student Research Committee, Hamadan University of Medical Sciences, Hamadan, Iran*
*3. Department of Cardiology, Ilam University of Medical Sciences, Ilam, Iran*
*4. Department of Pathology, Ilam University of Medical Sciences, Ilam, Iran. B. Biotechnology and Medical plants research Center, Ilam University of Medical Sciences, Ilam, Iran*

**Background**: Hs-CRP is an acute phase serum protein the synthesis of which is increased during tissue damage, inflammatory and infectious conditions by hepatocytes. High rate plasma levels of CRP indicates the risk of cardiovascular diseases. Diastolic dysfunction is one of the most basic cardiovascular complications in patients with hypertension, which may be diagnosed by screening hs-CRP.
**Methods**: This case-control study was conducted on 71 patients, diagnosed with primary HTN and between 20-55 years of age who visited the specialized cardiac clinic in the city of Ilam. Through a detailed history, physical examination and laboratory measures, those patients with effective factors in their serum levels of hs-CRP (kidney, liver and inflammatory diseases; infectious diseases; peripheral vascular or cerebrovascular disease; connective tissue disease; malignant tumor; trauma; drugs such as statins and aspirin) and diastolic function (e.g. ischemic cardiac disease; cardiomyopathy and pericardial disease; arrhythmias and valvular disease) were excluded from the study. Serum Hs-CRP were measured through Nephelometry method. Based on tissue Doppler echocardiographic findings, the patients were divided into two groups: with and without diastolic dysfunction. The serum levels of hs-CRP in these patients were also compared.
**Results**: 31% of the patients were male, and 69% were female with an average age of 45.42± 7.37. 54 patients (76.1%) had diastolic dysfunction. The results revealed that a close relationship existed between hs-CRP in the serum and diastolic dysfunction ($P<0.05$).
**Conclusion**: Based on the findings a close relationship existed between hs-CRP in the serum and diastolic dysfunction. The higher the rate of hs-CRP, the more likely the emergence of diastolic dysfunction. Therefore, inflammatory factors especially hs-CRP can be used as a diagnostic and prognostic marker for diastolic dysfunctions.

**Keywords**: hs-CRP, Biomarker, Diastolic dysfunction, Ilam

ICIA2018\Innate Immunity and Inflammation\Poster\7419
**7419**

# Correlation of Serum hs-CRP and Lipid Profile with Coronary Artery Disease

Fatemeh Khaki Khatibi[1]

*1. Drug Applied Research Center and Department of Clinical Biochemistry, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Coronary Artery Disease (CAD) is one of the major causes of morbidity and mortality in the world. Atherosclerosis is a cause of CAD. Atherosclerosis leads to the stenosis of the vessels and there are several causes. These factors include: inflammation, lipids and their peroxidation, and other risk factors. The aim of the present study is to evaluate serum levels of hs-CRP and lipid profile and the correlation of these parameters with extent of CAD. Measuring these parameters, can help prevent the progression of the disease.
**Methods:** In this study, 200 individuals including 160 CAD subjects as patient group and 40 age and sex matched healthy persons as control group were chosen. Patients were angiographically divided into four groups: patients with normal angiography (Non-CAD, n=40), one coronary artery occlusion (n=40), two coronary artery occlusion (n=40) and three coronary artery occlusion (n=40). Control group was selected from healthy persons with no history of other diseases. Hs-CRP serum level was measured by immunoturbidimetric method. Serum lipid levels were measured by standard methods.
**Results:** There was no significant difference between two groups in terms of age and sex, but a significant difference existed among familial history (p=0.03). Serum level of hs-CRP was significantly higher in the patient group than the control group (p=0.01). Also Serum levels of cholesterol, TG, HDL and LDL were significantly higher than the control group (p=0.001). Furthermore, serum concentration of hs-CRP in double-vessel disease was significantly higher as compare with control and no-vessel groups (p=0.003 and p=0.05, respectively). A significant correlation was found between some lipid parameters and hs-CRP.
**Conclusion:** Serum levels of hs-CRP and lipid parameters were increased in CAD patients compared to normal controls. These biochemical parameters may have roles in the progression of atherosclerosis. Timely measurement and control of these parameters in normal range may be helpful in preventing CAD and its development. It can also be used to diagnose and control the treatment of vascular disease.

**Keywords:** hs-CRP, lipid profile, coronary artery disease, Atherosclerosis

ICIA2018\Innate Immunity and Inflammation\Poster\7431
**7431**

# The associations between serum levels of vitamin D and TGF-β in coronary artery disease patients

Nadereh Naderi[1,2], Mahsa Rahimzadeh[3], Fereshteh Rasa[3], Ebrahim Eftekhar[3], Elahe Mansoori[3]

*1. Department of Immunology, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran*
*2. Food health research center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran*
*3. Department of Biochemistry, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran*

**Background:** The protective role of vitamin D in atherosclerosis and coronary heart disease is well stablished but the mechanism has not been fully elucidated. The aim of this study was to investigate the associations between vitamin D status and regulatory T cells (Tregs) inhibitory cytokine, TGF-β1, in patients suffering from coronary artery disease (CAD).
**Methods:** Vitamin D, and TGF-β1 were measured in 81 patients. The patients were divided into single (n= 20), double (n=20) and triple (n=20) vessel disease groups and compared to no vessel disease (n=21).
**Results**: Vitamin D and TGF-β1 concentrations in controls (32.4±15.2, 667.7±427.6 respectively) were significantly higher than CAD patients (18.1±9.8, 360.4±354.1 respectively, $p<0.05$). TGF-β1 was significantly higher in double vessel disease patients (591.9±465.7) compared to those with triple vessel disease (173.1±163.3, $p<0.05$). Vitamin D, TGF-β1 revealed a negative correlation (r= -0.36, r=-0.46 and r-0.024 respectively) with severity of CAD ($p< 0.05$). TGF-β1 in vitamin D deficient patients was significantly lower compared to normal serum vitamin D patients (326.6±351.7 pg/mL vs. 754.5±560 pg/mL, p=0.036 respectively).
**Conclusion:** We found *significant association* between TGF-β1 and vitamin D deficiency in CAD patients. It seems that the *anti-atherosclerotic* effect of vitamin D is attributed, *at least partly,* to the up-regulation of anti-inflammatory cytokines, TGF- β1.

**Key words:** TGF-β, coronary artery disease, vitamin D

ICIA2018\Innate Immunity and Inflammation\Poster\7438
**7438**

# Role of monocyte chemotactic protein-1 in irritable bowel syndrome

Arezoo Gowhari Shabgah[1,2], Mojgan Mohammadi[3]*, Mohammad Mahdi Hayatbakhsh[4], Saeid Pishgoee[5], Jalil Tavakol Afshari[1], Hadi Zeidabadi[5]

*1. Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
*2. Student research committee, Mashhad University of Medical Sciences, Mashhad, Iran*
*3. Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
*4. Gastroenterology and Hepatology Research Center, Institute of Basic and Clinical Physiology sciences, kerman University of Medical Sciences, Kerman, Iran*
*5. Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran.*

**Background:** Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal disorders worldwide, with the prevalence ranging from 1.1% to 29.2% in the general population diagnosed by the Rome III criteria. IBS causes digestive problems like cramping, abdominal pain, and bloating; and diarrhea (IBS-D), constipation (IBS-C), or both (IBS-A: alternating). Little is known about the immunopathogenesis of these three subtypes of IBS. Monocyte chemotactic protein-1 (MCP-1) is a cytokine and belong to chemokine family. MCP-1 causes infiltration of antigen presenting cells and memory T cells to the sites of inflammation. We aimed to evaluate the serum levels of MCP-1 in patients with IBS and compare it with healthy controls.
**Methods:** The serum concentration of MCP-1 was assessed by enzyme linked immunosorbent assay (ELISA) in patients with IBS (n=96) and healthy controls (n=44).
**Results:** Our results showed a significant decrease in serum levels of MCP-1 in patients with IBS as compared to controls (p= 0.001).
**Conclusion:** As all patients who participated in our study had taken medications for IBS, significant decline in the MCP-1 serum levels in comparison with controls might be due to the conventional therapy. Further investigations on the new case of IBS patients to evaluate the effect of MCP-1 in the immunopathogenesis of IBS is highly recommended.

**Keywords:** Irritable bowel syndrome, monocyte chemotactic protein-1, inflammation.

ICIA2018\Innate Immunity and Inflammation\Poster\7454
**7454**

# Stimulation of cholinergic anti-inflammatory system by nicotine ameliorates animal model of rheumatoid arthritis (RA)

Sara Golbahari, Seyyed Meysam Abtahi Froushani

*Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** It has been revealed that the signaling by α7 nicotinic acetylcholine receptor can promote the anti-inflammatory condition. This study was designed to evaluate therapeutic benefit of nicotine on the animal model of rheumatoid arthritis (RA).
**Methods:** Intra-footpad injection of complete Freund's adjuvant was used to induce RA in Wisatr rats. Then, rats were allocated in 3 groups: treated with nicotine (2.5 mg/kg-orally), treated with Prednisolone (2 mg/kg-orally) and un-treated group. All therapies were started at day 5 post-induction. The changes in the dorso-plantar diameter of hands and legs of each rat were monitored every day until the day 23 post-intrafootpad injection of complete Freund's adjuvant.
**Results:** Nicotine treatment after the occurrence of clinical symptoms significantly reduced the edema and swelling of the soles of the feet of RA rats in a similar manner with prednisolone. The serum levels of myeloperoxidase and nitric oxide concurrent with proliferation of spleen lymphocytes were significantly decreased in treatment groups compared to control rats. Albeit, the level of decrease in the splenocytes proliferation index and serum nitric oxide level was somewhat higher in the prednisolone group compared to the nicotine group.
**Conclusion:** This pharmacological approach may be as a useful strategy to control RA.

**Keywords:** Nicotine, Rheumatoid arthritis, Wistar rat.

ICIA2018\Innate Immunity and Inflammation\Poster\7467
**7467**

# Comparison of serum and tissue antioxidant changes in rat model of ulcerative colitis treated with hydroalcoholic extract of Cinnamon

Mojtaba Salamatian[1], Vahid Mohammadi[1], Seyyed Meysam Abtahi Froushani[2]

*1. Department of internal medicine and clinical pathology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*
*2. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Cinnamon is a spice obtained from the inner bark of the genus *Cinnamomum*. It has been used for a long time in folk medicine. Here, we investigated and compared serum and tissue antioxidant changes in rat model of ulcerative colitis (UC) treated with hydroalcoholic Cinnamon extract.
**Methods:** UC was induced in the male Wistar rats by luminal instillation of acetic acid. Animals in the treatment group treated with hydroalcoholic extract of Cinnamon (400 mg/kg PO-daily) for 10 consecutive days. Finally, the rats were sacrificed and the disease activity index, the levels of malondialdehyde (MDA) and total antioxidant capacity (TAC) were measured in the colonic homogenized tissue specimens and sera.
**Results:** Attained data indicated that hydroalcoholic extract of Cinnamon could reduce the clinical scores and the mortality rate of ulcerative colitis. The levels of TAC were significantly decreased and conversely the levels of MDA were increased in both sera and gut samples. Nevertheless, hydroalcoholic extract of Cinnamon, concurrent with a significant decrease in clinical scores, could regress the levels of MDA and conversely increased the levels of TAC in gut tissues more prominent than sera samples.
**Conclusion:** It seems that the changes in the antioxidant levels of the tissue samples is a more suitable criterion for the study of beneficial effects of a therapeutic agent compared to study of these change in sera.

**Keywords:** Hydroalcoholic extract of Cinnamon, Ulcerative colitis, Antioxidant.

ICIA2018\Innate Immunity and Inflammation\Poster\7470
**7470**

# A lower mRNA expression level of TLR2, 3, 4 in PBMCs isolated from non-responder children to HBV vaccine

Kousar Smailneja ganji [1, 2], Mehdi Shahbazi [2], Mousa Mohammadnia-Afrouzi [2],
Mojdeh Ghias Tabari[3],Motahareh Ehsani[2], Majgan Bagherzadeh[2]

1. *Student research committee, Babol University of medical sciences, Babol,Iran*
2. *Department of Immunology, School of Medicine, Babol University of Medical Sciences, Babol, Iran*
3. *Department of Biochemistry, School of Medicine, Babol University of Medical Sciences, Babol, Iran*

**Background:** Toll like receptors (TLR) have an important role in the innate immune system and also able to recognize Hepatitis B virus that effect on immune response. Many host related factors such as the expression of TLRs, contribute to vaccine response. This study was designed to determine the expression levels of TLR2, 3, 4 genes as indicator of innate immunity response to the hepatitis B vaccine among 3-5 years old children.
**Methods:** The study was carried out among 60 children with age 3-5 years old who received HBV vaccine (43 responders, 17 non responders). Blood samples were collected, then the expression of TLR2, 3, 4 genes in peripheral blood mononuclear cells (PBMCs) were analyzed by Real Time PCR method.
**Results:** The results of this study indicated that the average expression of TLR2, 3, 4 genes in the responder group was higher than the non-responder group. Additionally, the expression levels of TLR 3, 4 genes were significantly higher in responders compared to non-responders (P-Value $\leq$ 0.01).
**Conclusion:** This study suggest that the expression of TLR2, 3, 4 could be considered as a marker of protection along with serological markers. Indeed, evaluation of the HBs antibody titer and the expression level of immune response genes together can also be used to determine efficiency of hepatitis B vaccine in responder and non-responder individuals.

**Keywords:** Hepatitis B vaccine, TLR2, 3, 4 genes and Real Time PCR

ICIA2018\Innate Immunity and Inflammation\Poster\7499
**7499**

# The role of CCR1 and different monocyte subsets in progression of coronary atherosclerotic lesion

Saeid Taghiloo[1], Abolghasem Ajami [1,2]*, Hossein Asgarian Omran[1,3], Hamideh Rahimi[1], Reza Alizadeh Navaei[2], Seyed Mohammad Mehdi Peyghambari[4], Mahin Nikogoftar[5]

1. *Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
2. *Molecular and Cell-Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran*
3. *Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
4. *Department of Cardiology, School of Medicine, Tehran University of Medical Sciences, Sari, Iran*
5. *Department of Research Center of Iranian Blood Transfusion Organization Tehran, Iran*

**Background:** The inflammation underlying atherosclerosis is strongly related to monocyte action. Circulating monocyte divide into subpopulation, according to CD14 and CD16 expression. In this study, we investigated the relationship between the severity of coronary artery disease (CAD) and the two distinct monocyte subsets and CCR1 receptor.
**Methods:** We enrolled 88 patients who underwent diagnostic CAG (coronary angiography). Patients were divided into four groups. Those without CAD (normal group), those with 50% stenosis in one vessel (MVD), those with single vessel stenosis (1VD) and those with 2 or 3 vessels stenosis (2+3 VD). In addition, the severity of CAD was evaluated by Gensini score. The two monocyte subsets: classical ($CD14^{+}CD16^{-}$) and non-classical ($CD14^{+}CD16^{+}$) were measured by flow cytometry. Mean fluorescence intensity (MFI) for CCR1 marker was measured and compared between monocyte subset in different group of patients.
**Results:** Circulating non-classical monocytes were more frequently observed in patients with 2+3 VD than normal, minimal and 1VD groups, but this increase is not statistically significant P> 0.05). According to the results of the CCR1, both classical and non-classical monocytes were surface expressed, but a larger number of classical monocytes expressed this molecule. The number of non-classical monocytes that express the CCR1 molecule decreased with CAD, but not statistically significant.
**Conclusions:** This study showed that non-classical monocytes increased with increase CAD, and CCR1 is not associated with atherosclerosis, because of this molecule decreased at the level of non-classical monocytes.

**Keywords:** monocyte, CCR1, coronary artery disease, cardiovascular diseases

ICIA2018\Innate Immunity and Inflammation\Poster\7562
**7562**

# Anti-APOH IgG antibody in diabetic patients with atherosclerosis

Mohammad Rafiee[1], Hossein Golmoghaddam[1], Shahdad Khosropanah[2], Mehrnoosh Doroudchi[1]

*1. Memory T-cell Laboratory, Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
*2. Cardiovascular Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Self-proteins such as Apolipoprotein-H (APOH), Heat Shock Proteins, and Apolipoprotin-B100 may be involved in atherosclerosis. APOH is shown to be associated to vascular alterations in type-2 diabetes; however, the information regarding its antigenicity in these disorders is scarce. In this study, we assessed the anti-APOH-antibody levels in patients with atherosclerosis.
**Methods:** In total, 134 participants who underwent coronary artery angiography for diagnostic purposes were included in this study. Thirty-one subjects had normal/insignificant coronary artery disease (group 1). Twenty-eight subjects had diabetes type-2 but normal/insignificant coronary artery disease (group 2). Thirty-nine subjects had no diabetes but showed ≥50% stenosis in one or more coronary arteries (group 3) and thirty-six subjects had diabetes type-2 and showed ≥50% stenosis in one or more coronary arteries (group 4). Blood samples were obtained from all 134 individuals and IgG class anti-APOH-Ab was measured by a commercial ELISA in the sera.
**Results:** In total, 11 (8.2%) of subjects showed IgG anti-APOH-Ab in their sera. The frequency of anti-APOH Ab+ samples in group 4 was the highest of all (16.7%). In group 2, 7.1% and in group 3, 5.1% of subjects had anti-APOH-Ab in their sera. The least frequency of anti-APOH-Ab+ samples was found in group 1 in which only one subject was positive (3.2%). Statistical analysis between the 4 groups did not reach the significant level by Chi-square test (p=0.17) or by Kruskal-Wallis test (p=0.18). However, the diabetic patients were more likely to have IgG anti-APOH-Ab in their sera (p=0.08).
**Conclusion:** Our results suggest that anti-APOH-Ab is higher in type-2 diabetic patients and increases with the existence of artery stenosis. Therefore, not only APOH protein but also anti-APOH-Ab is produced in diabetic patients. The mechanism by which this self-antigen bypasses immune-tolerance is worth investigating in a larger group of patients.

**Keywords**: ApoH, Atherosclerosis, Diabetes type 2, IgG

ICIA2018\Innate Immunity and Inflammation\Poster\7591
**7591**

# Serum levels of interleukin (IL)-33 in patients with ischemic heart disease

Morteza Jafarinia[1] , Mohsen Khalili , Sina Vakili

*Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
*Department of Immunology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran*
*Department of Biochemistry, Shiraz University of Medical Sciences, School of Medicine, Shiraz, Iran*

**Background :** Objective: It has been reported that cytokines play a crucial role in pathogenesis of different diseases such as cardiovascular diseases. The aim of this study was to evaluated the serum level of IL-33 in patients with ischemic heart disease (IHD) and also clarify its association with traditional risk factors of the disease.
**Methods:** A total of 400 patients with IHD as having acute myocardial infarction (AMI; n=100), unstable angina (UA; n=100) or stable angina (SA; n=100) and 100 sex- and age- matched healthy subjects as control group were enrolled to this cross-sectional, case controlled study. Serum samples were collected from all participants (for AMI patients at 3-5 days after events and for UA or SA at admission time) and tested for IL-33 by use of ELISA method.
**Results**: The mean serum concentration of IL-33 in AMI (103.33 ± 19.29 Pg/ml), SA (157.60 ± 33.43 Pg/ml) and UA (122.21 ± 22.26 Pg/ml) was significantly higher than that observed in healthy control (61.85 ± 7.67 Pg/ml). There was no significant difference between patient with or without certain traditional risk factor including hypertensive patients, dyslipidemic patients, smoker, obese patients and diabetic patients in the mean serum level of IL-33.
**Conclusion:** These results showed that the higher serum concentration of IL-33 were associated with IHD. The presence or absence of certain traditional risk factors of IHD did not influence the serum level of cytokines.

**Keywords:** ischemic heart disease, acute myocardial infarction, unstable angina, stable angina, interleukin (IL)-33

ICIA2018\Innate Immunity and Inflammation\Poster\7605
**7605**

# Thymol ameliorates acetic-acid induced ulcerative colitis via selective COX-2 inhibition

Pourya Tahmasebi, Seyyed Meysam Abtahi, Nahideh Afzale Ahangaran

*Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Thymol, a natural monoterpene phenol derivative of Thymus, has anti-inflammatory properties. Here, we investigated the potential of Thymol in ameliorating animal model of ulcerative colitis (UC).
**Methods:** UC was induced in the male Wistar rats by luminal instillation of acetic acid. Rats in the treatment groups treated with Thymol (100 mg/kg PO) or Prednisolone (2 mg/kg PO) daily for 10 consecutive days. At the end, colon tissue specimens were used for Cyclooxygenase-2 expression by immunohistochemistry. Also, the levels of IL-1β and IL-6 were checked in colonic homogenates.
**Results:** The data indicated that both therapies with Thymol and Prednisolone could regress the clinical scores and the mortality rate of ulcerative colitis in a comparable manner. The levels of COX-2 was significantly decreased in the guts of Thymol treated rats more than Prednisolone groups. Nevertheless, Thymol somewhat decreased the levels of IL-1β and IL-6 more than Prednisolone.
**Conclusion:** Thymol can ameliorate acetic-acid induced ulcerative colitis via selective COX-2 inhibition compared to Prednisolone.

**Keywords:** Thymol, Ulcerative colitis, COX-2.

ICIA2018\Innate Immunity and Inflammation\Poster\7718
**7718**

# Effect of aqueous extract and saponin fraction of *Tribulus terrestris L.* on the expression of *ICAM1* and *VCAM1* genes in the human endothelial cell lines in vitro

Zahra Fereydouni[1], Elahe Amirinezhad Fard[1], Ali Mostafaie[1]

*1. Department of immunology, Kermanshah University of Medical sciences, Kermanshah*

**Background:** Atherosclerosis is a chronic inflammation during which low density cholesterol and lipid deposition on the wall of arteries are formed. *Tribulus terrestris L.* as a medicinal plant, due to its anti-inflammatory properties, has been used for treatment of various kinds of diseases such as atherosclerosis. These observations motivated us to investigate the effect of aqueous extract and saponin fraction of *T.terrestris* on the expression of Intracellular Adhesion Molecule-1 *( ICAM1*) and Vascular Cell Adhesion Molecule-1(*VCAM1)* genes in the Human Umbilical Vein Endothelial Cell (HUVEC) and Human Bone Marrow Endothelial Cell (HBMEC) lines in vitro during normal and LPS-induced conditions.
**Methods:** After the culture of HUVEC and HBMEC cell lines and induction with LPS, the cells were treated with aqueous extract and a saponin fraction of the *T.terrestris.* Then, the expression of *ICAM1* and *VCAM1* genes under normal and LPS-induced conditions was investigated using Real-Time PCR technique.
**Results:** The data obtained showed that the aqueous extract and saponin fraction of *T. terrestris* exerted a decreasing effect on mRNA expression of the investigated genes in the LPS-induced HUVEC and HBMEC lines.
**Conclusion:** As revealed by our study, anti-inflammatory activity of *T. terrestris* could be partly attributable to its potential to downregulate *ICAM1* and *VCAM1*. More reduction in the expression of above-mentioned genes by aqueous extract compared to saponin fraction may be due to the presence of other chemical constituents, such as flavonoids and alkaloids in the aqueous extract. In conclusion, *T.terrestris* could be considered as a candidate for the treatment of atherosclerosis.

**Keywords:** atherosclerosis, *ICAM-1*, *VCAM-1*, *Tribulus terrestris L.*

ICIA2018\Innate Immunity and Inflammation\Poster\7739
**7739**

# Macrophage function can alter toward anti-inflammatory phenotype after oral administration of Hypiran

Somayeh Ahmadiafshar, Mahsa Alem, Seyyed Meysam Abtahi Froushani

*Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** *Hypericum perforatum* is a medicinal herb with anti-inflammatory properties as an arachidonate 5-lipoxygenase inhibitor and COX-1 inhibitor. Hypiran is a commercial hydro-alcoholic extract of this plant. The current research was conducted to investigate the effects of oral administration of Hypiran on the some functions of the peritoneal macrophages in NMRI mice.
**Methods:** The study population consisted of 10 NMRI mice randomly allocated into the treatment and control groups. Hypiran was orally administered to treatment group in daily doses of 110 mg/kg from the beginning of the study and continued for 1 week. The control mice received PBS at the same volume. The resident macrophages were isolated from the peritoneal cavity of mice by injecting 20 ml of ice-cold PBS and used for experiments.
**Results:** The NBT reduction assay test showed that oral administration of Hypiran couldn't induce any change in the potential of reactive oxygen production by of macrophages. Moreover. MTT reduction assay test showed that Hypiran can increase vitality and biological activity of macrophages isolated from Hypiran treated mice compared to macrophages isolated from untreated mice.
**Conclusion:** Despite increase in biological activity of macrophages, potential harmful reactive oxygen substances production by macrophages couldn't showed any significant changes. Therefore, oral administration of Hypiran can alter the phenotype of macrophages toward anti- inflammatory phenotype.

**Keywords:** Macrophages, Hypiran, *Hypricum perforatum*.

ICIA2018\Innate Immunity and Inflammation\Poster\7862
**7862**

# Evaluation of interleukin-1 beta (IL-1β), before and after curcumin treatment in HAM/TSP patients and HTLV-1 carriers

Zahra Soleimani[1], Houshang Rafatpanah[2,*], Reza Boostani[3], Reza Farid Hosseini[4]

*1. Inflammation and Inflammatory Diseases Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*2. Inflammation and Inflammatory Diseases Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*3. Department of Neurology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*4. Allergy Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** Human T-cell leukemia virus (HTLV-1) is the first human lymphotropic retrovirus which is associated with adult T cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), a chronic neuroinflamatory disease that lead to destruction of central nerveous system. Some of the proinflammatory cytokines such as IL-1β, IL-6, IL-8 and TNF-α play important roles in the diseases progression. Curcumin, a bioactive polyphenol from turmeric, has wide range of biological activities including anti-inflammatory, anti-oxidant and anti-tumor properties. Curcumin mediates its anti-inflammatory effects through the downregulation of inflammatory transcription factors, protein kinases, enzymes and cytokines such as IL-1, -2, -6, -8, -12 and TNF-α. Thus the aim of this study was to evaluate the serum levels of IL-1β, before and after curcumin treatment in HAM/TSP patients. Furthermore we compared the levels of this cytokine between HAM/TSP patients and HTLV-1 asymptomatic carriers (ACs).
**Methods:** The Serum levels of IL-1β was measured by ELISA before and after curcumin treatment in 21 HAM/TSP patients. Furthermore, the Serum levels of this cytokine was also measured in 21 ACs.
**Results:** The serum levels of IL-1β was lower after treatment with curcumin in HAM/TSP patients. The serum levels of IL-1β was significantly higher in HAM/TSP patients compared with ACs.
**Conclusion:** The results of the present study showed that curcumin reduces the levels of IL-1β and could be useful as a supplement for treatment of HAM/TSP patients.

**Keywords:** HTLV-1, HAM/TSP, Curcumin, IL-1β, Proinflammatory cytokines

ICIA2018\Innate Immunity and Inflammation\Poster\7902
**7902**

**Dopamine modulates inflammatory functions of the monocytes.**

Hanieh Nikkhah, Seyyed Meysam Abtahi Froushani, Habbib Dastmalchi Saee, Nahideh Afzale Ahangaran

*Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Dopamine, produced in many cell types like immune cells, has autocrine and paracrine effects. The current research was designed to evaluate the effects of dopamine on the inflammatory functions of the monocytes.
**Methods:** The peripheral blood cells were collected by a Ficoll-Hypaque density gradient from the blood of Wistar rats. Then, non-adherent plastic cells were removed after 2 h. Monocytes pulsed with dopamine for 24h within the concentration of $5\times10^{-7}$ M (maximum physiologic concentration).
**Results:** The mRNA level of NF-κB p65 in monocytes treated with dopamine was reduced compared macrophages without treatments. MTT assay showed that dopamine treatment does not significantly change the viability of monocytes. Nevertheless, the potential of nitric oxide and reactive oxygen species production of monocytes stimulated with opsonized yeast were regressed after dopamine exposure.
**Conclusion:** Dopamine treatment of monocytes can regress the inflammatory function of monocyte without altering any changes in the vitality of monocytes.

**Keywords:** Monocytes, Dopamine, anti-inflammatory.

ICIA2018\Innate Immunity and Inflammation\Poster\7903
**7903**

**The effects of dopamine on the respiratory burst and nitric oxide production by macrophages.**

Hanieh Nikkhah, Seyyed Meysam Abtahi Froushani

*Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Dopamine is a neurotransmitter in both central and peripheral nervous system. Nowadays, its role in the neural-immune communication has been revealed. The current research was set out to investigate the effects of dopamine on the inflammatory functions of the peritoneal macrophages.
**Methods:** The resident macrophages were isolated from the peritoneal cavity of Wistar rats by injecting 20 ml of ice-cold PBS and pulsed with the maximum physiologic concentration of dopamine ($5\times10^{-7}$ M) for 24 h.
**Results:** MTT reduction assay test showed that dopamine couldn't alter the vitality of macrophages**.** Phagocytosis of opsonized yeast also couldn't show any changes after dopamine treatment of macrophages**.** Nevertheless, concurrent with the killing activity of macrophages after challenge with opsonized yeast, the potential of nitric oxide and reactive oxygen production by these cells were significantly reduced after treatment with dopamine.
**Conclusion:** Dopamine exposure of macrophages can reduce the inflammatory potential of macrophages without altering any changes in the vitality of these cells.

**Keywords:** Macrophages, Dopamine, Respiratory burst, Nitric oxide.

ICIA2018\Innate Immunity and Inflammation\Poster\8109
**8109**

# Curcumin can suppress experimental autoimmune encephalomyelitis via ameliorating oxidative stress

Laleh Mavaddatiyan[1], Shiva Khezri[1], S.Meysam Abtahi frushani[2]

1. *Department of Biology, Faculty of Sciences, Urmia University, Urmia, Iran*
2. *Department of Microbiology, Faculty of Veterinary, Urmia University, Urmia, Iran*

**Background:** Despite the advances made in recent years in controlling multiple sclerosis, many patients respond less well to existing treatments. Previous studies have pointed to the role of oxidative stress in the pathogenesis of multiple sclerosis. The aim of this study was to investigate the effects of curcumin on reducing the effects of oxidative stress in the animal model of multiple sclerosis.
**methods:** In this experimental study, Autoimmune encephalomyelitis was induced by immunization of wistar rats (12 vertebrae) with homogenized spinal cord of Indian guinea pigs and Compound Freund`s Adjuvant. Then, the animals were safely placed in two groups of 6 heifers. Six vertebras were considered as healthy controls. Treatment with curcumin (10 mg / kg daily) from the onset of clinical symptoms in the treatment group (day 12). At the same time, the control group received only the drug solvent. Treatment continued until 30 days after immunization.
**Results:** Administration of curcumin in rats with multiple sclerosis significantly reduced nitric oxide levels and serum myeloperoxidase activity. Both malondialdehyde and uric acid levels were reduced at the same time in the serum of affected and treated animals. However, there was no significant change in total antioxidant capacity of the serum.
**Conclusion:** Adding curcumin to a multiple sclerosis regimen may be helpful in reducing the harmful effects of oxidative and direct anti-inflammatory effects.

**Keywords:** Multiple Sclerosis, Experimental Autoimmune Encephalomyelitis, Curcumin, Oxidative Stress

ICIA2018\Innate Immunity and Inflammation\Poster\8133
**8133**

**Glycyrrhizin instructs M1 pro-inflammatory macrophages.**

Sahar etemadi[1], Seyyed Meysam Abtahi Froushani[1], Hadi smaili Govarchin Ghalee[1], negin mohammadpoor[1]

*1. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Glycyrrhizin is under preliminary clinical research for its activity against common viruses, like hepatitis C. This study was designed to evaluate the effects of Glycyrrhizin on the polarization of macrophages.
**Methods:** The resident macrophages were isolated from the peritoneal cavity of Wistar rats by injecting 20 ml of ice-cold PBS and treated with dopamine for 24h in the concentration of 100 µg/mL.
**Results:** MTT reduction assay and neutral red uptake assay indicated that Glycyrrhizin couldn't alter the vitality of macrophages. Nevertheless, concurrent with the killing activity of macrophages after challenge with opsonized yeast, the potential of nitric oxide and reactive oxygen production by these cells were significantly up-regulated after treatment with Glycyrrhizin.
**Conclusion:** Glycyrrhizin exposure of macrophages potentiates the inflammatory potential of macrophages without altering any changes in the vitality of these cells. These findings might offer new insight into the potential mechanisms that underlie the immunostimulatory effects of Glycyrrhizin.

**Keywords:** Macrophages, Glycyrrhizin, Inflammation.

ICIA2018\Innate Immunity and Inflammation\Poster\8527
**8527**

# Trimethylamine-N-oxide induce the expression of the TLR2 and NADPH oxidase in U937-derived macrophages

Zakaria Vahabzadeh[1], Abbas Ahmadi[2], Leila Farhadi[2], Mohammadraman Molodi[3], Sara Shirahmadi[1]

*1. Department of Clinical Biochemistry , Faculty of Medicine , Kurdistan University of Medical Science*
*2. Department of Molecular Medicine and Genetics, Faculty of Medicine , Kurdistan University of Medical Science*
*3. Department of Medical Physiology and Pharmacology, Faculty of Medicine , Kurdistan University of Medical Science*

**Background:** The exact mechanism by which gut microbiota-dependent trimethylamine-N-oxide (TMAO) induce inflammation and bring about atherosclerosis is not well understood. Engagement of toll-like receptors (TLRs) on macrophages and consequently its downstream NADPH oxidases (NOX) activates the signaling pathways that initiate pro-inflammatory cytokines as well as ROS production. The present study was designed to evaluate the expression of TLR2, TLR6 and NOX2 at mRNA levels in macrophages.
**Methods:** U937-derived macrophages were treated with different concentration (37.5, 75, 150 and 300 μM) of TMAO for 24 h. The cells were also treated with tunicamycin, as a positive control for stress. RT-qPCR was used to evaluate the expression of TLR2, TLR6, and NOX2 at mRNA levels.
**Results:** Unlike tunicamycin, high dose of TMAO significantly increased TLR2 and NOX2 mRNA levels compared to the control cells ($P<0.05$). Tunicamycin significantly increased the mRNA levels of TLR6 ($p=0.010$).
**Conclusion:** Our results provide findings to support the contribution of TLR2 and NOX2 in proatherogenic mechanism of TMAO during the foam cell formation and abnormal activation of macrophages.

**Keywords:** Toll-like receptors, Atherosclerosis, Trimethylamine-N-Oxide, Macrophages

ICIA2018\Innate Immunity and Inflammation\Poster\8532
**8532**

# The proinflammatory cytokine response of U937-Derived macrophages treated with Trimethylamine-N-oxide

Zakaria Vahabzadeh[1], Mohammad Abdi[1], Leila Farhadi[2], Amin Ghanivash[1], Javad Hosseini[3]

*1. Department of Clinical Biochemistry , Faculty of Medicine , Kurdistan University of Medical Science*
*2. Department of Molecular Medicine and Genetics, Faculty of Medicine , Kurdistan University of Medical Science*
*3. Cellular and Molecular Research Center, Kurdistan University of Medical Sciences*

**Background:** Atherosclerosis is known as an inflammatory disease. Trimethylamine N-oxide (TMAO) as a risk factor, has the potential to trigger or promotes the immune inflammatory reactions in atherosclerosis. The exact mechanism by which TMAO induce inflammation during atherosclerosis is not well understood. The present study was designed to evaluate the expression of IL-1β, IL-6, and TNF-α at mRNA levels in response to treatment of macrophages with TMAO.
**Methods:** U937-Derived macrophages were treated with different concentration of TMAO for 24 h. The cells were also treated with tunicamycin as a positive control for stress. RT-qPCR was used to evaluate the expression of IL-1β, IL-6, and TNF-α at mRNA levels.
**Results:** although TMAO induced the expression all IL-1β, IL-6, and TNF-α at mRNA levels, only 300 µM of TMAO significantly increased the expression of IL-1β at mRNA level compared to control cells. Tunicamycin increased the mRNA levels of IL-6.
**Conclusion:** The results of this study provide documentation to support the possible contribution of IL-1β as a proinflammatory cytokine, among the others, to involve in immune inflammatory reactions induced by TMAO during atherosclerosis.

**Keywords:** Cytokine, Atherosclerosis, Trimethylamine-N-Oxide, Tunicamycin, Macrophage

ICIA2018\Innate Immunity and Inflammation\Poster\9943
**9943**

# Increased concentrations of chemokines CXCL10 in patients with ischemic heart disease

Mojde Kazemi[1], Abdollah Jafarzadeh[1*], Amin Safa [3], Hamid Reza Rashidinejad[4], Maryam Nemati [1], Mohammad Mehdi Mohammadi [1]

*1. Department of Immunology, Medical School, Kerman University of Medical Sciences, Kerman, Iran*
*2. Department of Immunology, Medical School, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
*3. Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran*

**Background:** Recruitment of leukocytes is one of the earliest events in the pathogenesis of ischemic heart disease (IHD) and chemokines play an important role in the migration of these cells into the inflammation sites. The aim of this study was to evaluate the CXCL10 in patients with IHD.
**Methods:** A total of 300 patients with IHD as having acute myocardial infarction (AMI; n=100), stable angina (SA; n=100) or unstable angina (UA; n=100) and 100 healthy subjects as a control group were enrolled to study. Serum samples from all participants were tested for the CXCL10 levels by using ELISA.
**Results:** The mean serum concentrations of CXCL10 in AMI patients (395.97 ± 21.20 pg/mL), SA patients (405.48 ± 27.36 pg/mL) and UA patients (396.69 ± 22.79 pg/mL) were significantly higher than in the healthy group (179.38 ± 8.85 pg/mL, P<0.001). Similarly, the mean serum levels of CXCL10 in total IHD patients (399.38 ± 13.77 pg/mL) were also significantly higher as compared with healthy subjects (P<0.001).
**Conclusion:** These results showed that the higher levels of CXCL10 were associated with IHD. The serum levels of chemokines may influence by the certain traditional risk factors of IHD.

**Key words:** Acute myocardial infarction, Stable angina, Unstable angina, Chemokine, CXCL10

ICIA2018\Innate Immunity and Inflammation\Poster\9948
**9948**

**Does HLA-DR influence susceptibility to pulmonary cystic fibrosis?**

Esmaeil mortaz[1], alireza asef[2], Hossein Ali Ghaffaripouur[3], Ali Arabkhazali[1]

*1. Department of Immunology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Young Researchers Club, Rasht Branch, Islamic Azad University, Rasht, Iran*
*3. Pediatric Respiratory Diseases Research Center, NRITLD, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Cystic fibrosis (CF) is the most common lethal autosomal recessive disease in white Caucasians. It affects many organs including the lung, pancreas and liver. Whilst CF is a monogenic disease, several studies reveal a complex relationship between genotype and clinical phenotype. Objective of study: we examined the expression of HLA class II alleles among Iranian CF patients in relation to disease-related criteria namely infection with Pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans and with the serum total IgE level.
**Materials and Methods:** Our study was conducted on 50 CF patients (27 male, 23 female) hospitalized in the Masih Daneshvari Hospital in Tehran and 50 healthy age- and gender-matched control subjects from March 2016 to April 201. Individuals with a family background of CF were excluded. Clinical and demographic data including lung function and comorbidities such as diabetes and gallstones were evaluated. HLA-DRB1 subtypes were determined by Single Specific Primer Polymerase Chain Reaction (SSP-PCR).
**Results:** HLA-DRB1*09 and HLA-DRB1*10 were less frequent and HLA-DRB1*13 and HLA-DRB1*11 were the most frequent in CF patients compared with healthy controls although this did not reach significance. 16 CF patients had high serum IgE levels (430.25 ± 219.7 IU/ml) which was most closely associated with the HLA-DRB1*04 allele. Twenty seven CF patients were P. aeruginosa positive with the HLA-DRB1*04 being the most frequent allele. Thirty one CF patients were candida albicans colonized with HLA-DRB1*11 as the most frequent allele. The small numbers of patients with allergic bronchopulmonary aspergillosis (ABPI, n=3) or with diabetes (n=2) prevented further exploration of their data.
**Discussion**: Our data suggests that the DR4 and DR11 serotypes are the most prominent in the Iranian CF population and further research on should be conducted on DR4+ CF patients to understand the role in clinical phenotypes of CF.

**Key words:** HLA-DRB1, SSP-PCR, lung cystic fibrosis

ICIA2018\Innate Immunity and Inflammation\Poster\10009
**10009**

# Nicotine and Caffeine Alter the Effects of the Pro-Inflammatory Mesenchymal Stem Cells on the Co-Cultured Neutrophils Function

Rahmani Kukia Nasim[1], Abbasi Ardeshir [2*], Abtahi Froushani Seyyed Meysam[3], Hashemi Seyyed Mahmoud[4]

1. *Division of Immunology, Department of Microbiology, Veterinary Faculty, Urmia University, Urmia, Iran*
2. *Division of Immunology, Tarbiat Modares, University of Medical Sciences, Tehran, Iran*
3. *Department of Microbiology, Veterinary Faculty, Urmia University, Urmia, Iran*
4. *Department of Immunology, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background**: Mesenchymal stem cells (MSCs) express some of the nicotinic receptor subunits and adenosine receptors. Recent studies indicate that polarized MSC1 mostly elaborate pro-inflammatory mediators. The communication between pro-inflammatory MSCs with neutrophils has not been yet studied. Therefore, the main desire is to determine the role of nicotine or caffeine on MSC1s and its effects on neutrophils.
**Methods:** After isolation, characterization and differentiation of MSCs into osteocyte and adipocyte, they were pulsed with LPS (10 ng/ml) for 1 hour. MSCs were incubated with different concentrations of caffeine (0.1, 0.5 and 1 mM) and with different concentrations of nicotine (5, 25 and 50 µl) separately for 48 hours. Then the medium was aspirated and cells were used for co-culture experiment with neutrophil and the behavior of neutrophils was studied.
**Results:** Attained data indicated that when pro-inflammatory MSCs treated with nicotine or caffeine, increased significantly the vitality of co-cultured neutrophils. The rate of the respiratory burst of neutrophils after co-culture MSC1s, didn't show any significant change after challenging with non-opsonized yeast in non-treated groups .Nicotine or caffeine treatment, at a high dose, could increase the respiratory burst of neutrophils after challenging with non-opsonized yeast. Interestingly, after challenging with opsonized yeast, the rate of the neutrophil respiratory burst was decreased after co-culturing with pro-inflammatory MSCs comparing the respiratory burst of neutrophil alone. Nicotine and/or caffeine treatment could reverse this reduction.
**Conclusion:** Generally, these findings provide a new insight into understanding the anti-inflammatory and immunomodulatory effects of nicotine and caffeine.

**Keywords**: Mesenchymal stem cells, Caffeine, Nicotine, Neutrophil

ICIA2018\Innate Immunity and Inflammation\Poster\11068
**11068**

**Investigating level of hs-CRP in steatohepatitis patients**

**zivar zangeneh**

*Isfahan University of medical sciences, Isfahan, Iran*

**Background:** Non-alcoholic fatty liver disease (NAFLD) is characterized by triglyceride accumulation in hepatocytes, which occurs without alcohol abuse. It is one of the most commonly encountered chronic liver diseases. The clinical spectrum of NAFLD ranges from asymptomatic steatosis to steatohepatitis, fibrosis and cirrhosis. Most NAFLD patients have asymptomatic simple steatosis without adverse sequelae. Recently, studies have reported that NAFLD is an independent risk factor of numerous diseases, including diabetes, cardiovascular disease, hypertension, kidney disease and colon cancer. Therefore, interest is growing with regard to NAFLD**.** C-reactive protein (CRP) is a biomarker of inflammation. Plasma CRP concentrations increase rapidly and dramatically (100-fold or more) in response to tissue injury or inflammation. High-sensitivity CRP (hs-CRP) is more precise than standard CRP when measuring baseline (ie, normal) concentrations and enables a measure of chronic inflammation. The aim of this study is to investigate relationship between CRP and fatty liver diseases.
**Methods:** 113 patient with steatohepatitis were determined based on the results of abdominal ultrasonography grade I and II. Hs-CRP was measured by kit and auto-analyzer and TG and cholesterol levels were determined. Statistical analysis was done by prism gragh-pad.
**Results:** Seventy eight patients had hs-CRP level average of more than 3mg/dl with grade II. But others had an average level of less than 3mg/dl. Seventy eight patients had higher levels of TG and cholesterol.
**Conclusion:** Hs-CRP is suggested as a biomarker for diagnosis and also prognosis of steatohepatitis.

**Keywords**: hs-CRP, steatohepatitis

# Monoclonal Antibody Diagnostic and Therapeutic

# Poster Presentation

ICIA2018\ Monoclonal Antibody Diagnostic and Therapeutic\Poster\8583
**8583**

# Expression and purification of Recombinant scFv antibodies against EpEX in *Escherichia coli* Rosetta™(DE3) Rosetta-Gami™, Origami™ and SHuffle™

Mohammadi, S.A [1], Hashemi, A[1], keramati, M[2]

1. *Pharmaceutical Biotechnology Department, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
2. *Pilot of nanobiotechnology Department, Pasteur institute of IRAN, Tehran, Iran*

**Background:** Overexpression of some cell surface antigens in malignancies make them good targets for active and passive immunotherapy of cancers. EpCAM (epithelial cell adhesion molecule) is an antigen that over expressed in many types of epithelial cell cancers. Recently, several monoclonal antibodies have been developed to directly interfere with EpCAM extracellular domain (EpEX) antigen. Single-chain variable fragment (scFv) is a class of engineered antibodies generated by the fusion of the heavy (VH) and light chains (VL) of immunoglobulins through a short polypeptide linker. Now, scFvs have been developed as potential diagnostic and/or therapeutic agents. *Escherichia coli (E.coli)* is one of the most widely used hosts in recombinant proteins production.
**Methods:** This report describes the production and purification of a scFv specific for EpEX in periplasm of *E.coli* strains containing Rosetta™(DE3) Rosetta-Gami™, Origami™ and SHuffle™.
**Results:** The majority of expressed anti-EpEX-scFv protein was produced in soluble form. A Ni-NTA affinity column was used to purify the anti-EpEX scFv protein. The molecular weight of anti-EpEX-scFv protein was estimated to be approximately 30 kDa, as confirmed by SDS-PAGE and western blotting assay. The anti-EpEX-scFv showed near 95 % purity.
**Conclusion:** Our results revealed that *E.coli* is a well-established host system for the production of antibody fragments such as anti-EpEX-scFv. This novel targeting agent can be used in naked or conjugation forms in the treatment of the wide range of tumor cells with epithelial origin including colon cancer.

**Key words:** ScFv, EpEX, EpCAM, *E.Coli*

# Mucosal and Gastrointestinal Immunology

# Poster Presentation

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster\6314
**6314**

**The differences between bovine and human sIgA in binding to gut-associated bacteria**

Milad Ameri[1], Vedad Alkhamis [2]

1. *Department of Pathobiology ,University of Bu Ali Sina, Hamedan*
2. *Department of Microbiology University of Shahid Chamran Ahvaz*

**Background:** In our study, we tested the hypothesis that human and bovine sIgA have similar innate binding activity for bacteria
**Methods:** SIgAs, isolated from human and bovine milk, were incubated with a selection of commensal, pathogenic and probiotic bacteria from gastrointestinal tract (gut). Using flow cytometry, we measured numbers of bacteria binding SIgA and their level of SIgA binding
**Results:** The percentage of bacteria bound by human and bovine SIgA varied from 30 to 90% depending on bacterial species and strains, but was remarkably consistent between human and bovine SIgA. The level of SIgA binding per bacterial cell was lower for those bacteria that had a higher percentage of SIgA-bound bacteria, and higher for those bacteria that had lower percentage of SIgA-bound bacteria. Overall, human and bovine SIgA interacted with bacteria in a comparable way
**Conclusion:** The in vitro data have shown that milk-derived hSIgA and bSIgA have a similar propensity to bind a range of gut-associated bacteria. Further studies are required to understand why the level of SIgA binding varies between different bacteria. These data will contribute to longer term research about the potential benefits of bovine SIgA for human consumers

.**Keywords:** Secretory IgA, Bovine IgA, Human IgA, infection, milk

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster\7459
**7459**

# Association between *helicobacter pylori* infection in Subjects with Gastritis and serum levels of LL-37, MBL and M–Ficolin

Mehdi soltanzadeh Yamchi[1], Mohammad mirzakhani[1], Mehdi shahbazi[1], Mousa Mohammadnia Afrouzi[1], Mojgan Bagherzadeh[1], Javad Shokri Shirvani[2]

1. *Department of Immunology, School of Medicine, Babol University of Medical Sciences, Babol, I.R. Iran*
2. *Department of Internal Medicine, School of Medicine, Babol University of Medical Sciences, Babol, I.R. Iran*

**Background:** *Helicobacter pylori* (*H. pylori*) can stimulate immune responses and lead to release of pro-inflammatory factors and antimicrobial peptides such as LL-37, MBL and M –Ficolin. The aim of this study was to determine the level of changes in serum levels of the three mentioned factors in people with gastritis and their association with the presence or absence of *Helicobacter Pylori.*
**Methods:** Study populations were divided in two groups of 35 patients with *H. pylori* and 25 patients without *H. pylori* infection. Biopsy and blood samples were collected from each subject. *H. pylori* positivity was investigated regarding the serum level of anti-*H.pylori* IgG by enzyme-linked immunosorbent assay (ELISA) kit and its presence in the tissue was examined by histopathology observations and rapid urease test (RUT). LL-37, MBL and M-Ficolin serum levels were measured in each group using the standard ELISA kit.
**Results:** It was revealed that 58% of the subjects were infected with *H. pylori*. Subjects with MBL levels lower than 500 ng/ml in the sera were significantly infected with *H. pylori* and subjects with MBL levels higher than 1000 ng/ml often did not have *H. pylori* infection. LL-37 had an increased level while M-Ficolin showed no significant change in the presence of H. pylori.
**Conclusion:** Our findings indicate that MBL with a lower presence and LL-37 with a higher presence might be involved in *H. pylori* infection while M-ficolin seems to be less effective in the infection.

**Keywords***: Helicobacter pylori***,** LL-37, MBL, M -Ficolin

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster\7514
**7514**

# Gene expression and serum concentration of Thioredoxin in patients with celiac disease is more than healthy controls despite gluten free diet

Manizhe Faghih[1], Mohammad Rostami Nejad[2], Davar Amani[1], Elham Aghamohamadi[2], Amir Sadeghi[1], Mohammad Reza Zali[2]

*1. Department of Immunology, School of medical science , Shahid Beheshti University of Medical Sciences,Tehran, Iran*
*2. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, shahid Beheshti University of Medical Sciences,Tehran, Iran*

**Background:** Thioredoxin (TRX) as a ubiquitous redox-active protein is induced in several cells against oxidative stress, as well as increasing in the extracellular matrix in various infectious and autoimmune diseases. Additionally TRX is a highly specific activator of oxidized human extracellular TG2 in small intestinal biopsies, thus it can play a role in the pathogenesis of celiac disease. We investigated the gene expression and serum level of TRX in celiac disease patient's compared to healthy group.
**Methods:** In this study, duodenal biopsies were collected from 60 confirmed celiac disease patients under gluten-free (between 6 months and 2 years) diet and 60 healthy subjects as control group. RNA was extracted from tissue according to the protocol of the commercial kits, cDNA was synthesis, primer pairs were designed and then TRX gene expression was run by using Real-time PCR technique. Also the serum concentrations of TRX was determined by using ELISA method.
**Results:** Out of 60 CD patients and 60 controls, with mean age of 38 and in control group with an average age of 35. The result of this study was showed that gene expression and serum concentration of TRX in GFD-patients was more than healthy controls ($P<0.4$ and $<0.001$), respectively.
**Conclusion:** TRX plays an important role in the onset of tissue damage in celiac disease, and the result of this study confirmed that thioredoxin gene expression and serum level is higher in CD patients than healthy group. According to the results of this study, thioredoxin may interfere with the pathogenesis or process of celiac disease, and further studies are needed to make this clear.

**Keywords:** Celiac Disease, TRX (Thioredoxin), Gene Expression, serum level

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster\8353
8353

# Serum mucosa-associated epithelial chemokine (MEC/CCL28) in COPD: a specific marker for severity

Erfan babaei[1], Ali jalili[2], Rasoul Nasiri kalmarzi[2], Naseh Sigari[3]

1. *Student Research Committee, Kurdistan University of Medical Sciences, Sanandaj, Iran*
2. *Cancer and Immunology Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran*
3. *Department of Internal Medicine Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran*

**Background:** Chronic obstructive pulmonary disease (COPD) is a complex inflammatory disease of the lung with systemic reflections. The disease is one of the major health problems around the world that affects 10 to 15 percent of the adult population of 40 and older, this disease is thought to be associated with local and systemic inflammation. However, the exact mechanism of inflammation in this disease has not been fully understood. The chemokine CCL28, known as mucosal epithelial chemokine, is shown to be produced by epithelial cells and chemoattracts inflammatory cells into musical tisues.
**Methods:** Blood sample were taken from 40 patients with COPD and 40 age matched normal individuals. Serum were collected and frozen at -80 ºC until use. CCL28 was measured by ELISA as described by manufacturer. All the data were analyzed by SPSS 24.
**Results:** Our data show that (i) the levels of CCL28 almost equal in both genders, (ii) CCL28 levels are significantly higher in the serum of patients with COPD than the normal group (p value <0.05) and (iii) treated patients showed to have a lower levels of CCL28 than untreated patients. In addition, spirometric findings show that, if the disease is more severe, the concentration of ccl28 is higher.
**Conclusion:** For the first time we demonstrate that CCL28 Is elevated in serum of patients with COPD and this inflammatory marker could be used as biomarker for diagnosis of COPD.

**Keywords:** CCL28, COPD, Spirometry

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster\8512
**8512**

# Evaluating of the expression level of CXCL12 and CXCL13 in animal model of Ulcerative Colitis disease

Mohammad amin Boshagh[1], Poorya Foroutan[1], Ali Jalili[2], Shohreh Fakhari[2]

1. *Student Research Committee, Kurdistan University of Medical Sciences, Sanandaj, Iran*
2. *Department of Immunology, Faculty of medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran*

**Background**: Ulcerative colitis (UC) and Crohn's disease (CD), two forms of inflammatory bowel disease (IBD) that are accompanied by the uncontrolled activation of effector immune cells in the mucosa. In recent years, experimental studies in rodents have led to a better understanding of the role played by these inflammatory mediators in the development and progression of colitis. Therefore, the aim of this study was to evaluate of the expression level of CXCL12 and CXCL13 in animal model of Ulcerative Colitis disease.
**Methods:** In this experimental study, 6 male Wistar rats were randomly divided into two groups: control and treated groups. In the treated group, colitis was induced by rectal injection of acetic acid. After 48hours, the animal was anesthetized and then the mucosa samples of its colon were separated in order to consider the histopathological indices and polymerase chain reaction.
**Results:** Based on PCR results the expression of these chemokine receptors in the treated group has changed in comparison with the control group. CXCl2 and CXCl13 up-regulated in treated group in comparison with the control group.
**Conclusion:** According to the information obtained from this study, the likely expression of these receptors in the animal model of the ulcerative colitis has changed. Given the fact that this study is part of my thesis, the final confirmation, and publication of exact information require more samples, as well as quantitative techniques such as Real-Time PCR, as well as verification methods such as Immunohistochemistry. Finally, in the current study, we intend to evaluate the expression of the whole CXCLs in this model.

**Keywords:** Inflammatory bowel disease, Ulcerative colitis, Chemokine, Acetic acid

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster\9825
**9825**

# The Effect of iFlora Probiotic on Hepatic Enzymes Serum Level NAFLD Patients

Zahra Asadi[1], Hossein Ghaedamini[2], Hamidreza mohajerani[1], Yasaman Alimohammadi[1]

1. *Islamic Azad University Arak, Arak, Iran*
2. *Kerman University of Medical Sciences, Kerman, Iran*

**Background:** Today there is evidence of using probiotic products on the improvement of non-alcoholic fatty liver disease (NAFLD). This study was conducted to consider the effect of iFlora probiotics on serum levels of liver enzymes in patients with NAFLD.
**Methods:** This randomized, double-blind clinical trial was performed on 40 patients (24 women and 16 men) with NAFLD. The intervention group (n=20) used probiotic powder iFlora in dairy products for 8 weeks. The percentage change in body mass index, blood pressure, and serum levels of fasting glucose, triglyceride, total cholesterol (total, LDL, HDL), AST and ALT were collected at the beginning and end of this research. Statistical analysis was performed by SPSS software using Chi-square, independent t-test, covariance analysis and paired t-test.
**Results:** The results showed that using probiotic iFlora caused a reduction of 3.45% of serum alanine aminotransferase ($p < 0.002$), a reduction of 4.65% in aspartate aminotransferase ($p < 0.001$) and a reduction of 7.8% in total cholesterol ($p < 0.001$) in the intervention group compared with the control group.
**Conclusion:** Using of probiotic iFlora improves the levels of liver enzymes and total cholesterol in patients and can be effective in treating NAFLD.

**Keywords:** Probiotics, iFlora, liver enzymes, NAFLD

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster\9928
**9928**

## Comparison of LncRNA13 gene expression in patients with celiac disease

Naghmeh Zamani, Farzaneh Tafvizi, Mohammad Rostami Nejad, Mohammad Reza Zali

*1. Basic Department of Biology, Parand Branch, Islamic Azad University, Parand, Iran*
*2. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran*
*3. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Celiac disease has been known to be one of the health concerns with an increasing trend worldwide. This autoimmune disease is due to a reaction to gluten resulting in destroyed intestinal villi. Recent studies have shown that LncRNA13 affects the pathogenicity of the disease. This part of genome does not result in production of protein and were previously known as junk DNA. However, recent studies showed that this group of genes can affect the inflammation due to the disease. We aimed to evaluate the expression of LncRNA13 in a group of celiac patients.
**Materials and Methods:** In a case-control study, LncRNA13 was evaluated in a total of 50 celiac patients and 50 healthy controls. To evaluate the expression of target gene, qRT-PCR method was applied.
**Results:** No significant difference was detected between the LncRNA13 expression between the cases and controls (P=0.27). No such significant difference was detected between males and females, either (P=0.99). In the celiac patients with positive and negative IgA tTg, LncRNA13 expression was significantly different (P=0.03)
**Conclusions:** LncRNA13 expression is recommended to be evaluated in celiac patients to determine the prognosis and treatment of choice. Evaluation of LncRNA13 expression is recommended in other gastrointestinal tract diseases.

**Keywords:** LncRNA13, gene expression, celiac disease.
.

ICIA2018\Mucosal and Gastrointestinal Immunology\Poster\9973
**9973**

# Investigation of Caspase 3 gene expression pattern in HepG2 cell line under Graphene Oxide exposure

Mahsa Saeedi Niasar[1&2], Seyed Reza Mohebbi[3], Mehrangiz Dezfoulian[2], Mohammad Reza Zali[3]

*1. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Department of Biology, Faculty of Science, Karaj Branch of Islamic Azad University, Karaj, Iran*
*3. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background:** Graphene based nanomaterials have been widely used as carrier for drug delivery, scaffold for tissue engineering purposes and clinical diagnosis during recent years due to their unique and attractive phycisal and chemical properties. However, evaluating the safety of these materials is necessary and a matter of great concern. The present thesis aimed to evaluate the possible cytotoxicity of graphene oxide nanomaterial on the HepG2 cell line.
**Methods:** Different concentrations of graphene oxide including 1, 5, 10, 20, 50, 100, 200,400 µg/ml have been utilized to treat the HepG2 cells as sample treated cells and compared to untreated cells. Cytotoxicity of these groups was determined by XTT assay. In addition, Caspase 3 gene expression was evaluated on different time 4 and 24 hours after cell exposure to graphene oxide using real time PCR technique.
**Results:** The results showed decreasing levels of cell viability in higher doses of graphene oxide 100µg/ml and more (P value=0.004). Inductions of apoptosis pathway and increasing of caspase 3 mRNA levels were also observed expression of caspase 3 was increased (RQ=3.921).
**Conclusion:** Based on the present results, graphene oxide at the low concentrations is a safe nanomaterial for cells with hepatocyte origin nevertheless its probable risk of graphene oxide in high concentrations should be considered prior to biomedical applications.

**Keywords:** Graphene oxide, cytotoxicity, Apoptosis, Cell viability

# Nanoimmunology

# Poster Presentation

ICIA2018\Nanoimmunology\Poster\5282
**5282**

# Nanoencapsulation of modified protective antigen in PLA-PEG nanoparticles

Seyed Masih E'temad aubi[1], Hosein Honari[1], Hamed Bagheri[2], Mojtaba Nofeli[3]

1. *Department of Biology, Imam Hosein University, Tehran, Iran*
2. *School of of Technology, Tarbiat Modarres University, Tehran, Iran*
3. *Department of Human Vaccine and Serum, Razi Vaccine and Serum Research Institute, Karaj, Iran*

**Background:** The *Bacillus anthracis* causes anthrax lethal disease. Traditional Anthrax vaccines require multiple injection doses due to their low stability to be fully effective. Polylactic acid (PLA) has been approved as a biodegradable and biocompatible polymer for drugs and vaccines delivery applications. The aim of this study is to encapsulate modified *Bacillus anthracis* protective antigen (mPA63) by PEG-PLA copolymers and characterizing the synthesized nanoparticles.
**Methods:** After expression of the PA63 in *E. coli*, the produced antigen was purified by affinity chromatography and the encapsulation in PLA-PEG copolymer was done using double emulsion solvent evaporation method. The morphological characteristics of produced nanoparticles were evaluated by scanning electron microscopy and particle size, polydispersity index and Zeta potential were measured using dynamic light scattering.
**Results:** The results of this study showed that the size of free protein-PLA-PEG nanoparticles and the protein-loaded nanoparticles were 162 nm and 233 nm, respectively and the polydispersity index for above mentioned particles were 0.034 and 0.340, respectively. Also, electron microscopic images showed spherical shape of nanoparticles. The Zeta potential of PLA-PEG nanoparticles was -32.2 and PLA-PEG-PA63 nanoparticles have an average peak at -10.8mV. The encapsulation efficiency of the PA63 recombinant antigen in nanoparticles was 34%.
**Conclusion:** From the result of this study it was showed that PLA-PEG copolymer has an appropriate ability to encapsulate the mPA63 and this system can be recommended as a candidate for an effective vaccine carrier in future investigation.

**Keywords:** Anthrax vaccine, Recombinant antigen, Nano-carrier, PEG-PLA copolymer.

ICIA2018\Nanoimmunology\Poster\7721
**7721**

# Targeting P68 and STAT3 molecules through Nanoparticles loaded with the siRNA in 4T1 and CT26 cancerous cells lines

Vida Hashemi[1,2,3], Ali Masjedi[2,3], Afshin Nikkhoo[2,3], Narges Rostami[2,3], Shima Bastaki[4,2], Farhad Jadidi Niaragh[2,3]

*1. Department of basic science, Faculty of Medicine, Maragheh University of Medical Sciences, Maragheh, Iran*
*2. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
*3. Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
*4. Department of Cellular and Molecular biology, Faculty of Basic Sciences, Azarbaijan University, Tabriz, Iran*

***Background*:** The ribonucleic acid (RNA) helicase P68 can acts as a coactivator of STAT3 and upregulate STAT3 target genes which are involved in various tumorigenic processes such as cellular survival, proliferation, angiogenesis and invasion. STAT3 is constitutively activated in breast and colon cancers.
**Methods:** P68- and STAT3- specific siRNA loaded chitosan-lactate (ChLa) nanoparticles (NPs) was produced by ionic gelation technique to suppress the expression of P68 and STAT3 molecule on 4T1 breast and CT26 colon cancerous cells lines, in vitro. All of physicochemical characteristics, serum stability, siRNA loading, siRNA release pattern, and cellular uptake of NPs were investigated. The expression of target genes was evaluated by real-time PCR. Anti-proliferative effect of NPs was assessed by MTT assay. Wound healing assay was performed to assay anti-metastatic effects.
**Results:** P68 and STAT3-siRNA-loaded ChLa NPs could efficiently suppress the expression of P68 and STAT3. Furthermore, downregulation of target molecules led to apoptosis of cancer cells and was associated with reduced expression of angiogenic and metastatic molecules. Consistently, migration capacity of cancer cells was significantly suppressed after treatment.
**Conclusion:** Combinatorial suppression of P68 and STAT3 by siRNA-loaded NPs may be considered as a promising therapeutic approach for cancer therapy.

**Key words:** P68, STAT3, Nanoparticle, siRNA, Cancer Therapy.

ICIA2018\Nanoimmunology\Poster\7801
**7801**

# Downregulation of PD-L1 and STAT3 in 4T1 breast and B16/F10 skin cancer cells through siRNA-loaded chitosan-lactate nanoparticles

Shima Bastaki[1,2] Narges Rostami[2,3], Afshin Nikkhoo[2,3], Ali Masjedi[2,3], Vida Hashemi[7,2,3], Fatemeh Atyabi[5,6] , Ali Rastegari[5] , Masoomeh Baghaei[6] , Farhad Jadidi Niaragh[2,3,4]

*1. Department of Cellular and Molecular Biology, Faculty of Basic Sciences, Azarbaijan Shahid Madani University, Tabriz, Iran*
*2. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
*3. Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
*4. Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
*5. Department of Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 1714614411, Iran*
*6. Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
*7. Department of Basic Science, Faculty of Medicine, Maragheh University of Medical Sciences, Maragheh, Iran*

**Background:** The immunosuppressive factors in tumor microenvironment play major role in tumor progression. One of the most critical factors is the programmed death-ligand1 (PD-L1), whose expression on tumor cells has been reported. The activation of STAT3 signaling is significantly associated with expression of PD-L1 in tumor cells, and blocking STAT3 signaling inhibits the PD-1/PD-L1 axis.
**Methods:** PD-L1 and STAT3-siRNA were encapsulated into chitosan-lactate (ChLa) nanoparticles (NPs) via ionic gelation strategy and were employed to decrease the expression of PD-L1 and STAT3 on 4T1 and B16/F10 tumor cells, *in vitro*. All of physicochemical characteristics, serum stability, siRNA loading efficiency, siRNA release pattern, and cellular uptake of NPs were assessed. The expression of target genes was evaluated by real-time PCR. Anti-proliferative effect of nanoformulation was assessed by MTT assay. Wound healing assay was performed to assay anti-metastatic effects.
**Results:** Generated NPs had about 100 nm size with a polydispersive index below 0.3 and a zeta potential about 12. Synthesized NPs had high siRNA loading, high serum stability, and efficient cellular uptake. Moreover, NPs had low toxicity during 72 hr cell culture. In addition, siRNA-loaded NPs significantly suppressed expression of PD-L1 and STAT3 in cancer cells. Downregulation of these molecules was associated with reduction of anti-apoptotic proteins, angiogenic, and metastatic molecules. Suppression of PD-L1 and STAT3 also led to decreased migration of cancer cells.
**Conclusion:** PD-L1 and STAT3 siRNA-loaded ChLa NPs can be considered as a promising therapeutic tool for cancer therapy; however it requires further *in vivo* investigations.

**Key words**: PD-L1, STAT3, cancer immunotherapy, chitosan-lactate

ICIA2018\Nanoimmunology\Poster\7803
**7803**

**Silencing S1PR1 and GP130 in 4T1 breast cancer cells and B16F10 melanoma cancer cells through siRNA-loaded trimethyl chitosan nanoparticles**

Narges Rostami[1,2], Farhad Jadidi iaragh[1,2,3], Afshin Nikkhoo[1,2], Ali Masjedi[1,2], Fatemeh Atyabi[4,5] , Ali Rastegari[4], Masoomeh Baghaei[5] , Vida Hashemi[6,1,2], Shima Bastaki[7,1]

*1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*
*2. Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
*3. Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
*4. Department of Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 1714614411, Iran*
*5. Nanotechnology Research Center , Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
*6. Department of Basic Sciences, Faculty of Medicine, Maragheh University of Medical Sciences, Maragheh, Iran*
*7. Department of Cellular and Molecular Biology, Faculty of Basic Sciences, Azarbaijan Shahid Madani University, Tabriz, Iran*

**Background:** There are several stimulating molecules in tumor microenvironment which enhance tumor growth. Among them, S1PR1 promotes cancer cell survival, invasion, metastasis and radio/chemo-resistance in various cancers via JAK/STAT3 and other signaling pathways. Glycoprotein130 is a shared subunit of IL-6 receptor which mediates tumor progression following ligation with IL-6 on tumor cells. IL-6 via its receptor, gp130, triggers STAT3 stimulation. There is an amplification loop between S1PR1 and IL-6 for stimulation of STAT3 signaling in tumor cells. Therefore combinatorial blockage of these molecules can be considered as an effective immunotherapeutic approach for cancer therapy.
**Methods:** S1PR1-siRNA and gp130-siRNA were encapsulated into trimethyl chitosan (TMC) nanoparticles (NPs) were generated through ionic gelation of TMC by hyaloronic acid and employed to suppress the expression of S1PR1 and gp130 molecules on 4T1 and B16F10 cancer cells, *in vitro*. All of physicochemical characteristics, serum stability, siRNA loading efficiency, siRNA release pattern, and cellular uptake of NPs were assessed. The expression of target genes was evaluated by real-time PCR. Anti-proliferative effect of nanoformulation was assessed by MTT assay. Wound healing assay was performed to assay anti-metastatic effects.
**Results:** SiRNA-loaded NPs had about 100 nm size with a polydispersive index below 0.3 and a zeta potential about 15. Generated NPs exhibited high siRNA loading capacity, high serum stability, and efficiently uptaken by cancer cells. The NPs demonstrated low toxic effect during 72 hr cell culture. In addition, S1PR1-siRNA and gp130-siRNA-loaded TMC NPs could efficiently suppress the expression of S1PR1 and gp130. Combination treatment of tumor cells also led to downregulation of anti-apoptotic proteins, angiogenic, and metastatic molecules. Suppression of S1PR1 and gp130 was also associated with decreased migration of cancer cells.
**Conclusion:** Combinatorial treatment of tumor cells with S1PR1-siRNA and gp130-siRNA-loaded TMC NPs may be considered as a promising therapeutic tool for cancer therapy.

**Key words**: S1PR1, gp130, cancer immunotherapy, Trimethyl chitosan

ICIA2018\Nanoimmunology\Poster\7871
**7871**

# Generation of M918/HIV-1 Nef nanoparticles and its delivery into mammalian cells

Bahareh Rostami[1], Azam bolhassani[2*], Shiva Irani [1], Reza Ahangari Cohan[3]

*1. Department of Biology, School of Basic Science, Science and Research Branch, Islamic Azad University, Tehran, Iran*
*2. Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran*
*3. Pilot Nano-Biotechnology Department, Pasteur Institute of Iran, Tehran, Iran*

**Background:** Among different HIV-1 proteins, Nef protein plays an important role in the down-regulation of CD4 and MHC class I as a virulence factor for AIDS pathogenesis. Therefore, Nef protein can be considered as a possible attractive target in development of therapeutic HIV vaccine. The most important limitation of protein-based vaccines is their low immunogenicity. Recently, a group of short, highly basic peptides known as cell penetrating peptides (CPPs) were used to carry polypeptides and proteins into cells. In this study, the production of HIV-1 Nef protein was performed in *E. coli* expression system for delivery into a mammalian cell line using a novel cell penetrating peptide, M918, in a non-covalent fashion.
**Methods:** The cloning of HIV-1 Nef was performed into the cloning site of pET23a expression vector. The *E.coli Rossetta* strain was applied for expression of the recombinant Nef using IPTG inducer. The Nef expression was detected by SDS-PAGE and purified using affinity chromatography. The morphological features of the M918/Nef and TurboFect/Nef complexes were studied by scanning electron microscopy (SEM) and SDS-PAGE. The efficiency of Nef transfection using M918 and TurboFect reagent was evaluated by SDS-PAGE and western blot analysis in HEK-293T cell line.
**Results:** Our results showed a clear band of ~ 27 kDa for Nef protein in SDS-PAGE and western blot analysis. SEM data confirmed the formation of discrete nanoparticles with a diameter of below 300 nm. The dominant band of ~ 27 kDa was detected in the transfected cells with Nef/M918 at molar ratio of 1:20 using the anti-HIV-1 Nef monoclonal antibody. The corresponding band was not detected in the un-transfected cells and transfected with Nef protein alone indicating that M918 could transfer Nef protein into the cells.
**Conclusion:** These data suggest that M918 peptide would indicate promising applications for improvement of HIV therapeutic vaccines.

**Keywords:** HIV, Nef antigen, Protein vaccine, M918

ICIA2018\Nanoimmunology\Poster\8034
**8034**

# Combinatorial Suppression of Interleukin-6 and STAT3 in breast, colon and melanoma cancer cells through siRNA-loaded trimethyl chitosan nanoparticles

Ali Masjedi[1,2], Fatemeh Atyabi[3,4], Narges Rostami[1,2], Afshin Nikkhoo[1,2], Vida Hashemi[1,2,5], Shima Bastaki[1,6], Ali Rastegari[3], Masoomeh Baghaei[4] , Farhad Jadidi Niaragh[1,2,7]

*1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
*2. Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
*3. Department of Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 1714614411, Iran*
*4. Nanotechnology Research Centre, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
*5. Department of basic science, Faculty of Medicine, Maragheh University of Medical Sciences, Maragheh, Iran*
*6. Department of Cellular and Molecular Biology, Faculty of Basic Sciences, Azarbaijan Shahid Madani University, Tabriz, Iran*
*7. Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Chronic inflammation in the tumor microenvironment enhances resistance against chemo- and radiotherapy, besides promoting growth. Interleukin-6 (IL-6) secreted by tumor cells is one of the main cytokines in the tumor microenvironment and its overexpression has been reported in most types of tumors. The role of IL-6 and its associated signaling pathway (IL-6/JAK/STAT3) has been proven in cancer growth, progression, metastasis and inducing therapeutic resistance.
**Methods:** IL-6 and STAT3s directed small interfering RNA (siRNA) molecules encapsulated into trimethyl-chitosan nanoparticles(TMC-NP) via ionic gelation of TMC by Dextran and used to suppress the expression of target molecules on murine 4T1(breast), CT26 (colon) and B16F10(melanoma) cancer cells, in vitro.
**Results:** Generated NPs exhibited optimum physicochemical characteristics associated with high siRNA loading, high stability and efficient cellular uptake. NPs could markedly suppress expression of target molecules which was associated with apoptosis induction. Moreover, expression of angiogenesis and metastatic related molecules was reduced following treatment. Accordingly, migratory function of cancer cells was significantly decreased after suppression of target molecules.
**Conclusion:** Combinatory suppression of IL-6 and STAT3 using siRNA-loaded TMC-NPs may be considered as a promising therapeutic approach for cancer therapy.

**Keywords:** IL-6, STAT3, cancer immunotherapy, Trimethyl chitosan, siRNA

ICIA2018\Nanoimmunology\Poster\8487
**8487**

# The Cytotoxic and Apoptotic Effects of Different Sizes of Copper Oxide Nanoparticles in B92 Cancer Cell Line

Rahmani Kukia Nasim[1], Abbasi Ardeshir [2*], Abtahi Froushani Seyyed Meysam[3]

1. *Division of Immunology, Department of Microbiology, Veterinary Faculty, Urmia University, Urmia, Iran*
2. *Division of Immunology, Tarbiat Modares, University of Medical Sciences, Tehran, Iran*
3. *Assistant professor of Immunology, Department of Microbiology, Veterinary Faculty, Urmia University, Urmia*

**Background**: Chemotherapeutic researches based on the use of copper oxide nanoparticles (CuO NPs) for new anticancer drugs with the potential of being less toxic and more cytotoxic against tumors have been developed. Therefore this study was done to investigate the concentration and size dependent of CuO NPs exposure in $B_{92}$ cancer cells.
**Methods:** CuO NPs were first green synthesized and characterized by typical scanning electron microscopy, the zeta sizer and X-ray diffractometer, then were cultured in 30 and 60 nm particle sizes at several concentrations (5, 10, and 20 µgr/ml) with $B_{92}$ glial cancer cells for 24 hr. The apoptosis and cytotoxicity of cells were estimated by acridin orange/ propidium iodide staining and MTT assay, respectively. Also, the RT-PCR method was used to evaluate the expression of $P_{53}$ and *Bax* expression.
**Results:** The images of synthesized CuO NPs confirmed the average sizes of 30 and 60 nm. The both size have cytotoxic effects, even with the lowest concentration, the cytotoxic effect accommodates the increasing percentage of cell apoptosis (32% at 30 nm size). Also, as the concentration increases, viability decreases and apoptosis increases. The $IC_{50}$ of CuO NPs with 30 nm size was (12.015±1.9) and for NPs with 60 nm was (9.17±3.3). However, the amount of $IC_{50}$ was decreased as the size of particles increase, but there was no significant change. Both mRNA level of $P_{53}$ and *Bax* was upregulated in treated cells.
**Conclusion:** Our results suggest that an exposure to CuO NPs had significant cytotoxic effects when compared to unexposed ones in a way that the smaller size and higher concentration displayed the maximum cytotoxicity. However, it seems that augmentation may not have any effect on CuO NPs cytotoxicity *in vitro* state.

**Keywords**: $B_{92}$ cell line, CuO NPs, Cytotoxicity, Apoptosis, Glioma

ICIA2018\Nanoimmunology\Poster\10049
**10049**

**New nanoformulation for artimisinin and evaluation of its effect on MCF7 cell line**

Yasaman salimi, zuhair saraf hasan

*Department of immunology, faculty of medical sciences, Tarbiat Modares University, Tehran, Iran*

**Background:** One major challenge to relieve cancer burden is to develop highly effective drugs with specificity on cancers but little or no side effects on normal mammalian cells. Because conventional therapies based on chemotherapy, surgery and radiotherapy and current anti-cancer regiment are in many cases of limited efficacy. Therefore, herbal antioxidants, including artemisinin, were expanded. Artemisinin is a sesquiterpene lactone with a 1, 2, 4-tiroxane ring system that extracted from the Chinese herb qinghaosu (Artemisia annua or annual wormwood). Despite its efficacy, artemisinin, has pharmacokinetic limitations. artemisinin has low solubility in water and biological fluids, poor bioavailability, and a short half-life in vivo (~2.5 h). To overcome these problems, we conjugated albumin with artimisinin and produced nano-artemisinin. The important thing is that the Nano substance must be harmless, protect the anti-cancer properties of artemisinin and also increase its solubility in water and its stability in the blood. In this study artemisinin had been conjugated with albumin.
**Methods:** After making the nano artemisinin, we used FTIR graph and scanning electerone microscopy (SEM) In order to confirm the combination of these two substances. Also the cytotoxicity properties of artemisinin and nano-artemisinin effect on mcf7 cell lines in Gradient of concentration within 24 hours, examined by mtt test.
**Results:** The FTIR confirmed that artimesinin and albumin bonded together. Particle size was reported 51.31 nm by SEM. The analysis of MTT tests indicated that the rate of killing of artemisinin and nano-artemisinin at 20µg dose was 77% and 86% respectively. Previous studies have been shown that this dosage almost has no killing effect on normal cells.
**Conclusion:** Due to the drug loading which is about 25% , Therefore, the amount of artemisinin used is a quarter of the amount of free artemisinin. This indicates that the killing ability was 4 times higher, with about no effect on normal cells.

**Key words**: artemisinin, MCF7 cell line, nanoformulation

ICIA2018\Nanoimmunology\Poster\9823
**9823**

# The effects of coq10 nanoparticle on inflammation markers in streptozotocin-induced diabetic rats

Azad bahrampour[1], Roonak azizbeigi[2]

*1. DVM Student, Faculty of Veterinary Medicine, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran*
*2. Assistant of Physiology, Department of Physiology, Faculty of Veterinary Medicine, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran*

**Background:** Coenzyme Q10 is an intracellular antioxidant that protects cell membrane phospholipids against oxidative damage caused by free radicals. Diabetes and its complications have been related to increased levels of free radicals and systemic proinflammatory cytokines and to an abnormal lipid profile. The aim of this study was to investigate the effects of CoQ10 nanoparticle supplementation on some cytokine levels in streptozotocin-induced diabetic rats.
**Methods:** In this study, 40 healthy adult male rats were used. The rats were divided into 5 groups. The animals in group 1 was fed standard rat pellets for 4 weeks. It was administered at 0.3 mL corn oil intraperitoneally daily for four weeks in group 2 animals. The animals in group 3 was injected intraperitoneally with (0.3 mmol/lit) CoQ10 nanoparticle daily for 4 weeks. Group 4 was made diabetic by subcutaneous injections of streptozotocin at dose of 40 mg/kg in 0.1 M citrate buffer (pH 4.5) single daily dose for two days and group 5 was made diabetic by subcutaneous injections of streptozotocin at dose of 40 mg/kg in 0.1 M citrate buffer (pH 4.5) single daily dose for two days and then was injected intraperitoneal with (0.3mmol/lit) CoQ10 nanoparticle daily for 4 weeks.
**Result:** At the end of the study, blood samples were taken from all animals. In these blood samples, IL-6and TNF-α plasma levels were determined with ELISA using sandwich enzyme-linked immunosorbent method via commercial kits. In this study, IL-6 and TNF-α levels in diabetic group significantly increased ($P < 0.05$) in parallel with each other compared to control group levels. The same parameters were reduced ($P < 0.05$) by CoQ10 nanoparticle application in diabetic animals.
**Conclusion:** CoQ10 nanoparticle suppressed the increments in plasma pro-inflammatory cytokine levels and play important role in regulation of imbalance between inflammation markers in diabetes conditions.

**Keywords:** CoQ10 nanoparticle, cytokine, diabetes, rat

# Psychoneuroimmunology and Immunoendocrinology

# Poster Presentation

ICIA2018\Psychoneuroimmunology and Immunoendocrinology\Poster\7761
**7761**

# Relationship between serum levels of vitamin D and anti-Tpo in Hypothyroid patients in Urmia city

Asma khanzade[1], Yousef yousefzadeh[2], Roya khanahmadi[3], Amir asghari[4]

1. *Department of microbiology, Islamic azad university of urmia*
2. *Social security organization of urmia, iran*
3. *Faculty of medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
4. *Department of Biology, Faculty of Sciences, Urmia University, Iran*

**Background**: Vitamin D is nowadays regarded as a vitamin that is associated with several diseases. The aim of this study was to determine the relationship between serum vitamin D level and anti-TPO antibodies in hypothyroid patients. Thyroperoxidase (TPO) is an enzyme that is involved in the synthesis of the thyroid hormone. Vitamin D is a group of fat-soluble vitamins and a natural moderator of the immune system, then the serum levels of vitamin D and anti-TPO antibodies were then evaluated using ELISA (Monobain, USA).
**Methods:** In this study, 90 subjects with hypothyroidism and 60 healthy subjects were matched with age and sex, and blood samples were taken.
**Results:** from 90 patients, 66 had a vitamin D deficiency (> 30 ng) and had positive anti-TPO.The vit. D level in 32 patients was less than 30ng/ml (48.5% deficiency) and in 20 patients (30.3%) it was more than 30ng/ml. 14 patients had vit.D level under 10ng/ml (severe deficiency in 21.2%). while this was in the control group (1.75 ± 31.78) and the patients average control group was (27.31).
**Conclusion:** In people with vitamin D deficiency, the anti-TPO antibody level is high. The results showed that there is a significant relationship between vitamin D deficiency and anti-TPO antibodies and vitamin D deficiency effects hypothyroid. Further consideration is suggested in the other geographic regions.

**Keywords**: Vitamin D, Anti-TPO, Hypothyroid deficiency

ICIA2018\Psychoneuroimmunology and Immunoendocrinology\Poster\8291
**8291**

**Diminished circulating levels of interleukin-10 in patients with Parkinson's disease**

Javad Masoumi[1], Hossain Khorramdelazad[1], Mohammad Taghi Rezayati[1], Azam Bagherizadeh[1], Shekoofeh Moghadam[1], Alireza Vakilian[2], Abdollah Jafarzadeh[3]

1. *Department of Immunology, Medical School, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.*
2. *Department of Neurology, Medical School, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.*
3. *Department of Immunology, Medical School, Kerman University of Medical Sciences, Kerman, Iran.*

**Background:** Parkinson's disease (PD) is one of the most prevalent neurodegenerative disorders that is specified by the destruction of dopaminergic neurons. A number of genetic as well as environment parameters have been introduced as risk factors of PD. However, the neuro-inflammation is one of the most important characteristic of PD. The aim of this study was to investigate the levels of anti-inflammatory cytokine IL-10 in patients with PD.
**Methods:** The blood samples collected from 23 patients with PD and 24 age and gender-matched healthy subjects as a control group. The serum levels of IL-10 were measured by ELISA.
**Results:** The mean serum levels of IL-10 was 7.7± 3.89 pg/ml in PD group and 12.61 ± 9.9 in healthy subjects. The mean serum levels of IL-10 in PD patients was significantly lower than healthy group ($P<0.03$). No significant differences were observed between men and women neither in PD patients nor in healthy control group.
**Conclusion:** These results showed lower levels of IL-10 in patients with PD that represent the may plays an important role in pathogenesis of PD. The levels of IL-10 did not influence by gender of participants.

**Keywords:** Parkinson's disease, Interleukin-10, Serum, Gender

ICIA2018\Psychoneuroimmunology and Immunoendocrinology\Poster\8480
**8480**

**Evaluation of the effects *of Lactobacillus plantarum* on the acquired conditional learning of rats**

Minoo Mohamadi, Maryam Tajabadi Ebrahimi*, Bahareh Pakpour

*Department of Biology, Faculty of basic science, Islamic Azad University, Central Tehran branch, Tehran, Iran*

**Background:** Investigations have shown that foodstuffs can affect the brain and nervous system hormones through vagus nerve. Accordingly, probiotics can be effective in regulating activity of different parts of the brain by improving the function of the brain.
Aim: The aim of this study was to evaluate the effect of *Lactobacillus plantarum*, an indigenous probiotics isolated from traditional Iranian dairy products, on acquired conditional learning of Wistar rats.
**Methods:** In this study, 80 male Wistar rats with 200-250 grams weight range were used. For induction of amnesia, intra peritoneal pre-test injection of morphine (1 mg/rat) was performed in rats, and $1\times10^9$ cfu/ml probiotic bacteria (*Lactobacillus plantarum*) were administered to rats in the treatment group for nine month. The step-through passive avoidance used to examine long-term memory in control group and probiotic-treated group.
**Results:** In the control group, morphine significantly decreased learning. In the probiotic-treated group receiving morphine, there was a significant improving in the acquired conditional learning when compared with control group.
**Conclusion:** The findings of this study represented the additive effect of *Lactobacillus plantarum*, an indigenous Iranian probiotic isolated from dairy products, in acquired conditional learning of rats received intraperitoneal morphine, indicating beneficial effects of oral probiotics on memory and the learning ability of rats.

**Keywords:** Amnesia, Morphine, *Lactobacillus plantarum*, Rats

ICIA2018\Psychoneuroimmunology and Immunoendocrinology\Poster\8572
**8572**

# Circulating specific immunoglobulin G to *Helicobacter pylori* in patients with Parkinson's disease

Saeed Mirzaee[1], Hossain Khorramdelazad[1], Mohammad-Taghi Rezayati[1], Azam Bagherizadeh[1], Shekoofeh Moghadam[1], Alireza Vakilian[2], Abdollah Jafarzadeh[3]

1. *Department of Immunology, Medical School, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
2. *Department of Neurology, Medical School, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
3. *Department of Immunology, Medical School, Kerman University of Medical Sciences, Kerman, Iran*

**Background:** Parkinson's disease (PD) is a progressive neurodegenerative disease that is specified by damage of dopaminergic neurons. The neuro-inflammation is one of the main feature of PD and there are some reports regarding the association of infections with PD. The aim was to evaluate the seroprevalence of anti-H. Pylori IgG in patients with PD and healthy individuals from Rafsanjan, a city located in south-east of Iran.
**Methods:** The blood samples collected from two groups including patients with PD and age and gender-matched healthy subjects as a control group. The sera were tested for the anti-H. pylori IgG antibodies using ELISA method.
**Results:** The seroprevalence of anti-H. pylori antibodies in patients with PD (78.3%) was similar to that observed in healthy subjects (79.2%). There was no also significant difference between healthy control group and control group regarding the mean titer of anti-H. pylori antibodies (67.17 ± 8.74 U/ml vs 56.54 ± 9.38 U/ml; P =0.41).
**Conclusion:** These results show that H. pylori seropositivity rate was similar in PD patients and control group. No association was also found between H. pylori and PD in investigated population.

**Keywords:** Parkinson's disease, *Helicobacter pylori*, Seroprevalence

ICIA2018\Psychoneuroimmunology and Immunoendocrinology\Poster\9807
**9807**

# The Role of Cytokines in Autism Spectrum Disorder

Fatemeh Mohammadizadeh[1,2]

*1. Student Research Committee, Sirjan Faculty of Medical Sciences, Kerman University of Medical Sciences, Kerman. Iran*
*2. Department of Laboratory, Sirjan Faculty of Medical Sciences*

**Background:** Autism is a neurodevelopmental disorder characterized by deficits in communication and social skills as well as repetitive and stereotypical behaviors. While much effort has focused on the identification of genes associated with autism, research emerging within the past two decades suggests that immune dysfunction is a viable risk factor contributing to the neurodevelopmental deficits observed in autism spectrum disorders (ASD). Currently, it is estimated that ASD affects 1 in 68 children in the United States, with the average age of diagnosis at 4 years.
**Methods**: A systematic search was performed to identify studies published in multiple databases (Cochrane, Embase, Pubmed and google Scholar) up to 2018, and recently published abstracts were also reviewed. Using the key words Autism, Cytokines in Autism, Autism Spectrum Disorder, Neuroimmunology and pathophysiology of autism.
**Results:** There is strong evidence that disruption of normal cytokine levels has a significant role as a risk factor for neurodevelopmental defect, such as autism. Results showed that elevated mid-gestational levels of inflammatory cytokines and chemokines are more highly associated with the ASD subphentoype that presents with intellectual disability (ID), compared with ASD without ID, developmental delay (DD) without ASD, and typically developing controls. These included higher levels of GM-CSF, TNF-α, IFN-γ, IL-1α, IL-1β, IL-4, and IL-6.
**Conclusion:** Maternal inflammation history and the pro-inflammatory/anti-inflammatory cytokine balance are thus particularly important avenues for further study, especially at the interface between mother and fetus.

**Keywords:** Autism, Cytokine, Immune dysfunction, autism spectrum disorders

ICIA2018\Psychoneuroimmunology and Immunoendocrinology\Poster\7476
**7476**

# Dysregulation of peripheral Th17 cells in elderly patients with ischemic stroke

Farnaz Hajizadeh[1, 2], Sanam Dolati[1, 2], Mehdi Yousefi[1, 2]

1. *Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Ischemic stroke causes brain injury with activation of an inflammatory response that can contribute to clinical impairment. The involvement of IL-17A and IL-17-secreting T cell subsets in IS patients has not been verified. In the present study, we studied Th17 frequency, cytokine secretion, expression of transcription factors related to Th17 which is compared between IS patients and control group.
**Methods:** 30 patients with IS and 30 persons as control group were enrolled in this study.Blood samples were collected from IS patients at 24 hours, 5 and 10 days after stroke,Th17 was detected among PBMCs using flowcytometric analysis, SYBR Green method of polymerase chain reaction (PCR) was employed for the analysis ofRORγt mRNA levels, Quantification of IL-17 was carried out with the TaqMan Real-Time PCR. Secretion of IL-17, was evaluated in the supernatant acquired from cultured PBMCs using ELISA.
**Results:** The proportion of Th17 cells were significantly elevated in IS patients, RORγt mRNA expression levels were significantly increased in IS patients after 1 and 5 days compared to the controls 2.06 ±72 and 1.77 ±0.5 ($p<0.0001$), IL-17A levels were increased in IS patients at 1, 5 and 10 days after stroke compared with the controls from 118 ± 35.9 to 171 ± 34 ($p=0.0003$),147 ± 24.84 ($p=0.005$) and 146 ± 27.57 ($p=0.005$), respectively.
**Conclusion:** These studies suggest that the increase in proportion of Th17 cells might contribute to the pathogenesis of IS.

**Keywords:** Ischemic Stroke, Th17, Cytokines, Inflammation

# Reproductive Immunology

# Poster Presentation

ICIA2018\Reproductive Immunology\Poster\5289
**5289**

# Relation of Gene's expression Profile of IL-17 and IL-23 in Cumulus cells and their Follicular Fluid Concentrations in Infertile Patients at the Risk of Ovarian Hyperstimulation Syndrome (OHSS)

Leila Motamedzadeh [1, 3], Hossein Hadinedoushan [2], Ehsan Farashahi Yazd [3], Farzaneh Fesahat [3], Mohammad Mahdi Mohammadi [1, 4*]

1. *Department of Immunology & Allergy, Kerman University of Medical Sciences, Kerman, Iran*
2. *Department of Immunology, Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Science, Yazd, Iran*
3. *Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Science, Yazd, Iran*
4. *Physiology Research Center, Kerman University of Medical Sciences, Kerman, Iran*

**Background:** Ovarian hyperstimulation syndrome (OHSS) is one of major complications during assisted reproductive technology.
**Methods:** The cumulus cells of oocytes from 80 infertile women were divided into two groups of case (n=40) with an average age 29.6±1.4 years and control (n=40) with an average age 29.2±1.1 according to the patients' infertility etiologies. The control group was the infertile patients with male factor infertility etiologies while; infertile women at the risk of OHSS were defined as case group in this study. IL-17 and IL-23 concentrations in FF were determined. mRNA expression levels of IL-17 and IL-23 of samples were measured using real time PCR method in all subjects.
**Results:** The concentrations obtained from FF demonstrated the higher levels of IL-17 in case group (P=0.04), while, it showed no remarkable difference in IL-23 (P=0.3). The mRNA levels of IL-17 and IL-23 showed no significant differences between two groups. There was a positive significant correlation between FF concentrations of IL-23 and the oocytes maturation rates in case group (P=0.01).
**Conclusion:** Our finding showed the mRNA expression of IL-17 and IL-23 were similar in two groups, and IL-17 may be considered as risk factor for OHSS.

**Keywords:** OHSS, IL-23, IL-17

ICIA2018\Reproductive Immunology\Poster\7412
**7412**

# TH17 and Treg frequencies in treatment of RPL women with immune abnormalities after IVIG therapy

Golshan Sheikhansaria [1, 2], Leili Aghebati-Maleki [1, 2, 3], Mehdi Yousefi [1, 2]

1. *Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
3. *Immunology research center, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background**: Recurrent pregnancy loss (RPL) is a complication in pregnancy and is defined as three or more recurrent miscarriage in over 30 years of old women. Previous studies have described that immunological factors have essential role in RPL. After 1980 using of IVIG therapy for abortion associated with antiphospholipid antibodies (APA) have considered. In this study, we aimed to evaluate of immune cell (TH17, Treg) and pregnancy outcome after IVIG therapy in RPL patients.
**Methods**: 44 blood samples were taken from RPL women with immune abnormalities after positive pregnancy test. Simultaneously 400 mg/kg of IVIG was administered intravenously every 4 weeks until 28–30 weeks of gestation. Control group was included of 12 RPL patient with abnormal cellular immune profile without IVIG therapy. The frequency of Th17 and Treg cells, the expression of related transcription factors of these cells and the serum levels of associated cytokines were evaluated after IVIG therapy by flow cytometry, real-time PCR and ELISA, respectively.
**Results:** We have seen significantly reduced frequency of Th17 cells from 3.94 ±1.12% to 1.83 ± 0.56% and increased frequency of Treg from 3.55±1.65 to 9.13±1.23 in IVIG treated group. Significant decreased secretion of IL-17 and IL-23 and expression of RORγt and IL-17 mRNAs also significant increased secretion of IL10 and TGF-β and expression of Foxp3. Pregnancy outcome in IVIG treated group in Comparison to untreated group had increased 40.8 %.
**Conclusion:** These studies suggest that, IVIG therapy can be very helpful in establishing the balance between Th-17 and Treg in successful pregnancy. Also, the imbalance TH17/Treg in RPL women with immune abnormalities could be improve by decreased TH17 and increased Treg frequency following IVIG therapy.

**Keyword:** Intravenous immunoglobulin G, Recurrent pregnancy loss, TH17, Treg

ICIA2018\Reproductive Immunology\Poster\7416
**7416**

**Effect of IVIG on exhausted T cell and exhausted regulatory T cells in women with recurrent miscarriage**

Amin kamrani[1], Leili Aghebati Maleki[2], Mehdi Yousefi[3],

*1. a Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran b Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
*2. a Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Ta*
*3. briz, Iran b Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran c Immunology research center, Tabriz University of Medical Sciences, Tabriz, Iran*

*a Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran b Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.*

**Background:** Exhausted T cell, exhausted regulatory T cells and Treg cells have been suggested as novel main factors for recurrent miscarriage (RM). In current study, we studied the effect of IVIG on exhausted T cell and exhausted regulatory T cells and pregnancy outcome in women with RM.
**Methods:** 94 pregnant women with RM were participated in this study. Blood samples were taken. Then, IVIG (400 mg/kg) was administered for 44 patients, intravenously. 50 other RM patients were included as no IVIG interfering control group. Following the first administration, IVIG was given every 4 weeks through 32 weeks of pregnancy. Blood sample was collected after the last administration. Exhausted T cell, exhausted regulatory T cell and regulatory T cell population were assessed before and after treatment in the two groups.
**Results:** IVIG administration reduced the population and function of exhausted regulatory T cells and increased the population and function of regulatory T cell CD4 + CD25 + cells. Also IVIG treatment don't effect on population and function of Exhausted T cells. But, no significant difference in population and function of exhausted T cell, regulatory T cell and exhausted regulatory T cells was detected in the untreated groups before and after pregnancy. Pregnancy outcome in IVIG treated subjects was successful in 38 out of 44 RM women (86.3%). Nevertheless, pregnancy outcome was successful in 21 out of 50 untreated RM women (42%).
**Conclusion:** Administration of IVIG in RM women with cellular immune cells irregularities during pregnancy effects exhausted T cell and exhausted regulatory T cells and regulatory T cells in peripheral blood and improves Treg and decreases exhausted regulatory T cells responses. These studies suggest that, this immune modulatory influence of IVIG may be associated with effective pregnancy.

**Keywords:** IVIG; exhausted T cell; regulatory T cell; recurrent miscarriage

ICIA2018\Reproductive Immunology\Poster\7513
**7513**

**Investigation of T cell subtypes in women with recurrent pregnancy loss and control group**

Samaneh Abdolmohammadi Vahid [1, 2]- Majid Ahmadi [1,2] -Mehdi Yousefi [1, 2]

1. *Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran.*
2. *Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.*

**Background:** Recurrent pregnancy loss (RPL) includes approximately 15% to 25% of all pregnancies and is determined as 3 pregnancy losses before the twentieth week of gestation. Immunologic abnormalities, such as elevated rate of Th1, Th17 and reduced number of Th2 and Treg, are among the etiologies which are mentioned for RPL. Here, we evaluated T cell subtypes frequency and related genes expression level in RPL women and control group.
**Methods:** Totally 40, 20 RPL and 20 healthy women enrolled in the study. Evaluation of Treg and Th17 frequency was done by flowcytometry and mRNA expression level of T-bet (Th1 transcription factor), GATA-3 (Th2 transcription factor), GITR (Treg associated factor) and IRF-4 (Th17 related marker), the genes responsible for T cells subpopulation function, by real time PCR.
**Results:** Treg frequency was significantly decreased in RPL women compared with healthy women, while an increase was observed in Th17 level. Comparison of expression of GATA-3 and GITR in RPL patients, showed a significant decrease when compared with healthy women (p value=0.0008 and 0.0001, respectively), while expression level of IRF-4 and T-bet were higher in RPL women (p value=0.0001 and 0.011, respectively).
**Conclusion:** Increased rate of proinflammatory parameters such as Th17 frequency, T-bet and IRF-4 expression and reduction of suppressive parameters, Treg and expression of GATA-3 and GITR, in RPL women, represent the undeniable role of immune system in successful pregnancy. Immunologic factors may be used as helpful prognostic biomarkers in high risk women.

**Keywords:** recurrent pregnancy loss, T regulatory, T helper17

ICIA2018\Reproductive Immunology\Poster\7593
**7593**

# Modulatory effect of pioglitazone on pro-inflammatory cytokines (TNF-α and IL-1β) levels in testes of streptozotocin-induced diabetic rats

Farin Malekifard[1], Ali Soleimanzadeh[2]

1. *Department of Microbiology, Facutly of Veterinary Medicine, Urmia University, Urmia, Iran*
2. *Department of Theriogenology, Facutly of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Diabetes mellitus has adverse effects on the male sexual and reproductive functions. Oxidative stress is one of the major pathophysiological routes during diabetes Pioglitazone is high-affinity PPAR-γ agonists**.** Previous studies have demonstrated that pioglitazone is potent inhibitor of inflammatory and potent antioxidants. Oxidative stress causes the activation of various transcription factors including the nuclear factor-κB (NF-κB). NF-κB plays a crucial role in inflammation, immune function and expression of pro-inflammatory cytokines such as TNF-α ,IL-1β, and IL-6. IL-1β, alone or in combination with TNF-α and IFN γ induces transcription of the inducible nitric oxide synthase (iNOS) gene.
The present study was undertaken to examine the protective effect of pioglitazone on anti-inflammatory system in testis of streptozotocin induced diabetic rats.
**Methods:** Induction of experimental diabetes was done using single intraperitoneal injection of streptozotocin (STZ) dissolved in citrate buffer (pH 4.5) at the dose of 65 mg/kg to overnight fasted rats. Only rats with blood glucose concentrations above 250 mg/dl were considered as diabetic. Animals were randomly divided into four groups of eight rats: control group, STZ-induced diabetic group and STZ-induced diabetic groups treated with low or high doses of pioglitazone of 1 or 10(mg/kg/day, orally) for 5 weeks. Mice were euthanized on day 35 and pro-inflammatory cytokines (IL-1β and TNF-α) levels in testicular tissue was performed by ELISA kits.
**Results:** Testicular levels of pro-inflammatory cytokines including TNF-α and IL-1β were increased in diabetic rats compared to control animals ($P<0.05$). IL-1β and TNF-α levels significantly decreased only in high (10 mg/kg) dose of pioglitazone group when compared to diabetic rats ($p<0.05$)
**Conclusion:** Pioglitazone reduced testicular inflammation by decreasing pro-inflammatory cytokines levels.

**Keywords** Pioglitazone, IL-1β, TNF-α, Testicular damage

ICIA2018\Reproductive Immunology\Poster\7594
**7594**

# Effect of pioglitazone, a ligands of peroxisome proliferator-activated receptor gamma (PPAR-γ), on expression of NF-κB in testes of streptozotocin-induced diabetic rats

Farin Malekifard[1], Ali Soleimanzadeh[2], Navideh Mirakhani[3]

1. *Department of Microbiology, Facutly of Veterinary Medicine, Urmia University, Urmia, Iran*
2. *Department of Theriogenology, Facutly of Veterinary Medicine, Urmia University, Urmia, Iran*
3. *Department of Pathobiology, Facutly of Veterinary Medicine, Urmia University, Urmia, Iran*

**Background:** Diabetes mellitus caused testicular damage by increasing oxidative stress and inflammation. Pioglitazone is high-affinity PPAR-γ agonists with antioxidant properties and potential anti-inflammatory effects**.** Previous studies have suggested that the anti-inflammatory effect of pioglitazone is mediated by the inhibition of NF-κB activation. It has been shown that Native or synthetic PPAR-γ ligands inhibit the production of several inflammatory mediators, nitric oxide synthase and NF-κB transcription activity. Previous reports showed that diabetes is associated with up-regulations of iNOS and NF-κB-p65 expressions with a concomitant increase in the level of NO in testicular tissues of diabetic rats. The purpose of this study was to investigate the preventive effects of pioglitazone on expression of NF-κB of testicular tissues.
**Methods:** Induction of experimental diabetes was done using single intraperitoneal injection of streptozotocin dissolved in citrate buffer (pH 4.5) at the dose of 65 mg/kg to overnight fasted rats. Only rats with blood glucose concentrations above 250 mg/dl were considered as diabetic. Animals were randomly divided into four groups of eight rats: control group, STZ-induced diabetic group and STZ-induced diabetic groups treated with low or high doses of pioglitazone (Sigma) of 1 or 10(mg/kg/day, orally) for 5 weeks. Mice were euthanized on day 35 and expression of NF-κB in testicular tissue was performed by western blot.
**Results:** Diabetic rats exhibited significant high level in the expression of NF-κB-p65 in testicular tissues ($P < 0.05$). Administration of pioglitazone effectively inhibited expression of NF-κB-p65($P < 0.05$).
**Conclusion:** Pioglitazone attenuated testicular damage in diabetic rats by decreasing expression of NF-κB.

**Keywords:** Diabetes, Pioglitazone, NF-κB, Testicular damage

ICIA2018\Reproductive Immunology\Poster\7595
**7595**

# Effect of hydro-alcoholic garlic extract on serum level of testosterone of streptozotocin induced diabetes in C57BL/6 mice

Farin Malekifard[1], Ali Soleimanzadeh[2], Yaser jafarikhataylou[3]

1. *Department of Microbiology, Facutly of Veterinary Medicine, Urmia University, Urmia, Iran.*
2. *Department of Theriogenology, Facutly of Veterinary Medicine, Urmia University, Urmia, Iran*
3. *Department of Microbiology, Facutly of Veterinary Medicine, Tabriz University, Tabriz, Iran*

**Background:** Previous studies have shown that diabetes induces changes in reproductive functions especially on steroidogenesis, histology of testes, spermatogenesis, sperm quality and fertility both in diabetic men and experimental diabetic animals. Oxidative stress is one of the major pathophysiological routes during diabetes. It has been reported that hyperglycemia causes dysfunction of leydig cells thus these cells cannot secrete testosterone. The aim of present study was to examine the antioxidative effects of garlic extract in diabetic mice.
**Methods:** Diabetes was induced by multiple low-dose of streptozotocin injection (40 mg/kg/day for 5 consecutive days) in male C57BL/6 mice (15-20 gr body weight). After induction of diabetes, mice were divided into 6 groups: group 1 (normal control group); group 2 (diabetic control group); group 3 (treatment with garlic extract 200 mg/kg for 35 days) and group 4 (treatment with garlic extract 400 mg/kg for 35 days). Mice were euthanized on day 35 and serum testosterone levels were estimated by ELISA kit.
**Results:** In the present study, significant decrease in serum testosterone level was observed in diabetic control group ($P < 0.05$). Garlic treatment effectively increased ($p<0.05$) serum level of testosterone
**Conclusion:** Garlic treatment protects against diabetesinduced testicular damage in diabetic mice by increasing serum testosterone level.

**Keywords:** Diabetes, Garlic Extract, Testosterone

ICIA2018\Reproductive Immunology\Poster\7643
**7643**

# Effect of pioglitazone, a ligands of peroxisome proliferator-activated receptor gamma (PPAR-γ), on expression of inflammatory marker (iNOS) in testes of streptozotocin-induced diabetic rats

Farin Malekifard[1], Ali Soleimanzadeh[2], Yaser jafarikhataylou[3]

1. *Department of Microbiology, School of Veterinary Medicine, Urmia University, Urmia, Iran*
2. *Department of Theriogenology, School of Veterinary Medicine, Urmia University, Urmia, Iran*
3. *Department of Microbiology, School of Veterinary Medicine, Tabriz University, Tabriz, Iran*

**Background:** Impaired homeostasis under diabetic conditions is connected with the increased production of free radicals and defiency of antioxidant systems. Pioglitazone is potent inhibitor of inflammatory and potent antioxidants. Biological markers of inflammatory responses are elevated level of NF-κB and the associated inflammatory mediators such as iNOS. iNOS catalyzes the production of large amount of NO from L-arginine, and importantly, NO inhibited insulin secretion. The purpose of this study was to investigate the preventive effects of pioglitazone on expression of inflammatory marker (iNOS) of testicular tissues.
**Methods:** Induction of experimental diabetes was done using single intraperitoneal injection of streptozotocin dissolved in citrate buffer (pH 4.5) at the dose of 65 mg/kg to overnight fasted rats. Only rats with blood glucose concentrations above 250 mg/dl were considered as diabetic. Animals were randomly divided into four groups of eight rats: control group, diabetic group and treated with low or high doses of pioglitazone of 1 or 10(mg/kg/day, orally) for 5 weeks. Mice were euthanized on day 35 and expression of iNOS in testicular tissue was performed by western blot.
**Results:** Expression of iNOS in testicular tissue was significantly increased in diabetic rats compared with normal control group. Administration of pioglitazone for five consecutive weeks markedly reduced expression of iNOS in testicular tissue.
**Conclusion:** Our data suggest that pioglitazone attenuated testicular damage in diabetic rats by decreasing expression of iNOS.

**Keywords:** Diabetes, Pioglitazone, iNOS, Testicular damage

ICIA2018\Veterinary Immunology\Oral\8689
8689

**Soluble expression of IHNV immunogenic glycoprotein in the periplasm of Escherichia coli**

Seyed Amir Hossein Jalali[1,2], Rezvan Mohammadi Nezhad[3]

*1. Department of Natural Resources, Isfahan University of Technology, Isfahan 84156–83111, Iran*
*2. Research Institute for Biotechnology and Bioengineering, Isfahan University of Technology, Isfahan 84156–83111, Iran*
*3. Department of Biotechnology, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran*

**Background:** Glycoprotein of the infectious hematopoietic necrosis virus (IHNV), is the only structural protein of the virus capable of inducing neutralizing antibodies in the fish host. Special attention has been paid for recombinant production of this vaccine candidate. However, due to the highly hydrophobic nature and the presence of several disulfide bonds, IHNV-G expression in Escherichia coli (E. coli) is associated within complete protein folding and aggregation. In the present study, we have explored the soluble expression of heterologous IHNV-G in the periplasm of E. coli using Pel B signal peptide.

**Methods:** The full coding sequence (CDs) of IHNV-G was amplified from infected tissues of rainbow trout (Oncorhynchus mykiss) using reverse transcription-polymerase chain reaction (RT-PCR). After cleavage with appropriate restriction enzymes, the PCR product was cloned in the bacterial plasmid pET22b containing Pel B signal sequence for periplasmic expression. Simultaneously, a truncated fragment of IHNV-G lacking N- and C-terminal hydrophobic parts was cloned in pET19b for cytoplasmic expression. Both constructs were used to transform the expression host E. coli Rosetta (DE3).

**Results:** Considerable amount of truncated protein was produced in E.coli cytoplasm after induction with IPTG, but predominantly in the insoluble fraction. However, targeting protein expression to the periplasm resulted in the production of soluble IHNV-G, as detected by both SDS-PAGE and western blotting analysis. The soluble IHNV-G was successfully purified in an optimized stabilizing buffer using affinity chromatography.

**Conclusion:** The expression procedure described in this study can provide sufficient amount of recombinant IHNV-G to immunize rainbow trout fish or to be used for producing specific antibodies for diagnostic or therapeutic purposes.

**Keywords:** Periplasmic expression, Glycoprotein, Recombinant vaccine, IHNV

ICIA2018\Veterinary Immunology\Oral\9978
9978

**The lysozyme cross reactivity, pH-optimum activity and similarity between animal species**

Mohammad Khosravi[1],Shokooh Salimi Moghadam[2]

1. *Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran.*
2. *Student of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran.*

**Background:** Lysozymeis distributed in serum, plasma, amniotic fluid, stool, saliva, tears, urine and other biological fluids. The most commercially available egg-white lysozyme and detection methods routinely used for creating standard curves and detection tools on other species; this experiment investigated sequences similarity, cross-reactivity and pH-optimum activity of the lysozyme in different animal species.

**Methods:** Polyclonal antibodies against chicken's lysozyme were obtained from the immunized rabbits and the IgGs were purified using ion exchange chromatography. An in-house ELISA was developed for detection of the lysozyme cross reaction between different animal species. The optimum pHs for the lyses of Micrococcus cell wall were detected using turbidimetric analysis. The lysozyme amino acid sequences were retrieved and analyzed using NCBI data base.

**Results:** The linearity of developed ELISA was evaluated in the range of 1mg–7µg. The coefficients of regression 0.98 confirmed that the method employing detection was linear for the determination of chicken lysozyme; however, the test has unacceptable results for the other species samples. The optimum pH of the buffer on turbidimetric method was determined as 5.6, 5, 5, 5, 5 and 5.6 in human, cattle, sheep, horse, dog and fish samples, respectively. The chicken's lysozyme amino acid sequence has the identity as 0.60, 0.61, 0.55, 0.50, 0.58, 0.54, 0.60, 0.59 and 0.61 with cattle, fish, sheep, horse, human, goat, buffalo, dog and donkey sequence, respectively.

**Conclusion:** It's suggested to use a species-specific lysozyme and detection tools for measurement of the lysozyme.

**Keywords:** Lysozyme, ELIZA, Cross- reactivity

ICIA2018\Reproductive Immunology\Poster\7644
**7644**

# Effect of pioglitazone, a ligands of peroxisome proliferator-activated receptor gamma (PPAR-γ), on antioxidant status marker (SOD) levels in testes of streptozotocin-induced diabetic rats

Farin Malekifard[1], Ali Soleimanzadeh[2], Nowruz Delirezh[1]

1. *Department of Microbiology, Urmia University, Urmia, Iran*
2. *Department of Theriogenology, Urmia University, Urmia, Iran*

**Background:** Diabetes can cause sexual dysfunction in people including spermatogenic disorders. Enhanced oxidative stress and changes in antioxidant capacity have important roles in the pathogenesis of chronic diabetes mellitus. Previous studies have demonstrated that pioglitazone treatment could reduce superoxide radical generation in different tissue types. The aim of present study was to examine the antioxidative effect (SOD levels) of pioglitazone in diabetic rats.
**Methods:** Induction of experimental diabetes was done using single intraperitoneal injection of streptozotocin dissolved in citrate buffer (pH 4.5) at the dose of 65 mg/kg to overnight fasted rats. Only rats with blood glucose concentrations above 250 mg/dl were considered as diabetic. Animals were randomly divided into four groups of eight rats: control group, diabetic group and treated with low or high doses of pioglitazone of 1 or 10(mg/kg/day, orally) for 5 weeks. Mice were euthanized on day 35 and testicular SOD (Superoxide dismutase) activity was estimated following the method described by Kono. One unit of SOD is described as the amount of enzyme required to cause 50% inhibition of pyrogalol auto-oxidation at alkaline pH.
**Results:** Diabetic animals treated with pioglitazone showed the activity of anti-oxidative enzyme. Pioglitazone had enhanced the level of SOD. The improvement in the level of SOD indicated that pioglitazone could has either increased the proliferation of the antioxidant enzyme or reduced the formation of ROS.
**Conclusion:** Pioglitazone could reduce excessive production of ROS with a resulting decrease in the anti-oxidative defense.

**Keywords:** Diabetes, Pioglitazone, Superoxide dismutase, Testicular damage

ICIA2018\Reproductive Immunology\Poster\7884
**7884**

# Sjögren's Syndrome Related Infertility: Case Report in Iran

Fatemeh Rezayat[1], Sadegh Feyzollahzadeh[2], Abbas Rezaei[1]

1. *Department of Immunology, School of Medicine, Isfahan University of medical Science, Isfahan, Iran*
2. *Department of Immunology, School of Medicine, Urmia University of medical Science, Urmia, Iran*

**Background:** Infiltration in the glands. The etiology of Sjögren's syndrome has been remained unknown. The chronic immune system stimulation mediators may play an important role in the pathogenesis of this disorder. This includes various autoantibodies, especially anti-Ro/SS-A and anti-La/SS-B. These autoantibodies are involved to immunopathogenesis of infertility and recurrent spontaneous abortion. Moreover high levels of antinuclear antibodies in pregnant women may induce fetus rejection. Most individuals with Sjögren's syndrome have systemic symptoms, such as dry eyes, xerostomia (dry mouth) and parotid gland enlargement. Primary Sjögren's syndrome occurs in the absence of the other rheumatic disorders; however, secondary Sjögren's syndrome is associated with rheumatic disease, such as SLE, rheumatoid arthritis, or scleroderma.
**Methods:** A 42 years old woman with 10 years history of infertility referred to Dr. Rezaei's Immunological Laboratory. Routine and related immunological tests were performed and the presence of antinuclear antibodies (ANA) was detected. ANA profile test determined the reactivity of these autoantibodies with nuclear Ro/SS-A antigen. Other known causes of infertility and/or miscarriage were rule out by specific tests such as lupus anti-coagulant, Antiphospholipid antibodies, complement regulatory molecules (CD55, CD46 and CD59) and CD16/56.
**Results:** Moreover the infertility associated infections such as HIV, HCV, HTLV, CMV, Toxoplasma and rubella were negative. Symptoms of Sjögren's syndrome such as dry eyes with foreign body sensation, xerostomia and facial erythema confirmed.
**Conclusion:** According to these results this patient may be suffering from an early (Rheumatoid factor (RF) negative) primary Sjögren's syndrome and her infertility may be the cause of high levels anti-Ro/SS-A auto-antibodies.

**Keywords:** Sjögren's syndrome, Antinuclear antibody, anti-Ro/SS-A, Infertility.

ICIA2018\Reproductive Immunology\Poster\7915
**7915**

# Effect of Cyclosporine on Th1 and Th2 Lymphocytes and Improvement of Pregnancy Outcome in Recurrent Pregnancy Loss (RPL)

Ramyar Azizi[1], Majid Ahmadi[2], Mehdi Yousefi[3]

1. *Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran*
3. *Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Recurrent pregnancy loss (RPL) occurs in approximately 2-5% of clinically recognized pregnancies in reproductive-aged women. Cyclosporine is an immunosuppressant medication and decrease function and frequency of lymphocytes. In the present study, the effect of cyclosporine treatment on Th1/Th2 cell ratio and function is compared between cyclosporine treated and untreated RPL patients.
**Methods:** A total of 129 patients with a history of RPL were enrolled in this study. The frequency of Th1 and Th2 lymphocytes and the expression of related transcription factors were assessed by flow cytometry and real-time PCR respectively. 76 patients that have increased Th1/Th2 cell ratio selected for this study. 38 patients since the β-HCG test has been positive received oral CsA three times a day in a dose of 50mg for 6 months. Other 38 RPL patients were included as no CsA interfering control group. The dosage of CsA sustained between 40-80 ng/ml. flow cytometry and real-time PCR Tests were accomplished in both group before and after 32 weeks treatment.
**Results:** In the treated groups Th1 cells population and function down-regulated but for Th2 up-regulated also T-bet transcription factor down regulated and GATA3 transcription factor up regulated. Th1/Th2 cell ratio decreased in the treated group compared with untreated group after 32 weeks (p value = 0.0001). 31 cases from 38 patient have successful Pregnancy outcome in the treated group (81.5%) compared with 16 cases from 38 patients (42.1%) in the untreated RM women.
**Conclusion:** During pregnancy administration of CsA in RPL women with increased Th1/Th2 cell ratio, raises frequency and function of Th2 and decreases in Th1 as well as influences Th1/Th2 ratio in peripheral blood. These results indicate that CsA can be an effective treatment strategy for RPL women with immunological abnormality.

**Keywords:** Recurrent pregnancy loss, Cyclosporine,Th1, Th2

ICIA2018\Reproductive Immunology\Poster\7933
**7933**

# Investigating the presence of autoantibodies against Glucose Regulated Protein78 (GRP78) in the sera of women with Recurrent Pregnancy Loss

Behrouz Gharesi fard[1, 3], Mohammad Hossein karimi[2], Leila Bidar L[3], Mohammad Ali-Hasanzadeh [1]
Moslem Ahmadi [1]

1. *Department of Medical Immunology, School of Medicine, Shiraz university of Medical sciences, Shiraz, Iran*
2. *Organ transplant research center, Shiraz University of Medical Sciences, Shiraz, Iran*
3. *Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Recurrent pregnancy loss (RPL) is defined as 3 or more abortion prior to 20th week of pregnancy, and occurs in 15-20 % of all pregnancies. Glucose Regulated Protein78 (GRP78) is an endoplasmic reticulum(ER) protein which is expressed on the trophoblast cells. The purpose of this study was to evaluate the presence of anti-GRP78 antibodies in sera of women with RPL.
**Methods:** In a case-control study, 38 women with unexplained RPL as patients group and 42 healthy pregnant women with no history of abortion as control group who referred to the Infertility Center, Shiraz University of Medical Sciences were selected. In the present study, we evaluated the presence of anti-GRP78 antibody in the sera of RPL patients and healthy pregnant women using a Lab ELISA assay. Furthermore, western blot technique was used to confirm the expression of GRP78 by placental tissues.
**Results:** We detected the presence of anti-GRP78 antibody in both healthy and patient women by ELISA technique. However no significant difference regarding the presence of anti-GRP78 antibody levels between patients and healthy women was observed *(P* value= 0.1)
**Conclusion:** The results of this study did not shown a significant relationship between anti-GRP78 antibody level and susceptibility to unexplained RPL.

**Keywords:** Pregnancy, RPL, Placenta, GRP78

ICIA2018\Reproductive Immunology\Poster\7960
**7960**

# Human amniotic epithelial cells (hAECs) regulates NK cells activation in women with recurrent spontaneous abortion (RSA)

Fahimeh Khadem[1], Nafiseh Esmaeil[1], Abbas Rezaei[1], Faezeh Abbasi Rad[1] , Behnaz khani[2]

*1. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
*2. Department of Obstetrics and Gynecology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*

**Background:** Unexplained Recurrent Spontaneous Abortion (URSA) is the most common immunological complication during pregnancy. In recent years, it has been found that the cells such as human amnion epithelial cells (hAECs) have the potency to modulate immune responses in vitro and in vivo. However, the immunomodulatory effect of hAECs on NK cells in women with URSA is largely unknown.
**Methods:** Blood mononuclear cells (PBMCs) were obtained from 14 URSA patients and co-cultured with isolated hAECs. NK cells were identified using anti-CD56 and anti-CD3 monoclonal antibodies (mAb).The expression of the activating receptor CD69 and the degranulation marker CD107a on NK cells was detected using specific mAb and analyzed by flow cytometry.
**Results:** In this study, we demonstrated that CD69+ activating receptor expression on NK cells was significantly decreased by incubation with hAECs in a dose-dependent manner. Also, the degranulation marker CD107a was significantly downregulated on NK cells following incubation with hAEC.
**Conclusion:** Our results suggest hAECs have immune regulatory effects on activation and cytotoxicity of NK cells. Potential therapeutic application of hAECs for dysregulated NK immunity should be investigated in the future.

**Keywords:** Human amnion epithelial cells (hAECs), Unexplained recurrent spontaneous abortion (URSA), Immunomodulation, Natural killer cell.

ICIA2018\Reproductive Immunology\Poster\7993
**7993**

# Altered frequencies of $CD4^+CD25^+Foxp3^+$ and $CD8^+CD25^+Foxp3^+$ regulatory T cells in pre-eclampsia

Maryam Zare[1], Mehrnoosh Doroudchi[1], Behrouz Gharesi Fard[1,2], Masha Hoseini[1]

1. *Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Regulatory T cells are of utmost importance for tolerating the fetus during a normal pregnancy. In some pregnancy complications such as pre-eclampsia, the frequency of regulatory $CD4^+CD25^+Foxp3^+$ regulatory T cells is altered, but there is no consistency regarding the results. Besides, little is known about the frequency of $CD8^+CD25^+Foxp3^+$ Treg cells in pregnancy complications. We aimed to investigate the frequency of both $CD4^+$ and $CD8^+$ regulatory T cells in the peripheral blood of women afflicted by pre-eclampsia.
**Methods:** Ten non-pregnant, ten healthy pregnant, and ten preeclamptic women participated in this study. Four colors flow cytometry method was used to identify the frequency of the $CD4^+$ and $CD8^+$ regulatory T cells in the peripheral blood.
**Results:** Results indicated that the frequencies of $CD4^+CD25^+Foxp3^+$ and $CD8^+CD25^+Foxp3^+$ cells were significantly lower in preeclamptic women compared to healthy pregnant and non-pregnant ones ($p < 0.05$). A positive correlation was also observed between $CD4^+$ and $CD8^+$ regulatory T cells ($R= 0.532$, $p = 0.002$). Moreover, $CD4^+$ regulatory T cells negatively correlated with systolic and diastolic blood pressures ($R= -0.760$ and $-0.753$, respectively; p values $< 0.001$). $CD8^+$ regulatory T cells also had negative correlations with systolic ($R= -0.503$, $p = 0.001$) and diastolic ($R= -0.590$, $p = 0.005$) blood pressures.
**Conclusion:** In conclusion, a reduction in the frequencies of both $CD4^+CD25^+Foxp3^+$ and $CD8^+CD25^+Foxp3^+$ regulatory T cells might be important in the pathogenesis of pre-eclampsia.

**Keywords:** $CD4^+CD25^+Foxp3^+$ regulatory T cells, $CD8^+CD25^+Foxp3^+$ regulatory T cells, Pre-eclampsia, Pregnancy

ICIA2018\Reproductive Immunology\Poster\8020
**8020**

**The effects of maternal immune system deviation on development of the neonate's spleen**

Mohammad Khosravi[1], Kaveh Khazaeel [2], Mahmoud Khaksary Mahabady[2], Mohammad Amin Behmanesh[3], Shokooh Salimi Moghadam[4]

1. *Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran*
2. *Department of Basic sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran*
3. *Department of histology, Faculty of medicine, University of Dezful, Dezful, Iran*
4. *Student of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran*

**Background:** Various organs derived from fetal stem cell's development; these processes are highly dependent on the endometrial niches. Circulating hormones and cytokines are released mostly, by maternal and fetal immune system. This experiment evaluated the effects of maternal T lymphocytes deviation on development of the neonate's spleen.
**Methods:** Total of the 20 female rats divided in 4 groups (L, S, T and C). The L and S groups were treated using *Leishmania major* and *Salmonella typhimurium*, respectively. Tacrolimus (2mg/kg/day) orally administrated to group T in continuous 5 days. The group C, as control, treated using sterilized normal saline. The animals were mated after 3 times treatments at 2 week intervals. The newborns were scarified, according to animal's welfares, at one and two months of age. The total body and spleen weight of the neonates were determined; the prepared samples were analyzed using routine histological methods.
**Results:** The neonates of the group S showed significant reduction ($P \leq 0.05$) on the spleen's average weight at two months of age; also, the mentioned group had a significant elevation on the white pulp's number than the other groups. The neonates of the groups L and T showed no significantly difference on weight or pulps number, despite the higher levels than group C.
**Conclusion:** Imbalanced responses of the Th1<Th2, even occurrences before pregnancy, have significant effects on development of the neonate's spleen.

**Keywords:** Maternal, Neonate, Immune system, Spleen.

ICIA2018\Reproductive Immunology\Poster\8021
**8021**

**Evaluation of the effects of maternal immune response deviations on neonate's immune response**

Mohammad Khosravi[1], Mahmoud Khaksary Mahabady[2], Kaveh Khazaeel [2], Shokooh Salimi Moghadam[3]

1. *Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran*
2. *Department of Basic sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran*
3. *Student of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran*

**Background:** Development of the fetal immune system originated from stem cells expansion. The maternal circulating hormones and cytokines, which are released mostly by immune system components, may be transferred to uterine cavity and alter the endometrial niches. This experiment evaluated the effects of maternal immune response deviations on neonate's immune responses.
**Methods:** Total of the 20 female rats divided in 4 groups (L, S, T and C). The L and S groups were treated using *Leishmania major* and *Salmonella typhimurium*, respectively. Tacrolimus (2mg/kg/day) orally administrated to group T in continuous 5 days. The group C, as control, treated using sterilized normal saline. The animals were mated after 3 times treatments at 2 week intervals. The newborns were treated by injection of sheep Red Blood Cells (SRBC) at 4 weeks of age. The neonate's humoral immune responses to maternal treated antigens and SRBC were evaluated at 5 weeks of age using micro-agglutination test.
**Results:** The neonates of S and L groups have detectable antibodies titer against *S. typhimurium* and *L. major* respectively. Also, the neonates of S and T groups showed significant reduction ($P \leq 0.05$) on the anti-SRBC antibodies titer.
**Conclusion:** Stimulation of the Th2 and suppression of the immune responses, even occurred before pregnancy, have significant effects on neonate's immune responses.

**Keywords:** Maternal, Neonate, Immune response, Rat.

ICIA2018\Reproductive Immunology\Poster\8258
**8258**

# Comparison of the therapeutic effect of syngeneic, allogenic, and xenogeneic mesenchymal stem cells on the reduction of abortion rates in murine model

Fatemeh Rezaei, Seyed Mohammad Moazzeni

*Immunology Department, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

**Background:** Introduction: mesenchymal stem cells (MSCs), due to their immunomodulatory functions, are an ideal candidate for the treatment of immune related diseases. Recurrent spontaneous abortion (RSA) is one of the most common complications of pregnancy which in many cases is related to the immune system disorders. Our previous study has shown that syngeneic MSCs therapy could reduce the abortion rate in abortion prone mice. In this study the therapeutic effect of syngeneic, allogenic, and xenogeneic MSCs were compared in a mouse model of RSA.
**Methods:** MSCs were isolated from adipose tissue of CBA/j, BALB/c mice and human. After characterization, MSCs were injected (IP) at day 4 of gestation to female CBA/J mice following their mating with DBA/2 male. In control group PBS was injected and CBA/J x BALB/c mating was also used as normal pregnancy control. On day 14.5 of pregnancy embryo resorption rate was determined.
**Results:** All syngeneic, allogeneic, and xenogeneic MSCs therapy reduced the abortion rates compared to MSCs non-treated group, however the therapeutic potential of xenogeneic MSCs was less than syngeneic and allogeneic MSCs in this model.
**Conclusion:** Intraperitoneal administration of MSCs from various source could significantly reduce the abortion rate in abortion prone mice, this results indicates that the immunogenicity of allogeneic and xenogeneic MSCs is not a contradictory problem for their therapeutic effects on RSA.

**Keywords:** Recurrent spontaneous abortion, mesenchymal stem cells, syngeneic MSC, allogenic MSC, and xenogeneic MSC

ICIA2018\Reproductive Immunology\Poster\8324
**8324**

# The effect of leukemia inhibitory factor (LIF) on gene expression of vascular endothelial growth factor A (VEGF-A) in trophoblast tumor Cell Line (JEG-3)

Khodakaram Jahanbin[1], Mehri Ghafourian [1*], Mohammad Rashno[1], Afshin Amari[1], Iman Naamipouran[1],

*1. Department of Immunology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

**Background**: Several studies have shown that leukemia inhibitory factor (LIF) one of the most important cytokines in the process of embryo implantation and pregnancy, but so far the role of this cytokine in angiogenesis process have not been fully investigated. Our goal in this study is to evaluate the effect of LIF on gene expression of vascular endothelial growth factor (VEGF) in choriocarcinoma cell line (JEG-3) as a trophoblast cells.
**Methods**: JEG-3 choriocarcinoma cells stimulated with different concentrations of LIF for 6, 12, 24, 48 and 72 hours. Expression of VEGF analyzed by real-time PCR.
**Results**: In stimulated cells, different concentrations of LIF caused the significant decrease of VEGF gene expression ($p<0.05$) at 12, 24 and 48 hours but in 72 hours there was an increase in VEGF gene expression and at 6 hours at concentrations of 1 and 10 ng, there was also an increased expression of the gene, but the expression of gen reduced at 50 ng concentration.
**Conclusion**: Gene expression of VEGF in trophoblast cells treated with LIF decreased, indicating that this cytokine plays an important role in angiogenesis in placenta. Therefore, LIF may have effects in pregnancy-related disorders in related to angiogenesis.

**Keywords**: JEG-3, leukemia inhibitory factor, trophoblast, VEGF-A.

ICIA2018\Veterinary Immunology\Poster\5264
**5264**

# Survey Epidemiological of Anthrax in Kermanshah Province, West Iran During 2010- 2015

Pooyanmehr Mehrdad[1], Momeni Mohamad[2]*, Zangeneh Mohamad Mahdi [2]

1. *Department of Pathobiology, microbiology section, Faculty of Veterinary Medicine, Razi University,*
2. *Veterinary Student, Faculty of Veterinary Medicine, Razi University*

**Background and Aim:**Zoonotic diseases constitute a public health problem throughout the world. Anthrax is a potentially fatal zoonotic disease and is an important zoonotic disease which is primarily a job-related infection caused by Bacillus anthracis. The diagnosis of cutaneous anthrax (CA) may be very difficult, particularly in atypical presentations and non-endemic regions. Aim this study, anthrax cases seen between 2010 –2015 (six years) in Kermanshah Province.
**Materials and Methods:** Medical records of infected in patients with CA (Clinical, Para clinical information and pathological diagnosis of disease CA such as microbiologic procedures) who had been operated in referral all Health Centers Kermanshah Province were collected in the period six years (2010 – 2015). Twenty-five patients with CA were included in this study.
**Results:** Results indicated that All patients' referent Twenty-five patients were who diagnosed CA in medical center registries, 2010(3case), 2011 (4case), 2012 (7case), 2013 (7case), 2014(3case) and 2015(1case) patient) at six years' period (0,025%), per 100,000 persons respectively. Each Twenty-five patients with a diagnosis of CA were followed up. All patients had a history of animal contact. The clinical presentation of CA was typical in all patients were initially misdiagnosed with insect bites or angioedema. Cultures from the lesions were positive for *Bacillus anthracis* in Twenty-five cases. Gram stain from the lesions revealed Gram-positive rods in 25 cases. Patients were diagnosed by clinical presentation and a history of contact with sick animals or contaminated animal products.
**Conclusion:** CA is a very contagious and important infectious disease worldwide. Early and accurate diagnosis dramatically affects the prognosis of the disease. The diagnosis of CA may be difficult, especially in atypical presentations and non-endemic areas. Thus, CA should be kept in mind, especially in these situations.

**Keywords:** Bacillus anthracis, Zoonotic diseases, Kermanshah Province

ICIA2018\Transplantation\Poster\5266
**5266**

# Gene Expression patterns of Toll Like Receptor (TLR)-2, TLR-4 and (MYD88) in renal transplant patients developing allograft dysfunction; A cohort study

Morteza Hosseinzadeh[1], Pedram Ahmadpoor[2], Mir Saeed Yekaninejad[3] , Farshad Foroughi [4], Mehri Barabadi[5], SanazKeshavarz shahbaz[5], Ehsan Soltaninejad[5], Aliakbar Amirzargar[6]

*1. Department of Immunology, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran*
*2. Department of Nephrology, Shahid Labbafi-nejad Medical Center and Chronic Kidney Disease Research Center, University of Shahid Beheshti, Tehran, Iran*
*3. Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
*4. Department of Immunology, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran*
*5. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
*6. Molecular immunology research centre, Tehran university of medical sciences, Tehran, Iran*

**Background:** Innate immune responses play an essential role in the development of (IRI) and subsequent impaired graft function. This cohort intends to determine the sequential dynamic changes in (TLR)-4, TLR-2, and (MYD88*)* mRNA expressions in (PBMCs) and biopsy samples from kidney allograft recipients in relation to graft function within the first year post-transplant.
**Methods:** This study enrolled 52 renal transplant patients, 27 with well graft function (WGF) and 25 with graft dysfunction (GD). We collected peripheral blood samples from participants, pre- and post-transplantation (days 2, 90 and 180) in order to analyze mRNA expression levels of the TLR-2, TLR-4, and MYD88 genes in relation to allograft function during a one-year follow up period. Expression patterns of these molecules were evaluated in samples obtained by either protocol biopsy (PB,n=24) or cause biopsy (n=6) at the sixth month post-transplantation.
**Results:** There were significantly higher mean dynamic changes of post-transplant TLR-2, TLR-4, and MYD88 mRNA expressions in GD patients compared to WGF patients (P=0.001). (ROC) curve analysis based on (GFR) index showed the (AUC) values for the genes: TLR-2 (0.89; P<0.001), TLR-4 (0.86; P<0.001), and MYD88 (0.75; P=0.003) in the third month after transplantation for a GD diagnosis. Similar analyses showed the following AUC values at six months post-transplantation: TLR-2 (0.73; P=0.006), TLR-4 (0.73; P=0.007), and MYD88 (0.70; P=0.02). Also, the calculated AUCs for the expressions of these genes in allograft biopsies were 0.94 (TLR-2), 0.95 (TLR-4), and 0.98 (MYD88) in the sixth month post-transplant based on the pathology report (P<0.001).
**Conclusion**: Sequential monitoring of the expression patterns of TLR-2, TLR-4, and MYD88 in PBMCs as well as in biopsy samples could be considered predictive biomarkers for early and late kidney allograft outcomes.

**Keywords**: Kidney transplantation, acute rejection, Toll like receptors, Allograft Outcome.

ICIA2018\Transplantation\Poster\5267
**5267**

# Increased Expression of Toll-Like Receptors 2 and 4 markers on Peripheral Blood Mononuclear Cells (PBMC) in Renal Transplant Recipients that Develop Allograft Dysfunction

Morteza Hosseinzadeh[1], Mohsen Nafar[2], Pedram Ahmadpoor[2], Mir Saeed Yekaninejad[3], Mohammad Hossein Niknam[1,4], Aliakbar Amirzargar[1,4]

*1. Department of Immunology, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran*
*2. Chronic Kidney Disease Research Center, Shahid Beheshti University of Medical Sciences*
*3. Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
*4. Molecular Immunology Research Center, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** The incidence of ischemic reperfusion injury (IRI) in early phase post transplantation and activation of toll-like receptor (TLR-2) and TLR-4 remarkably impacts the outcome of a renal allograft. To investigate whether the expression of TLRs in peripheral blood mononuclear cells (PBMCs) can predict the clinical outcome of kidney allografts.
**Methods:** We obtained blood samples from 52 renal transplant patients before transplant, and 2, 90, and 180 days post-transplantation in order to analyze the surface expressions of TLR-2 and TLR-4 on peripheral blood monocytes. The expression patterns of TLR-2 and TLR-4 were compared between patients with graft dysfunction (GD) and those with well-functioning graft (WFG).
**Results:** Significantly different mean dynamic changes in surface expression of TLR-2, according to percentage of TLR-2+ cells, between (the GD and WFG) groups existed at most time-points before and after renal transplantation (p=0.007) with the exception of day 2 post-transplantation. We observed significantly higher mean fluorescence intensities of TLR-2 and TLR-4 on CD14+ cells in the GD group compared to the WFG group. This finding was particularly observed 180 days post-transplantation (p=0.001). Based on TLR-2 and TLR-4 protein expression for each step, multiple logistic regression and ROC curve analysis revealed that an increase in CD14+ TLR-2+ monocytes within the 90 days post-transplantation was associated with increased risk of GD at 180 and 365 days post-transplantation [odds ratio (OR)=1.27, p=0.005)].
**Conclusion:** Sequential monitoring of TLR-2 and TLR-4 expression patterns in peripheral blood monocytes appear to be prognostic and predictive biomarkers for early and late kidney allograft outcomes.

**Keywords:** Allograft Function, Kidney Transplant, Toll-Like Receptors

ICIA2018\Vaccine\Poster\5271
**5271**

# Predicted B- and T-cell epitopes analysis of the genes encoding the malaria vaccine candidate, *Plasmodium falciparum* Cell Traversal Protein for Ookinetes and Sporozoites

Sakineh Pirahmadi[1], Sedigheh Zakeri[1*], AkramAbouie Mehrizi[1], NavidDinparast Djadid[1]

*1. Malaria and Vector Research Group (MVRG), Biotechnology Research Center (BRC), Pasteur Institute of Iran, Tehran, Iran, Pasteur Avenue, P.O. Box 1316943551, Tehran, Iran*

**Background:** Malaria still remains one of the most prominent public health problems. In modern malaria elimination and eradication strategies, a great deal of attention has been paid to the development of novel intervention strategies such as an effective malaria vaccine. However, one of the major concerns in malaria vaccine development is the antigenic diversity of the vaccine candidate antigens, since antigenic diversity limits the efficacy of acquired protective immunity to malaria. Cell-traversal protein for ookinetes and sporozoites (CelTOS) is a promising malaria vaccine candidate. Therefore, in the present investigation the antigenic diversity of CelTOS in global isolates of *P. falciparum* was assessed and the predicted immunodominant regions in PfCelTOS in relation to polymorphisms were analyzed.
**Methods:** The extent of sequence diversity of the *pfceltos* were assessed among both natural *P. falciparum* isolates collected from endemic areas of Iran (n = 93) and available global *pfceltos* sequence data retrieved from Plasmodb database (n = 159). Furthermore, PfCelTOS structure analysis in relation to antigenicity was done by using *in silico* approaches.
**Results:** The present results revealed that low-level diversity were observed in *pfceltos*gene. Also, most of the predicted B- and T-cell epitopes were located in the conserved region of the gene.
**Conclusion:** The present analysis demonstrates that there is a limited antigenic diversity in global PfCelTOS and most of the detected polymorphisms are located on C-terminal region. Also, most of the predicted B- and T-cell epitopes were located in the conserved region of the gene, but the limited detected polymorphisms in these regions should be considered for evasion of *P. falciparum* from immune responses in CelTOS-based vaccine. This study is the first investigation on the antigenic diversity of PfCelTOS, and the obtained results could provide knowledge for a better design of PfCelTOS-based malaria vaccine.

**Key words:** *Plasmodium falciparum*, CelTOS, immunodominant epitope, vaccine

ICIA2018\Research and Development\Poster\5323
**5323**

# Effects of Monochromatic Different Colored Lights on Melatonin Secretion from in vitro Peripheral Blood Mononuclear Cells, Polymorphonuclear Cells and Whole Blood Cultures

Mehrnoosh Zagheh,[1] Rostam Golmohammadi,[2] Mahdi Alahgholi Hajibehzad[1,3], Roya Najafi Vosough,[4] Zohre Zaerieghane,[1] Alireza Zamani[1,3,*]

*1. Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.*
*2. Department of Occupational Hygiene, School of Health, Hamadan University of Medical Sciences, Hamadan, Iran*
*3. Molecular Immunology Research Group, Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
*4. Department of Biostatistics, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran*

**Background:** The melatonin is mainly produced by pineal gland and controls the circadian rhythms. Recent studies have shown melatonin is also secreted from peripheral blood mononuclear cells (PBMCs). Therefore, we investigated whether melatonin could secrete from polymorphonuclear cells (PMNs) and then measurement monochromatic different colored lights effect on in vitro production of melatonin by PBMCs, PMNs and whole blood cultures.
**Methods:** Blood samples were obtained from an apparently healthy volunteer. PBMCs and PMNs were isolated by ficoll-paque and dextran solutions respectively. PBMCs, PMNs and whole blood cultures were exposed to white, red, blue and green lights (100 lux) and to darkness in with/without of phytohemagglutinin (PHA, 50 µg/ml) and phorbol myristate acetate (PMA, 10 ng/ml) activation for 8 hours. The melatonin secretion in the supernatants was measured by Enzyme-Linked Immunosorbent Assay (ELISA). Data were analyzed by multi-factorial, Kruskal-Wallis and Mann-Whitney tests.
**Results:** Results indicated in addition to PBMCs, PMNs also produce melatonin. The level of melatonin in the supernatant of PBMCs, PMNs and whole blood cultures was increased due to PHA (134.3± 29.31pg/ml) and PMA (141.1± 31.4 pg/ml) activation comparing with no activated (92.30± 22.5 pg/ml) ($P<0.001$) .The monochromatic different colored lights have no effect on the melatonin production by PBMCs, PMNs and whole blood cultures.
**Conclusion:** Conclusion: It concluded PBMCs, PMNs and whole blood cells produce melatonin in vitro and mitogens activation caused to higher production. Moreover, although white light suppressed melatonin secretion but there was not difference between various types of lights.

**Keywords:** Peripheral Blood Mononuclear Cells, Polymorphonuclear Cells, Whole Blood, Melatonin, Light.

ICIA2018\Vaccine\Poster\6335
**6335**

# CpG ODN and MPL are more efficient adjuvants than naloxone for induction of Th1 immune response against *plasmodium vivax* recombinant TRAP in C57BL/6 mice

Saeed Nazeri[1], Sedigheh Zakeri[1], Akram Abouie Mehrizi[1], Navid Dinparast Djadid[1]

*1. Malaria and Vector Research Group (MVRG), Biotechnology Research Center (BRC), Pasteur Institute of Iran, Tehran, Iran*

**Background**: *Plasmodium vivax* is the major cause of malaria outside Sub-Saharan Africa and it has been indicated that the elimination of *P. vivax* is often technically challenging. A key tool for the control, elimination and eradication of *P. vivax* is the development of a broadly protective vaccine. The thrombospondin-related adhesion protein (TRAP) is one of the major sporozoite antigens that plays an important role in the invasion of mosquito salivary glands and hepatocytes by sporozoites and it is a promising malaria vaccine candidate. The main aim was to investigate the role of antibodies and cellular immune responses induced by purified recombinant PvTRAP delivered in individual adjuvants, naloxone (NLX), CpGoligodeoxynucleotides ODN1826 (CpG ODN), and 3-O-decylated monophosphoryl lipid A (MPL), in immunized C57BL/6 mice.
**Methods**: The recombinant PvTRAP alone or combined with NLX, MPL or CpG adjuvants were applied for immunization of mice. IgG, IgG1, IgG2b, IgG2c, and IgG3 antibody responses as well as the IFN-γ and IL-4 cytokines were determined in post-immunized mouse plasma.
**Results**: The significant highest level of anti-rPvTRAP IgG, IgG2b and IgG2c were identified in the groups received rPvTRAP/MPL (mean $OD_{490}$ nm: 2.41, 1.50, 1.30, respectively) and rPvTRAP/CpG (mean $OD_{490}$ nm: 2.40, 1.44 and 1.27 respectively) in comparison to the group received rPvTRAP/NLX (mean $OD_{490}$ nm: 2.01, 1.40, 1.158, respectively) ($P< 0.05$, independent sample *t*-test). Also, mice receiving rPvTRAP/MPL (2164 pg/ml) and rPvTRAP/CpG (2029 pg/ml) induced significantly the higher levels of IFN-γ than mice receiving rPvTRAP/NLX (1615 pg/ml) ($P< 0.05$, independent sample *t*-test) and no detectable IL-4 production in all mouse groups.
**Conclusion**: The present result revealed that the administration of rPvTRAP with CPG and MPL induced higher Th1 immune response than NLX adjuvant in mice and had more potential to increase the level of anti-TRAP antibodies as well as the high production of INF-γ.

**Keywords:** *Plasmodium vivax*, TRAP, Adjuvant, Vaccine

ICIA2018\Veterinary Immunology\Poster\7353
**7353**

# Evaluation of some of the factors associated with immune system following effect of magnetic field in rats

Amin Lafzi[1], Mansour Khakpur[2]

1. *Dvm, Veterinary Medicine Clinician , Tabriz ,Iran*
2. *Department Of Pathobiology, Tabriz Branch, Islamic Azad University, Tabriz, Iran*

**Background:** This study was designed to investigate the effects of electromagnetic fields (EMF) uniformly on the immune system of 12 Norwegian male Wistar rats were used as experimental model.
**Methods**: These rats were divided into three control, treatment one and treatment two groups. The first treatment group was subjected to electromagnetic field for four hours and fifteen days. The second group received this treatment for eight hours and fifteen days. At the end of the fifteenth day, blood samples from rats were taken and were prepared to be analyzed. Then, blood cells were counted and after conducting biochemical tests, the data were analyzed using SPSS computer application, one-way ANOVA and Tukey range tests.
**Results:**The results revealed that the level of WBC increased in the first experimental group compared to control group; yet, it decreased in the second experimental group. The level of total protein relatively decreased compared to control group; however, level of albumin decreased significantly and at the same time Gamma globulin increased.
**Conclusion**: Eventually, the increase in the number of lymphocytes and decrease in the number of neutrophils and monocytes are the most significant effects of electromagnetic field on immune system.

**Keywords:** Immune system; Magnetic field; Rats.

ICIA2018\Tolerance and Autoimmunity\Poster\7413
**7413**

# Preserving Th17/Treg Balance to be Considerable in the Pathogenesis of Behçet's Disease

RaminPourakbari[1,2], Leili Aghebati-Maleki[1,2,3], Mehdi Yousefi[1,2*]

1. *Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
3. *Immunology research center, Tabriz University of Medical Sciences, Tabriz, Iran*

**Background:** Behçet's disease (BD) is a systemic inflammatory disorder with a various range of clinical appearances, containing mucocutaneous, ocular, vascular, gastrointestinal, musculoskeletal, and central nervous system contribution. The BD pathophysiology is remarkable as it shares several features with both autoimmune and inflammatory syndromes, perhaps prompted by infections or additional environmental factors. In this study, we aimed to assess Th17 and Treg cell frequency, cytokine secretion, and expression of transcription factors relevant to Th17 and Treg cells of patients with BD.
**Methods:** Blood samples from 47 patients with Behçet's disease and 58 healthy individuals were taken, and then PBMCs were isolated by ficoll separation method. The frequency of Th17 and Treg cells were evaluated by flow cytometry. Then, the expression of related transcription factors were assessed by real-time PCR. Also, the serum levels of associated cytokines were determined using ELISA Technique.
**Results:** The ratio of Treg frequency and levels of IL-10, FOXP3 expression were significantly decreased in BD patients. In contrast, the proportion of Th17 was significantly increased accompany with increase in the levels of IL-17, IL-23, and RORγt expression in patients with BD.
**Conclusion:** These studies suggest that, the increase in ratio of Th17 cells and decrease in Treg cells might collaborate to the pathogenesis of BD. Therefore, controlling balance between Tregs and Th17 could be useful for the treatment of the BD patients.

**Keywords:** Behçet's disease; Peripheral blood; Treg; Th17

ICIA2018\Transplantation\Poster\7433
**7433**

# Association of Delayed Graft Function with serum levels of soluble Fibrinogen-like protein 2 in patients with kidney transplantation

Ali Salmani [1], Majid Faridgohar [2], JavadAbsari [3], Hassan Nikoueinejad[*4], Hossein Akbari [5], BehzadEinollahi[6]

*1. Medical Student; Student Research Committee, Kashan University of Medical Sciences, Kashan, Iran*
*2. Master Student; Department of Microbiology and Immunobiology, Kashan University of Medical Sciences, Kashan, Iran*
*3. Master Student; Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
*4. Medical doctor & Immunologist; Nephrology and Urology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*
*5. Statistician; Trauma Research center, Department of Community medicine, Kashan University of medical sciences, Kashan, Iran*
*6. Nephrologist; Nephrology and Urology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*

**Background:** Delayed graft function (DGF) is an inflammatory complication of kidney transplant which means needing dialysis throughout the first week after transplantation. Considering the effects of regulatory T cells (Tregs) on DGF, we aimed to ascertain the relationship between soluble Fibrinogen-like protein 2 (sFGL-2), as Tregs' secretory factor, and DGF after kidney transplantation.
**Methods:** This Nested case-control study was done in a 6-month period among 2 groups of each 58-member of kidney transplanted patients with and without DGF. sFGL-2 serum level in all blood samples was measured by Elisa.
**Results:** The serum level of sFGL-2 in recipients with DGF was significantly higher than that in those without DGF ($P<0.001$). There was a strong meaningful correlation between the serum level of sFGL-2 and the risk of GDF ($P<0.001$). We found no significant effects of different variables of rejection, HLA mismatch, type of medication, family relation between recipient and donor, age, and sex on such correlation.
**Conclusion:** Our study showed a significant relationship between sFGL2 and DGF. Therefore, plasma levels of sFGL2 may be used as a diagnostic tool to determinate the risk of DGF.

**Key words:** Kidney Transplantation, Delayed Graft Function (DGF), Soluble Fibrinogen-Like protein 2 (sFGL-2)

ICIA2018\Tolerance and Autoimmunity\Poster\7434
**7434**

# The Increased Expression of Integrin β7 Gene in Peripheral Blood Leukocytes of the Newly Diagnosed Rheumatoid Arthritis Patients

Mahdi Taghadosi[1], Adel Fekri[1], Parisa Zafari[2], Nasrin Iranshahi[1]

1. *Department of immunology, school of medicine, Kermanshah University of Medical Sciences*
2. *Department of immunology, school of medicine, Mazandaran University of Medical Science*

**Background**: Integrins and chemokines play critical role in Lymphocytes and leukocytes migration and recirculation during autoimmune disorders. Normally gut-homing T cells express CCR9 and integrin β7 in order to home back to lamina propria of intestine from peripheral blood. Our study was conducted to evaluate expression levels of the chemokine receptor CCR9 and the integrin β7 in circulating leukocytes and lymphocytes from newly diagnosed Rheumatoid arthritis (RA) patients and compare these values with the same data obtained from healthy individuals without inflammatory disease.
**Methods**: Total RNA was extracted from peripheral blood samples immediately cDNA was synthesized and finally gene expression of CCR9 and β7 evaluated by quantitative Real time PCR. The T-test and ANOVA were used in order to compare the differences between two groups.
**Results**: The β7 gene expression showed significant difference between RA patients and healthy controls (P value= 0.007).
**Conclusion**: In our newly diagnosed patients who did not received any medication for RA the gene expression of integrin β7 was higher than healthy controls. CCR9 gene expression didn't reach significant different between two groups. Our results bring up disposability that over expression β7 gene may have a role in pathogenesis of RA.

**Key words:** Rheumatoid arthritis, CCR9, Integrin *β7*, MALT

ICIA2018\Veterinary Immunology\Poster\7456
**7456**

# Possible allergic reactions, blood factors and the immune system alterations in response to ingestion of oral sumac (Rhuscoriaria L) Compared to Levamisole in dogs.

Ali Vahid Dastjerdi[1], Mohsen Jafarian dehkordi[1], saam torkan[1]

*1. Department of Clinical Science, faculty of veterinary medicine, Islamic azad university of Shahrekord, Shahrekord, Iran*

**Background:** Since before any research on drugs we should be aware of its allergic dose, and given the importance of hematology system that implies the changes in the body, in this study, we decided to investigate the hematologic effects of sumac plant and compare it with a chemical agent (levamisole). In future studies, we'll use its valuable properties especially antibacterial and may replace it by some drugs including levamisole.
**Methods:** In this study, 8 native breed dogs were used for ten days, the dogs were divided into four groups of two, a group of control, a group receiving levamisole, a group receiving sumac at a rate of 10 mg per kg, and one group receiving 5 mg per kg sumac, and after the completion of prescribed period of sumac plant powder and oral levamisole powder, blood samples were taken and then the relevant tests were carried out.
**Results:** Given that in the majority of allergies and poisonings, increased eosinophils and basophils as well as red blood cell lysis, and decreased hematocrit can be seen, and because no study has been done so far on allergic factors such as eosinophils and basophils, and also due to the dosage of sumac plant used in this study that there was no significant difference between treatment and control group ( ), you can ensure the absence of allergies and sensitivities to this plant.
**Conclusion:** According to the same hematologic results of sumac plant and levamisole, valuable properties of sumac plant especially its antimicrobial and immune boosting effects used in future research without worrying about allergic reactions in certain doses of this research and without hematologic side effects.

**Keywords:** allergy, dog, Hematology, levamisole, sumac

ICIA2018\Tolerance and Autoimmunity\Poster\7464
**7464**

# The Effect of Vitamin D3 on Proliferation of CD4+ T Lymphocytesin Patients with Type 2 Diabetes Mellitus

ElhamMahabadi-Ashtiyani[1,2], Vida Sheikh [3], Shiva Borzouei[3], AlirezaZamani[1], IrajSalehi[4], Mahdi Alahgholi Hajibehzad[1,*]

1. *Department of Immunology, Hamadan University of Medical Sciences, Hamadan, Iran*
2. *Student Research Committee, Hamadan University of Medical Sciences, Hamadan, Iran*
3. *Department of Internal Medicine, Clinical Research Development Unit of Shahid Beheshti Hospital, School Of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
4. *Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran*

**Background:** It has been demonstrated that T helper cell subsets have a fundamental role in pathogenesis of type 2 diabetes mellitus (T2DM). Active form of vitamin D (1, 25 dihydroxyvitamin D3, VitD3) has an immunomodulatory effects on immune system. The aim of this study was to investigate the proliferation level of $CD4^+$ T cells and effect of VitD3 on $CD4^+$ T cells proliferation in T2DM compared to healthy controls (HC).
**Method:** Blood sample of18 patients with T2DM (mean: 51.68 ± 8.62; female=13, male=5) and 21 healthy control (mean: 42.80 ± 1.19; female=12, male=9) were collected. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient method. CFSE-labeled PBMCs were cultured and stimulated with PHA in presence/ absence of VitD3 for 5 days. The proliferation of $CD4^+$ T cells were analyzed using flow cytometry.
**Result:** The addition of VitD3 to the culture of PBMCs significantly decreased the proliferation of $CD4^+$ T cells in both T2DM and HC, respectively ($p= 0.001$ and $p=0.0001$). The % proliferation was significantly lower in the HC group compared with T2DM patients in cultures with VitD3. There was no significant changes in the % proliferation of $CD4^+$ T cells between T2DM and HCs in cultures without VitD3.
**Conclusion:** The data suggest potential insight into the consideration of VitD3 in the control of T cells proliferation in T2DM disorders.

**Keywords:** T2DM, Proliferation, Lymphocyte, Vitamin D

ICIA2018\Tolerance and Autoimmunity\Poster\7518
**7518**

**Gene Expression of CD226 and Its Serum Levels in Patients with Multiple Sclerosis**

Fereshteh Alsahebfosul[1,2], Nahid Eskandari[1,] Shokofeh Rahimpourkoldeh[1], Vahid Shayeghanejad[3], Mazdak Ganjalikhani Hakemi[1], Arezo Dabiri[4]

1. Immunology department, *Faculty of Medicine, Isfahan University of medical sciences, Isfahan, Iran*
2. *Isfahan Research MS and Neuroimmunology Center (IRMN), Isfahan, Iran*
3. *Neurology Department, Faculty of Medicine, Isfahan University of medical sciences, Isfahan, Iran*
4. *Immunology department, Shahid Sadoughi University of Medical Sciences, International Campus, Yazd, Iran*

**Background**: Multiple sclerosis (MS) is a chronic disabling inflammatory demyelinating disease that is caused by immune responses directed against myelin proteins and progressive axonal loss of the central nervous system (CNS).CD226 one of T cell co-stimulatory molecule is suggested as genetic risk factors for TH1 dependent autoimmune disease.CD226 co-stimulatory signals potentially promote Th1 differentiation. We hypothesized that the CD226 gene expression and serum level can increase in MS patients.
**Methods**: The type of study is case-control. The qRT-PCR (qualitative real-time polymerase chain reaction) and ELISA (Enzyme-Linked Immunosorbent Assay) methods were applied for determining CD226 gene expression and serum protein level, respectively.
**Results**: We found that the gene expression and mean serum protein level of CD226 no significant differences was found in the MS patient and control groups .In addition, there was no significant difference between age and the CD226 gene expression and its protein in the individual patients .In the patients group also no significant differences in the mean the of CD226 gene expression and protein between men and women was observed, although the mean of CD226 gene expression in women was higher than CD226 gene expression in the men, but this difference was not significant.
**Conclusion**: the gene expression and mean serum protein level of CD226 had no significant differences in the studied MS patient and control groups. Taken together, these data showed that there is no pathological relation between CD226 molecule and multiple sclerosis. It seems that CD226 can not be considered as a diagnostic marker for MS disease.

**Key words**: Multiple sclerosis, CD226, Gene expression, PCR

ICIA2018\Vaccine\Poster\7631
**7631**

# Cloning of Immunogenic Moiety of Fusion Protein of NDV in a Plant Expression Vector

NargesArkian Boroujeni[1], Atena Mozafari[1], Mohammad Javad Motamedi[1], Somayeh Behjat Khatouni[1], Mahyat Jafari[1], Ali Hatef Salmanian[1*]

*National Institute of Genetic Engineering and Biotechnology (NIGEB), Department of plant Biotechnology, Tehran, Iran*

**Background:** Newcastle disease (ND) is a highly contagious respiratory disease in poultry that affects many domestic and wild species. Outbreaks of virulent ND have a great economic impact on poultry industry in developing countries. The surface of Newcastle disease virus (NDV) particles containing two important functional antigens: the fusion (F) and hemagglutinin-neuraminidase (HN) glycoproteins which are considered as the main targets for the immune system against NDV. There are many killed or live-attenuated vaccines to prevent infection by NDV but they have many limitations which lead to develop novel vaccines having flexibility in administration, storage, transportation and cost-effective, also one of the promising platforms is using the .genetic engineering tools and production of recombinant proteins in plant based systems such as hairy roots
**Methods:** In the current study, a chimeric construct containing two antigens (F and HN), with the codon preference of plant which has already designed was used for further immunological analysis. The fusion (F) fragment was amplified from the synthetic HN-F gene by PCR using specific primers and cloned in pTZ57R/T vector. Then, the authentic fragment was subcloned into a plant expression vectors (pBI121), under the control of general (CaMV35S) and the specific hairy root (NtREL1) promoters, respectively. The recombinant vectors (pBI121-CAMV35s-*f*, pBI121-NtREL1-*f*) were transformed to an *Agrobacterium* host (LBA4404) and finally introduced to tobacco plant.
**Results:** The authentic fragment of *f* (~ 909 bp) in pTZ57R/T, pBI121-CAMV35s and pBI121-NtREL1 analyzed and confirmed by PCR, digestion and sequencing. Presence of *f* genein recombinant *Agrobacterium* host confirmed by PCR method.
**Conclusion:** The hairy roots have several advantages such as genotype and phenotype stability, fast and indefinite in vitro growth. Analysis of expression level and immunological response of the recombinant constructs will be evaluated in an animal model are in progress.

**Keywords:** Newcastle Disease Virus, Fusion protein, Hairy root, Recombinant edible vaccine

ICIA2018\Tolerance and Autoimmunity\Poster\7635
**7635**

# Frequencies of Antiphospholipid Antibodies in Women with Recurrent Abortion in Northeast of Iran

Tayebeh Sohrabi[1], Zahra Badiei[2], Mohammad Reza Keramati[2], AzamMoradi Zarmehri[2], Mohsen Rokni[3], Mohammad Hadi Sadeghian[2], Hamid Farhangi[2], Masoud Shahdroodian[2], Geshvad Nasiri Pirbazari[1]

*1. Department of Hematology & Blood Bank, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
*2. MD, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
*Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.*

**Background:** Antiphospholipid Syndrome (APS) is taken into account as one of the most important immunologic cause of recurrent abortion, which is mostly not diagnosed. APS is an autoimmune hypercoagulable disorder caused by antiphospholipid antibodies. The majority of women with antiphospholipid antibodies show complications such as recurrent fetal abortions even after treatment. Due to the lack of accurate and reliable statistical data and the necessity of further research in this field in Northeast of Iran, this study aimed to inves-.tigate the rate and relationship of these antibodies with recurrent abortions
**Methods:** This cross-sectional study was performed on 239 patients with recurrent abortion who were referred to the Sarvar Thalassemia and Hemophilia Center between 2012 and 2014 years. Venous blood samples were collected to determine the serum levels of protein C, protein S, and antiphospholipid syndrome panel; including anti-cardiolipin, anti-β2 glycoprotein I, and lupus anticoagulant.
**Results:** In 239 patients with a mean age of 30±5.4 years, the average rate of abortions was 2.75±1.04 times. Anti-β2 glycoprotein I was detected in 5% (12), anti-cardiolipin and lupus anticoagulant identified in 4.6% (11) and 3.3% (8) of patients, respectively. The levels of anti-β2 glycoprotein I and anti-cardiolipin showed a .(remarkable relationship with the frequency of abortion (*P-value*=0.048 and 0.004, respectively
**Conclusion:** The frequency of antiphospholipid antibodies among women with recurrent abortion was low in Northeast of Iran. The frequency of abortion was significantly linked to the levels of anti-cardiolipin and anti-β2 glycoprotein I antibodies. The presence of more than one antibody is considered as a risk factor for increasing the frequency of abortions.

**Keywords:** Antiphospholipid antibody, Anti-cardiolipin, Anti-β2 glycoprotein I, Recurrent abortion

ICIA2018\Vaccine\Poster\7651
**7651**

# Molecular Analysis of Chimeric Haemagglutinin-Neuraminidase Protein (HN) of Newcastle Disease Virus in Hairy Roots of Tobacco Plant (*Nicotiana tabacum L.)*

Somayeh Behjat Khatouni[1], Atena Mozafari[1], Mohammad Javad Motamedi[1], Narges Arkian Boroujeni[1], Mahyat Jafari[1], Ali Hatef Salmanian[1*]

*National Institute of Genetic Engineering and Biotechnology (NIGEB), Department of Plant Biotechnology, Tehran, Iran*

**Background:** Newcastle disease (ND) is an important avian infectious agent that causes large economic losses to the poultry industry in developing countries. The Newcastle Disease Virus has two envelope glycoproteins, the fusion (F) and hemagglutinin-neuraminidase (HN) protein which considered as the main antigenic targets of the immune system. The HN protein plays an important role in attachment of the virus to host cell membrane. Due to a presence of various epitopes within the HN protein, it could be considered as an appropriate target for the immune system. Developments in genetic engineering have led to establishing a plant-based platform for production and oral delivery of candidate vaccine. The main benefits of edible vaccines are the facility of administration, as well as high-level expression of immunogens in different organs such as seeds and hairy roots via ordinary *Agrobacterium*-mediated transformation.
**Methods:** In the present study, a chimeric construct composed of F-HN epitopes with plant codon preference was designed. The HN fragment was amplified by PCR using specific primers and cloned in pTZ57R/T cloning vector. The fragment (HN) was subcloned into two plant expression vectors, one harboring a root-specific promoter "NtREL1" (pBI121-NtREL1-HN) and another with the CaMV35S promoter (pBI121-CaMV-HN). The recombinant vectors were transformed to the LBA4404 strain of *A.tumefaciens* and finally introduced to tobacco (*Nicotianatabacum L.)* plant.
**Results:** The authentic fragment of *hn* (~1038 bp) in pTZ57R/T, pBI121-CaMV and pBI121-NtREL1 analyzed by PCR, digestion and sequencing. Presence of recombinant vector in *Agrobacterium* and regenerated plants were confirmed by PCR method.
**Conclusion:** Production of recombinant antigenic proteins in hairy root platforms has several advantages such as high growth rate, easy genetic manipulations, high levels expression, and the potential for large-scale production in bioreactor. The next step, the analysis of expression level and immunological response of the recombinant construct will be evaluated in an animal model.

**Keywords:** Hemagglutinin-Neuraminidase protein, Newcastle Disease Virus, Hairy roots, Edible candidate vaccine

ICIA2018\Vaccine\Poster\7664
**7664**

# Development of two novel chimeric VK210/VK247CSP based antigens for vaccine design against *Plasmodum vivax* malaria

Samaneh H. Shabani[1,2], Sedigheh Zakeri[1*], Ali H. Salmanian[3], Jafar Amani[4], Akram A. Mehrizi[1], Georges Snounou[5], Yousef Mortazavi[2], Navid D. D jadid[1]

1. *Institute, Iran*
2. *Zanjan University of Medical Sciences, Iran*
3. *National Institute of Genetic Engineering and Biotechnology, Tehran, Iran*
4. *Baqiyatallah University of Medical Sciences, Iran*
5. *Pasteur institute, France*

**Background:** *Plasmodium vivax* is the most widespread species of *Plasmodium* outside Africa and is predominant in countries where malaria is in pre-elimination or elimination phase. As *P. vivax*cannot be maintained in *in vitro*. a sporozoite-based vaccine is not a practical option, and most current efforts have been focused on developing a vivax-based subunit vaccine. The circumsporozoite protein (CSP) of the *P. vivax* is a major target of pre-erythrocyte vaccine. In this work, we designed and constructed two novel synthetic chimeric genes of *P. vivax* CSP by using computer modelling. Subsequently, the expression and purification of both chimeric recombinant proteins were carried out, and their biological and functional properties were evaluated.
**Methods:** The tow constructs were designed based on reference sequences (Sal-1 and PNG) encompassing different repeats from the two major alleles, VK210 and VK247 and entire N- and C-terminal to compare their structure, antigenicity and their biological activities. DNA encoding the selected chimeras were synthetically constructed based on *E. coli* codons and then cloned and expressed. Characterization of both constructs were performed by western blot, ELISA, heparin and HepG2 binding assay as well as IFA.
**Results:** Design, expression and characterization of both constructs were performed successfully. Strain specific mouse monoclonal antibodies recognized the chimeric antigens in ELISA, indicating correct conformation and that the B-cell epitopes are accessible to the antibodies. Heparin and HepG2 binding assay showed dose depended manner.
**Conclusion:** Although both designed constructs had structurally different characteristics at the molecular level, the expression levels were satisfactory, and chimeras had a conformational structure with biological function that signified their potential use in the development of vivax-based vaccine.
**Keywords:** *Plasmodium vivax*, malaria, subunit vaccine, circumsporozoite protein

ICIA2018\Veterinary Immunology\Poster\7692
**7692**

# Horse Toxoplasmosis Seroprevalance in three Northwest Provinces of Iran

Golbahar Hosseini[1], Reza Rezaie[2], Diba karimi[3]

1. *Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz*
2. *Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz*
3. *Department o*f Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz

**Background:** Toxoplasma gondii is one of the most important Zoonotics in the world. Despite many studies on human toxoplasmosis and ruminants, there are few to be found in Iran.
**Methods:** Ten ml of blood samples were collected from jugular vein of 385 horses among 68000 population in three northwest provinces of Iran. The sera were centrifuged at 3000 rpm for 10 minutes. To determine the prevalence of age, livestock was divided into three age groups ≤2, 10-2, and 10. Rh antigens of Toxoplasma gondii were from prestigious research centers (such as the Pasteur Research Institute or the Razi Vaccine and Parenting Institute) for use in Modified Agglutination Test (MAT) Toxoplasma gondii antibodies were evaluated using the modified (MOD) agglutination test (MAT) according to the described methodology of Tavalla et al. (2015). Sera were diluted in binary dilutions (dilution) using PBS containing 2-mercaptoethanol. Fifty µl of each dilution in each well was planted into 96 plates of U-shaped Eliza. Then, 50 µl of whole formalin-preserved T. gondii Tachyzoites taxicides will be added to each serum dilution.incubated at 37 ° C overnight.
**Results:** Fifteen of 385 horses were identified positive which included 9 stallion and 6 mare. Of the total, 5 were horses less than 5 years old and 10 horses were above 5 years old.
**Conclusion:** Studies on seroprevalence of the disease with various methods of serology in the human population, indicated the presence of Toxoplasma gondii with different prevalence among humans and Livestock.since Equine are considered as interface hosts of Toxoplasma gondii and there is no comprehensive information on seroprevaleance of the disease in the population we aimed to do this.

**Keywords**: Toxoplasma gondii. Horse, Ardabil, East/West Azerbaijan

ICIA2018\Vaccine\Poster\7775
**7775**

**A quaternary vaccine against Enterohaemorrhagic *Escherichia coli* (EHEC)**

Emad kordbacheh[1], Shahram Nazarian[1], Javad Fathi[1], Amin Farhang[1]

*1. Imam Hossein University, Faculty of Science, Department of Biology, Tehran, Iran.*

**Background**: Enterohaemorrhagic *Escherichia coli* (EHEC) are a subset of *E. coli*patovars and a frequent agent of diarrhoealdisease which can contaminate by food and water sources. Especially O157:H7 are most important serovar of EHEC and carry potent infectious agent; so it is associated with many outbreak that influenced on global human health. EHEC can leads to haemolytic uremic syndrome (HUS). this development kidney disease (HUS) 5% leads to death despite treatment with antibiotic or rehydration. type-III secretion system made this patotype formidable and help bacteria to attach and transfer virulence protein into host cell. bacteria first attach to host cell by flagella temporarily and HcpA play an important role in this stage, afterward Tir protein as a specific receptor make this adhesion permanent. Also EspA which is a part of needle apparatus help to translocate enterotoxins (like Stx) into host cell.
**Methods**: A chimeric protein inclusive of mentioned protein (HETS) was constructed and subcloned into PET32a Bl21(DE3) vector. After western blot which confirmed expressed protein, it purified by Ni-NTA column. Mice vaccinated with HETS protein and immune sera assessed for IgG. Finally neutralization and inhibition assays was performed versus O157.
**Results**: 1721bp DNA fragment encoding HETS cassette were successfully subcloned into pET32a vector. This sequence expressed by induced T7 promotor and associated 80kDa protein monitored on SDS-PAGE. Western blotting confirmed protein by using anti-His tag antibody. Immunological analyses showed production of a high titer of specific immunoglobulin in immunized animals. Adherence inhibition and neutralization assessments were shown immune sera are considerable functional in this regard.
**Conclusion**: development of this chimeric vaccine that focus on various virulence agent of only one bacteria can be literally functional and leads to broad vaccine coverage intention.

**Keywords:** Enterohaemorrhagic *E.coli*, chimeric protein, Immunization

ICIA2018\Tolerance and Autoimmunity\Poster\7781
**7781**

# The Influence of PCSK9 on CD36 Expression on Monocytes in Patients with Hashimoto's Thyroiditis

M.S.Fazaeli[1], A.R.Khoshdel[2], M.R.Shafipoor[3], M.Mahmoodi, M.R.Hajizade, M.A.Fahmidekar, M.Rohban

*Department of Clinical Biochemistry Faculty of Medicine University of Medical Sciences*

**Background:** Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease and a secreted protein which increases cholesterol levels in plasma via inducing degradation of low-density lipoprotein receptor (LDLR). So PCSK9 serum levels correlate positively with the plasma LDL-C. Some studies have reported that PCSK9 levels increase in hypothyroidism. Cluster of differentiation 36 (CD36) is a member of a family of cell surface proteins in many cells. CD36 is known as fatty acid translocase (FAT) because it imports fatty acids inside cells. Therefore CD36 involves in regulation of lipid metabolism by facilitating cellular uptake of fatty acids and participating in triglyceride storage. It has been suggested that PCSK9 regulates CD36 in some tissues.
**Methods:** Data and serum levels of TSH, FT4 and PCSK9 and expression level of CD36 on monocytes from 20 new untreated patients with Hashimoto's thyroiditis in subclinical stages and 20 age- sex- and BMI-matched euthyroid controls were analyzed in a cross-sectional study. Quantification of CD36 expression on monocytes was done by flow cytometry. Then the relationships between these parameters were examined.
**Results:** PCSK9 was significantly higher in patients ($p = 0{\cdot}001$). CD36 expression was lower in the patient group than in the control group, but this difference was not significant. There was no significant relationship between PCSK9 and CD36.
**Conclusion:** Currently, PCSK9 inhibitors are used to reduce blood cholesterol levels as drugs. If it will be proven that PCSK9 can reduce the level of CD36, taking these drugs may have unwanted side effects. This study showed that there is no relationship between PCSK9 and CD36 and PCSK9 antibodies can still be used as an effective way to treat hypercholesterolemia.

**Keywords:** Hashimoto's thyroiditis, Proprotein convertase subtilisinkexin type 9, Cluster of differentiation 36, Thyroid stimulating hormone.

ICIA2018\Vaccine\Poster\7791
**7791**

# Design, expression and purification of recombinant EtpA-LTB chimeric protein from Enterotoxigenic *Escherichia coli*

Moslem Kulivandi[1], Shahram Nazarian[2], Yousef Tarverdizadeh[3]

1. *Department of Biology, Faculty of Science, Imam Hossein University, Tehran, Iran*
2. *Corresponding author: Department of Biology, Faculty of Science, Imam Hossein University, Tehran, Iran. Tel: +98 21 77104934. E-mail address: kpnazari@ihu.ac.ir*
3. *Department of Biology, Faculty of Science, Imam Hossein University, Tehran, Iran*

**Background:** Enterotoxigenic *Escherichia coli* (ETEC) is the most common bacterial causes of children's diarrhea Because there are no effective vaccines against ETEC infection, investigating on vaccines against that consider necessary. In the classic paradigm of ETEC infection, these organisms adhere to the small intestinal mucosa via fimbrial colonization factor (CF) molecules, where they elaborate heatlabile enterotoxin and/or heat-stable enterotoxin. Recently novel antigens include adhesions EtpA and EaeH, and mucin enzymes are identified. Fusion of these novel antigens with classic antigens may help to producing effective candidate vaccines against ETEC infection. In this study we focused on expressing EtpA-LTB chimer as candidate immunogen for ETEC infection.
**Methods:** For optimum level expression of protein, the gene was synthesized with codon bias of *E. coli*. EtpA-LTB chimeric gene was subcloned in pET28 and expression of recombinant protein was induced by IPTG. The protein was purified with Ni-NTA column and confirmed by western blot.
**Results:** Percentage of codon having a frequency distribution of 91–100 in the native chimeric gene was 56%, which was significantly improved to 78% in the optimized gene sequence. Increasing CAI index to 81% means that appropriate codon is used for expression in *E. coli* SDS-PAGE analysis showed the presence of a 44 KD recombinant chimeric protein. Purification of the proteins was achieved by Ni-NTA affinity chromatography. Western blot analysis by anti LT antibody, confirmed a single band of recombinant chimeric protein.
**Conclusion:** The chimeric protein can be considered as a candidate immunogen against ETEC infection.

**Keywords:** Enterotoxigenic *Escherichia coli*, LT toxin, Chimeric protein,Bioinformatic

ICIA2018\Stem Cells Based Immunotherapy\Poster\7824
**7824**

# Exosomes from mesenchymal stem cells as a microRNA delivery system to mouse splenocytes

Zohreh Bolandi[1, 2], Seyed Mahmoud Hashemi[3], Sara Soudi[4], Hossein Ghanbarian[1, 2*]

*1. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*3. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*4. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

**Background:** MicroRNAs (miRNAs) are a type of small non-coding RNA that control gene expression at the posttranscriptional level. miRNAs have a critical role in many biological activities including cell proliferation, apoptosis and inflammation. Recent studies showed that miRNAs stably present in different extracellular biofluids (e.g., blood or urine) and vesicles (e.g., exosomes or microvesicles). Exosomes protect miRNAs from digestion and exosomal miRNAs can be taken up by cells and subsequently modulate their gene expression. Due to these properties, exosomes have attracted attention as an efficient cell-free vector for gene delivery. The aim of this study was to determine whether the exogenous miRNAs could be transferred to mouse splenocytes via adipose-derived mesenchymal stem cells (AD-MSCs) exosomes.
**Methods**: AD-MSCs were cultured in DMEM with 1% ITS (insulin-transferrin-Selenium) and culture media was harvested after 72hrs. Exosomes were then isolated and characterized by dynamic light scattering (DLS) and scanning electron microscopy (SEM). Exosomes were loaded with miRNA mimic by electroporation in 0.4cm electroporation cuvettes. Splenocytes were isolated from 8 weeks old female C57BL/6 and were cultured in RPMI-1640 plus 10% FBS in presence of the loaded exosomes. After 24 or 120 hrs, RNA was extracted and qPCR was performed.
**Results:** For transfer of miRNA to splenocytes, exosomes were isolated from AD-MSCs. SEM and DLS analysis revealed spherical structures with a diameter of 117nm. After exosomes electroporation, splenocytes were cultured with the loaded exosomes. RNA was extracted and qPCR determination showed that miRNA were overexpressed significantly in splenocytes.
**Conclusion:** Here, we showed that AD-MSCs exosomes can deliver exogenous miRNA to mouse splenocytes. Loaded Exosomes provides a new platform for genetic information transfer and gene therapy of immune cells or modulate immune responses including immune stimulation and tolerance induction.

**Keywords:** Splenocytes, Exosomes, miRNA.

ICIA2018\Vaccine\Poster\7881
**7881**

**Determination of tetanus immunization in pregnant woman in Zanjan province, Northwest of Iran**

SafaTahmasebi[1], Sousan Ghasemi[2], Maryam Erfanmanesh[3], Abdolreza Esmaeilzadeh [*4, 5]

*1. MSc student of Immunology, Tehran University of Medical Sciences, Tehran, Iran*
*2. Medical Laboratory of Shaheed Beheshti Hospital, Zanjan University of Medical Sciences, Zanjan, Iran*
*3. Young Researchers Club, Zanjan Branch, Islamic Azad University, Zanjan, Iran*
*Department of Immunology,[5]CancerGeneTherapyResearch Center, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran*

**Background:** Despite the fact that neonatal Tetanus has been eradicated in all over the world, it is still a significant health problem in some developing countries. It could be even resulted in death. This disease is a worrying problem but it could be easily prevented by vaccination of mother. Vaccinating every pregnant woman with at least one dose of TT would be an affordable way to protect against neonatal tetanus, and would be a step toward eliminating the deaths that continue to occur due to this preventable disease.
**Methods:** The purpose of this study was evaluation of immunity in 576 pregnant women in Zanjan province, Iran. These women's serums were separated. Then tetanus antibody was measured in their serums using Immunoaffinity Chromatography Assay (IACH).
**Results**: Results indicated that 95 percent of these pregnant women which had received vaccine had acquired immunity and the remained 5 percent of them had no immunization.
**Conclusion:** In conclusion, not only vaccination is important in women, but also effectiveness of vaccination is vital for successful immunization. It is proposed that more attempts should be done in order to gain affordable immunization.

**Keywords:** Tetanus antibody, vaccination, pregnant women, Immunoaffinity Chromatography Assay, Iran

ICIA2018\Vaccine\Poster\7898
**7898**

# A novel cell penetrating peptide for efficient delivery of HPV16 E7 antigen *in vitro*

Sepideh Shahbazi[1], Azam Bolhassani[1*], Fatemeh Motevalli[1]

*Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran*

**Background:** The E7 protein has been suggested as a candidate for generating protein vaccines against human papillomavirus infections. The cell penetrating peptides has been suggested for delivery of proteins and peptides *in vitro* and *in vivo*. In this study, the efficiency of a amphipathic peptide, hPP10, for delivery of the fluorescent HPV16 E7 protein was evaluated in HEK-293T cells.
**Methods**: Cloning of the recombinant hPP10-E7-GFP DNA performed in the pET-28a vector. After confirmation of the recombinant clones using digestion and PCR. The expression of hPP10-E7-GFP protein was induced by IPTG and purified by affinity chromatography and confirmed using SDS-PAGE and western blotting. HEK-293T cells were transfected with hPP10-E7-GFP and E7-GFP/TurboFect. The efficiency of hPP10 CPP and TurboFect reagent was compared by fluorescent microscopy and flow cytometry at 3 and 24 hours after transfection.
**Result:** The recombinant hPP10-E7-GFP gene construct was confirmed by PCR and digestion. The results showed a ~ 1100 bp band in gel electrophoresis. The purified hPP10-E7-GFP protein with apparent molecular weight of ~ 50 KDa, was recognized by SDS-PAGE and Western blotting. Flow cytometry results showed that the transfection efficiency of E7-GFP protein was ~ 63.66% and ~ 32.95% for hPP10 CPP and TurboFect, respectively. The cells transfected by hPP10-E7-GFP and E7-GFP/TurboFect demonstrated the green regions with different distribution using fluorescent microscopy.
**Conclusion:** The expression of the hPP10-E7-GFP protein was performed in *E. coli* system. *In vitro* transfection results showed the transfection of hPP10 was significantly higher than TruboFect commercial reagent. Thus, hPP10 can be considered as a promising delivery system for therapeutic purposes in Future.

**Keywords***:* HPV16, E7, CPP, hPP10

ICIA2018\Tolerance and Autoimmunity\Poster\7945
**7945**

**Survey of Alpha-1 Antitrypsin Activity in Patients with Multiple Sclerosis**

Giti Farsi[1], Alireza Khoshdel[2], Abbas Lotfi[3], Amir Moghadam Ahmadi[4], Mahmood Sheikhfatolahi[5], Mohammadreza Hajizadeh[6], Mehdi Mahmoodi[6], Fatemeh Ayoobi[7]

*1. Department of Clinical Biochemistry, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
*2. Department of Clinical Biochemistry, Faculty of Medicine, and Pistachio Safety Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
*3. Department of Clinical Biochemistry, Faculty of Medicine, Tarbiat modares University of Medical Sciences, Tehran, Iran*
*4. Neurologist, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
*5. Department of Epidemiology and Biostatistics, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
*6. Molecular Medicine Research Center and Department of Clinical Biochemistry, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
*7. Physiology-Pharmacology Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

**Background**: Alpha-1 Antitrypsin (AAT) is the most abundant serine-protease inhibitor in blood which impedes degradation of the tissues by its anti-protease activity. Any lack or imperfection of this protein remarkably intensifies the risk of various diseases including pulmonary diseases, hepatitis, cirrhosis, autoimmune diseases such as multiple sclerosis. Multiple sclerosis (MS) is an inflammatory, degenerative disorder with biological associations. Hypothetically, AAT may play an essential role in mentioned disorder. The most important objective of the present study is to determine the association between AAT activity and Multiple Sclerosis degree.
**Materials and Methods**: In this descriptive study, the activity of AAT was determined using sera, by Trypsin Inhibitory Capacity (TIC). Patients with MS were identified from 42 patients included in the specific diseases center. Also, 42 healthy volunteers (controls) were recruited. Data was analyzed by SPSS software.
**Results**: The average activity of AAT (TIC degree) in MS patients was significantly lower than controls (2.23 µmol/min/ml). In addition, TIC levels in MS patients in accord with age and sex was meaningfully lower than healthy individuals ($p<0.001$). Moreover, there was a reverse correlation between disease duration and AAT activity ($r=-0.481$, $p=0.001$).
**Conclusion**: Alpha-1 Antitrypsin activity in patients with MS was lower than the controls. Since alpha-1 antitrypsin is important in controlling inflammation process in response to environmental exposure (free radicals, smoking, etc.), such disorders may be due to abnormalities of this protein. Efforts should focus on early recognition and effective interventions for AAT activity in individuals with MS. Detection and changing of lifestyle as soon as possible continues decisive to the management off AAT deficiency.

**Keywords**: Alpha-1 Antitrypsin, Multiple sclerosis, Trypsin Inhibitory Capacity

ICIA2018\Vaccine\Poster\7972
**7972**

# Formulation of polio vaccine deactivated by gamma irradiation and comparing its immunogenicity with an Iranian commercial vaccine

Maryam mollaee alamooti[1],mehrdad ravanshad[2], farahnaz motamedi[3]

1. *Department of virology, School of medical science, University of tarbiat modares*
2. *Department of virology, School of medical science, University of tarbiat modares*
3. *Research Institute of Beam, Research Institute of Nuclear science and technology*

**Background**: poliomyelitis (polio) is caused by various poliovirus serotypes. A live vaccine that is weak commercially exists, in some cases as a result of vaccine-induced paralysis. Therefore, the production of low-cost inactivated vaccines is important and necessary to eradicate polio. In this study, the inactivated vaccine by gamma-ray radiation was compared with the common polio vaccine in the mouse model.
**Methods:** First, the virus titre of the oral polio vaccine was determined, then deactivated by the appropriate dose of gamma radiation and formulated to form a radio vaccine. BALB/c mice received two different formulations of the intramuscular vaccine in two week intervals. Two weeks after the final injection, blood samples were collected from the mice and the humoral and cellular immune responses were evaluated.
**Results: Humoral** immune responses using neutralizing anti-serum titers of mice vaccinated with Vaccine conventional, Radio Vaccine showed a significant difference in some dilutions. Also, the study of cellular immunity using MTT test and statistical analysis of the data by Anova showed that the induction of cellular immunity in the radio vaccine group was significantly increased.
**Conclusion:** The anti-serum titre of the inactivated vaccine by gamma irradiation showed that it could produce proper humoral immunity in mouse groups.

**Key words:** Polio, vaccine, gamma radiation, immune responses

ICIA2018\Tolerance and Autoimmunity\Poster\7990
**7990**

# Effects of Gliadin Administration on Gene Expression Profile of Chemokines in Central Nervous System of Mice

Parisa Esmaeilie[1], Hamed Bashiri, Shohreh Fakhari[1,2] , Maryam Moradi[1]

*1. Department of Immunology and Microbiology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran*
*2. Cellular and Molecular Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran.*

**Background**: Gluten is the most widely used food to trigger development of multiple autoimmune diseases and neurological disorders. Recently, an association between anti-gliadin serum antibodies and CNS autoimmune diseases such as MS has been reported. Concerning the involvement of chemokines synthesized in activated astrocytes and microglial cells in cellular infiltration and brain defense mechanisms, the present study was conducted to investigate chemokines gene expressione in CNS of mice immunized with gliadin.
**Methods:** Healthy female 6-8 weeks old C57BL/6 mice were assigned into 3 groups (N=6). Mice in group 1 were injected subcutaneously (sc) with PBS (400µl). In group 2, mice were immunized sc with complete freundadjuant (CFA;400µl). In group 3, mice were immunized sc with peptic-tryptic-gliadin (300µg) emulsified in CFA (400µl). Boosters containing the same amount of antigen were injected on days 7 and 14.On day 28, mice were sacrificed and brain and spinal cord tissue were removed**.** RT-PCR was used to evaluate the mRNA expression of MCP-1, CXCL2, and CXCL10 chemokines.
**Results:** mRNA expression for MCP-1 and CXCL2 were increased significantly ($p<0.05$) in group 3 in comparison to control groups (group 1 and 2). Conversely, the level of CXCL10 expression in group 3 in comparison to group 1 and 2 was significantly ($p<0.05$) up-regulated and down-regulated respectively.
**Conclusion:** The changes in gene expression profile of chemokines may be involved in gliadin induced CNS pathology.

**Keywords:** Gliadin, MCP-1, CXCL2, CXCL10

ICIA2018\Research and Development\Poster\8010
**8010**

# Gene expression and levels of IL-6 and TNFα in PBMCs correlate with severity and functional class in patients with chronic heart failure

V. Eskandari[1], A. A. Amirzargar[1] , M. J. Mahmoudi[2] , Z. Rahnemoon[3] , F. Rahmani [4,5] , S. Sadati[1] , Z. Rahmati [1] , F. Gorzin[1] , M. Hedayat[6,7] , N. Rezaei[1],[4,5]

*1. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
*2.. Department of Cardiology, Amir Alam Hospital, Tehran University of Medical Sciences, Tehran, Iran*
*3. Cardiac Heart Center, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
*4. Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran*
*5. Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Tehran, Iran*
*6. Division of Immunology, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA*
*7. Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Boston, MA, USA*

**Background:** Evidence shows that proinflammatory cytokines are important determinants of assessment of severity and prognosis of chronic heart failure (CHF).
**Methods**: In this report, we used real-time PCR assay to compare relative gene expression of TNFα and IL-6 in PBMC from CHF patients with various heart diseases (n = 42, EF < 45%, NYHA I to IV) and matched healthy control subjects (n = 42).We also determined the TNFα and IL-6 concentrations of cell culture supernatant of PBMCs with ELISA.
**Results**: There was a significant negative correlation between gene expression of TNFα and LVEF($r = 0.4$, $p < 0.05$). Patients with CHF had increased gene expression of TNFα and IL-6 in PBMCs ($p < 0.05$). They also had elevated the supernatant levels of these cytokines in cultured PBMCs ($p < 0.001$). Levels of TNFα and IL-6 were increased in ischemic heart disease compared to non-ischemic heart disease. There was a positive correlation between TNFα and IL-6 levels in CHF patients and severity of CHF in patients. Levels of these cytokines were higher in patients with NYHA III-IV than in NYHA I-II and normal subjects.
**Conclusions**: Results of this study indicate that peripheral expression of proinflammatory cytokines, TNF-α and IL-6, is important indicators of severity and prognosis in patients with chronic heart disease.

**Keywords**: Heart failure; Proinflammatory cytokines; Tumor necrosis factor alpha; Interleukin 6

ICIA2018\Vaccine\Poster\8044

**8044**

# Development of a multi-epitope peptide vaccine inducing robust T cell responses against Brucellosis using immunoinformatics based approaches

Ahmad Karkhah[1, 2], Mahdiye Saadi[1, 2], Hamid Reza Nouri[1*]

*1. Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran*
*2. Student Research Committee, Babol University of Medical Sciences, Babol, Iran*

**Background:** Current investigations have demonstrated that a multi-epitope peptide vaccine targeting multiple antigens could be considered as an ideal approach for prevention and treatment of brucellosis. According to the latest findings, the most effective immunogenic antigens of brucella to induce immune responses are included Omp31, BP26, BLS, DnaK and L7-L12. Therefore, in the present study, an *in silico* approach was used to design a novel multi-epitope vaccine to elicit a desirable immune response against brucellosis.
**Methods:** First, five novel T-cell epitopes were selected from Omp31, BP26, BLS, DnaK and L7-L12 proteins using different servers. In addition, helper epitopes selected from Tetanus toxin fragment C (TTFrC) were applied to induce CD4+ helper T lymphocytes (HTLs) responses. Selected epitopes were fused together by GPGPG linkers to facilitate the immune processing and epitope presentation. Moreover, cholera toxin B (CTB) was linked to N terminal of vaccine construct as an adjuvant by using EAAAK linker. A multi-epitope vaccine was designed based on predicted epitopes which was 377 residues in length. Then, the physico-chemical properties, secondary and tertiary structures, stability, intrinsic protein disorder, solubility and allergenicity of this multi-epitope vaccine were assessed using immunoinformatics tools and servers.
**Results:** Based on obtained results, a soluble, and non-allergic protein with 40.597 KDa molecular weight was constructed. Expasy ProtParam classified this chimeric protein as a stable protein and also 89.8% residues of constructed vaccine were located in favored regions of the Ramachandran plot. Furthermore, this multi-epitope peptide vaccine was able to strongly induce T cell and B-cell mediated immune responses.
**Conclusion:** In conclusion, immunoinformatics analysis indicated that this multi-epitope peptide vaccine can be effectively expressed and potentially be used for prophylactic or therapeutic usages against brucellosis.

**Keywords:** Brucellosis, multi-epitope vaccine, Omp31, BP26 BLS, Dnak, L7-L12

ICIA2018\Tolerance and Autoimmunity\Poster\8131
**8131**

# Association between gene expression of Interlukine-35 subunits with plasmaneopterin and disease activity in rheumatoid arthritis patients

Nasrin Iranshahi[1], shirin Assar[2], Seyed Mojtaba Amiri[3], Parisa Zafari[4,5] , Mahdi Taghaddosi[*]

*1. Department of Immunology, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*2. Assistance professor of Rheumatology in Clinical Research Development Center, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*3. Department of Biostatistics and Epidemiology, School of health, Kermanshah University of Medical Sciences, Kermanshah, Iran*
*4. PhD student in department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
*5. Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran*
**Assistance professor in department of Immunology, Kermanshah University of Medical Sciences, Kermanshah, Iran*

**Background:** Interleukin-35 (IL-35), is the member of IL-12 family that produced by T regulatory (Treg) cells. This cytokine has immunosuppressive properties which can inhibit overt inflammatory responses such as those associated with rheumatoid arthritis. This study aims to determine the correlation between gene expression of IL-35 subunits in peripheral blood with immunological parameters and disease activity in rheumatoid arthritis patients.
**Methods:** Peripheral blood samples were collected from 47 patients and 44 of healthy subjects. Plasma levels of Neopterin and Anti cyclic citrullinated peptide (Anti-CCP) have been evaluated using ELISA method. Gene expression of FoxP3, IL-35 subunits (IL-12A/EBI-3) analyzed by qPCR. Also RF detected by agglutination test and clinical variables were extracted by physical examination.
**Results:** In this study, there was no correlation between gene expression of EBI-3, IL-12A and FoxP3 with disease activity score-28 (DAS-28), (P=0.234), (P=0.634) and (P=0.457) respectively but the significant negative correlation was observed between the plasma levels of neopterin and EBI-3 gene expression (P=0.004).
**Conclusion:** With respect to negative correlation between EBI-3, the subunit of immunosuppressive cytokine IL-35 with plasma neopterin, the inhibitory effect of this cytokine on inflammatory process in RA patients can be concluded.

**Keywords:** Rheumatoid arthritis, Interlukine-35, Neopterin, DAS-28, FoxP3

ICIA2018\Tolerance and Autoimmunity\Poster\8137
**8137**

# Cold Autoimmune Hemolytic Anemia due to High-grade non-Hodgkin's B cell Lymphoma with Weak Response to Rituximab and Chemotherapy Regimens

Behzad Nazel Khosroshahi,[1,2], Mohammad Jafari,[3] Hossein Vazini,[4] Alireza Ahmadi [5] , Keivan Shams[6], Mahdi Kholoujini[7]

*1. Emam Reza Hospital, Hamadan University of Medical Sciences, Hamadan, Iran*
*2. Blood transfusion research center, High institute for research and Education in Transfusion Medicine, Tehran, Iran*
*3. Department of Pathology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
*4. Department of nursing, Hamedan branch, Islamic Azad University, Hamedan, Iran*
*5. Department of Hematology and Oncology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*
*6. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
*7. Department of Mycology, School of Medicine, Tarbiat Modares University, Tehran, Iran*

**Background:** Autoimmune hemolytic anemia (AIHA) is characterized by shortening of red blood cell (RBC) survival and the presence of autoantibodies directed against autologous RBCs. Approximately 20% of autoimmune hemolytic anemia cases are associated with cold-reactive antibody. About half of patients with AIHA have no underlying associated disease; these cases are termed primary or idiopathic. Secondary cases are associated with underlying diseases or with certain drugs. We report herein a rare case of cold autoimmune hemolytic anemia due to high-grade non-Hodgkin's lymphoma of B-cell type with weak response to rituximab and chemotherapy regimens. For treatment B cell lymphoma, Due to lack of treatment response, we used chemotherapy regimens including R- CHOP for the first time, and then Hyper CVAD, R- ICE and ESHAP were administered, respectively.
**Methods:** For treatment of autoimmune hemolytic anemia, we have used the corticosteroid, rituximab, plasmapheresis and blood transfusion and splenectomy.
**Results**: In spite of all attempts, the patient died of anemia and aggressive lymphoma nine months after diagnosis.
**Conclusion:** To our knowledge, this is a rare report from cold autoimmune hemolytic anemia in combination with high-grade non-Hodgkin's lymphoma of B-cell type that is refractory to conventional therapies.

**Keywords:** Cold autoimmune hemolytic anemia, High-grade, non-Hodgkin's lymphoma, Rituximab, Chemotherapy

ICIA2018\Tolerance and Autoimmunity\Poster\8160
**8160**

# Investigation of Chosen Polymorphisms in Forkhead box P3 (FoxP3) Gene in Patients with Hashimoto's Thyroiditis.

Mahsa Eshkevar Vakili[1], Solmaz Khansalar[1], Danial Ghasemi[1], Farshid Mossallaie[1], Zahra Amirghofran[1,2], Kurosh Kalantar[1]

1. *Department of Immunology, School of medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
2. *Autoimmune Disease Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

**Background:** Loss of immune tolerance to thyroid autoantigens leads to autoimmune thyroid diseases such as Hashimoto's thyroiditis (HT). A subset of T cells that promote immune tolerance to autoantigens, called regulatory T cell (Treg). Reduced number and function of Tregs result in abnormal responses against autoantigens and subsequently cause autoimmune diseases. These cells modulate the immune responses. Forkhead box P3 (FoxP3) is a master transcription factor of Treg cells which is pivotal for maintaining and function of these cells. In this study, we investigated the association of two polymorphisms of FoxP3 gene in Hashimoto's thyroiditis (HT).
**Methods:** The study was performed in the group consisting of 110 patients with Hashimoto's thyroiditis (HT) recruited from the Motahari Clinic which is affiliated to Shiraz university of medical science, (mean age: 38.1±01years) and 105 healthy subjects (mean age: 44.4±2.2years). DNA was extracted from the peripheral blood leukocytes using the salting-out method. The two single nucleotide polymorphisms (SNPs) including rs3761549 (-2383C/T) and rs376154 (-3279A/C) in the FoxP3 gene. Genotyping were done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).
**Results:** About rs3761549 (-2383C/T), there wasn't a significant difference in frequency of alleles ($v_{alue}$>0.05) and genotype ($v_{alue}$>0.05) between patients and control. In regards to the rs3761548 (-3279A/C) the same results obtained and no difference observed respect to the allele and genotype among studied subjects.
**Conclusion:** We have not shown association between FoxP3 polymorphisms and susceptibility to Hashimoto disease.

**Keywords:** Autoimmune thyroid diseases, Regulatory T cell, Forkhead box P3 (FoxP3), Single nucleotide polymorphism (SNPs)

ICIA2018\Tolerance and Autoimmunity\Poster\8221
**8221**

# The Association Circulating IL-17 Levels and IL-23 Receptor Gene Polymorphisms with Multiple Sclerosis

Seyed Ali ghaffarikhalafmohammadi[1,2], AbdollahJafarzadeh[1,2], Abdolkarim Sheikhi[3]

1. *Department of Immunology, Medical School, kerman University of Medical Sciences, kerman, Iran*
2. *Neurology Research Center, Department of Neurology, Kerman University of Medical Sciences, Kerman, Iran*
3. *Department of Immunology, Medical School, Dezful University of Medical Sciences, Dezful, Iran*

**Background:** IL-17/IL-23 axis plays a prominent role in the pathogenesis of a number of autoimmune diseases. The aim of this study was to investigate the IL-17 levels in MS patients and its association with gender, treatment, disease patterns and single nucleotide polymorphisms (SNP) in *IL-23R* gene, including rs11209026 and rs1004819.
**Methods:** The blood samples were collected from 135 MS patients and 135 healthy subjects. The patients have relapsing-remitting (RRMS; n=65), primary progressive (PPMS; n=19), secondary progressive (SPMS; n=35) or progressive relapsing (PRMS; n=14) patterns. The DNA analyzed for SNPs using PCR-RFLP and IL-17 levels measured by ELISA.
**Results:** The serum IL-17 levels in MS patients was significantly higher than in control group ($P<0.001$). The IL-17 levels were significantly higher in MS men as compared to women patients ($P<0.05$). Untreated patients had significantly higher IL-17 levels than healthy group and treated patients ($P<0.001$ and $P<0.01$, respectively). The IL-17 levels in treated patients with interferon-β (IFN-β), methylprednisolone (MP) or both IFN-β and MP were significantly lower than untreated MS patients ($P<0.05$, $P<0.02$ and $P<0.05$, respectively). Patients with RRMS and PRMS, had significantly higher IL-17 levels than healthy group ($P<0.005$ and $P<0.01$, respectively). No significant differences were observed between patients and controls regarding the genetic variations at rs11209026 and rs1004819. The levels of IL-17 did not influenced by genetic variations at investigated SNPs.
**Conclusion:** These results indicated higher levels of IL-17 in MS patients that may be influenced by disease patterns, treatment and gender. There was no any association between investigated SNPs and MS.

**Keywords:** Multiple sclerosis, IL-17, IL-23 receptor, Gene polymorphisms.

ICIA2018\Vaccine\Poster\8250
**8250**

# Naloxone reinforced immune response in compared to HBS antigen formulated with Montanide ISA-720

Mina Mirzaee[1], Setareh Haghighat [2], Mehdi Mahdavi [3,*]

*1. Department of Microbiology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, IR Iran*
*2. Department of Microbiology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, IR Iran*
*Department of Immunology, Pasteur Institute of Iran, Tehran, Iran*

**Background:** Utilization of immunostimulatory factors to promote the Immune system have captured the attention of the scientists in the field of vaccine and have resulted in improvement of vaccines potency. Hepatitis vaccine and its development is one of the ongoing projects of the country carried out by scientists and injected clinically, but it has a main problem being its deficiency to follow a positive response to all people. In this study, Naloxone as an immunopotentiatoris used in an oil-based Montanide ISA-720 formulation of hepatitis vaccine to promote higher cellular immune responses**.**
**Methods:** The HBs Ag (5µg/dose) was formulated in Montanide ISA-720 adjuvant by hemojenizer and Naloxone was added to the Formulation with final concentration of 5 mg/kg and 10 mg/kg of experimental mice. Then, the prepared formulation was injected to the BALB/C mice three times with two week interval and the cytokine responses of IL-4, IFN-γ, TNF-α, IL-2, total IgG antibody and IgG1 and IgG2a isotypes were assessed via ELISA method.
**Results:** The results indicated that addition of Naloxone to the final Formulation was followed by an enhancement of IFN- γ, IL-2 responses against control groups ($P<0.05$) and versus commercial vaccine.
**Conclusion:** Naloxone could be used as immunopotentiator in mixture adjuvant for induction of Th1 immunologic platform

**Keywords**: HBS-Ag1, vaccine2, Adjuvant3, Naloxane4, Montanide, ISA7205

ICIA2018\Stem Cells Based Immunotherapy\Poster\8275
**8275**

# Developing Rat Bone Marrow derived mast cells by the Splenic Cells Culture Supernatant of rat and mouse

Amani Saeede [1], Shahrooz Rasoul [2,*], Karimi Ali[1], Bakhtiari Zahra [1], Asl iranifam Neda [1] , Mortaz Esmaeil [3,4,5,*]

*1. PhD student, Comparative Histology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*
*2. Associate Professor, Anatomy sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*
*3. Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*4. Department of Immunology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*5. Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands*

**Background:** Mast cells are playing a crucial role in the pathogenesis of diverse diseases that include MC-driven disorders. The aim of the current study was to develop simple and cost-efficient method for differentiation and culture of rat mast cells from bone marrow by using rat and mouse spleen supernatant.
**Methods:** Bone marrow cells from male 10 to15-weeks-old rats was obtained through femur bone and cultured for three weeks at complete cell culture medium. After that, the purity of cells was confirmed by flowcytometry, toluidine blue and immunohistochemistry (IHC).
**Results:** After 3 weeks continuous culturing rat bone marrow cells with spleen supernatant of rat and mice, we found high purity of cells in expression of specific lineage markers of cells. Results similar to each other be derived for both spleen supernatants of rat and mouse Specific markers for differentiated bone marrow stromal stem cells. CD117, CD34 markers and tryptase were positive 80.1%, 76.89% and 87.9%, respectively, with rat splenic supernatant whereas were positive 85.4%, 83.07% and 82.1%, respectively for cells which cultured with mouse splenic supernatants. IHC result is confirmed by flowcytometry analysis. In this case with rat supernatant of spleen (89.1%) and with mouse splenocyte supernatant (91.8%) double positive for FCεRI and CD117 markers were obtained.
**Conclusion**: By modification rat standard rat/mouse bone marrow isolation by supernatant spleen of both animal, mast cells were obtained. Finding the differences of two cells from two specious is important in mast cell biology. In the current study, by modification of protocol we achieved high-yield mast cells by using rat splenic cells mitogens and changing concentration with culturing cells in density of $3 \times 10^5$-$1\times 10^6$ cells/ml whereas other data of old yield which published were $1*10^5$, $1* 10^6$.

**Keywords**: Bone Marrow derived mast cells**,** Cells Culture Supernatant**,** flowcytometry analysis

ICIA2018\Veterinary Immunology\Poster\8308
**8308**

**Experimental study immunomodulatory effects of Rosa canina L. on male rats**

Saeede Sadat Mirbageri[1], Hamidreza Hakkakifard[2], Pouyan houshmandpour[3]

1. *Young Researchers and Elite Club, Tabriz Branch, Islamic Azad University, Tabriz, Iran*
2. *DVM, Department of Veterinary Clinical Sciences, Tabriz Branch, Islamic Azad University, Tabriz, Iran*

**Methods:** In this study, 30 male rats were obtained and divided into three groups of 10 rats. First group received 0.9% saline (10 mg/kg), the second group Rosa canina L. fruit extract (250 mg/kg) and third group Rosa canina L. fruit extract (500 mg/kg) as oral gavages every day for a period of one month. After obtaining blood samples, differential white blood cell (WBC) counts, phagocyte activity (number) of samples were obtained.
**Results:** Neutrophil and monocyte counts and phagocyte activity significantly increased in comparison with the normal saline group.
**Conclusion:** The data suggest that the Rosa canina L. fruit extract used in traditional medicine might have immunomodulatory effects.

**Keywords:** Rosa canina, Rat, immunomodulator, phagocyte, white blood cells.

ICIA2018\Stem Cells Based Immunotherapy\Poster\8350
**8350**

# Evaluation of Mesenchymal Stem Cells Immunomodulatory Effects on Cytokine Production in Experimental Chronic Colitis

Maryam Heidari[1], Seyed Mahmoud Hashemi[1, 4*], Sedigheh Pouya[1], Kaveh Baghaei[2], Mohammad Reza Zali[3]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Basic and Molecular Epidemiology of Gastrointestinal Disorder Research Center, Research Institute for 3. Gastroenterology and Liver Disease, Shahid Beheshti University of Medical Science, Tehran, Iran
4. Gastroenterology and Liver Disease Research Center, Research Institute for Gastroenterology and Liver 5. Disease, Shahid Beheshti University of Medical Science, Tehran,Iran
6. Department of Applied Cell Sciences, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

**Background**: Inflammatory bowel disease (IBD) as a chronic recurrent disorder is characterized by mucosal immune response dysregulation; mesenchymal stem cells (MSCs) are the multipotent cells, which can be effective in immunomodulation via release of anti-inflammatory mediators. In this study, the effects of MSCs were evaluated in Dextran Sodium Sulfate (DSS) induced colitis.
**Methods**: Chronic colitis was induced in female C57BL/6 mice using 2% DSS in drinking water for 3 cycles, in each cycle, there were 4 days of DSS solution administration followed by 7 days of DSS-free water administration. MSCs, $10^6$ cells per mouse, were injected intraperitoneally (IP) on the last day of DSS in cycles 2 and 3. Colitis Clinical symptoms including body weight changes, bleeding, stool consistency were daily recorded. The colon macroscopic and pathological changes were then analyzed. The IL-17, IL-10 and TGF-β cytokine levels were also evaluated in spleen, mesenteric lymph nodes and serum of mice.
**Results**: After receiving MSCs in colitis mice, the clinical symptoms and disease activity index (DAI) were improved and the survival rate was increased. In MSCs-treated group, the colon length reduction was mild. The histopathological examination also showed tissue healing and less leukocyte infiltration in comparison to the non-treated group. Additionally, ELISA results indicated, increased level of transforming growth factor-beta (TGF-β), increased level of interleukin (IL)-10, and decreased level of IL-17 following the treatment.
**Conclusion**: This study demonstrated the ability of MSCs to regulate the inflammatory immune responses in chronic colitis. Production of anti-inflammatory cytokines by MSCs lead to immunosuppression, tissue healing and inflammatory cytokines reduction.

**Keywords**: Inflammatory Bowel Disease, Dextran Sodium Sulfate, Mesenchymal Stem Cell, Colitis Mouse Model.

ICIA2018\Reproductive Immunology\Poster\8355
**8355**

# Evaluation of TH1 & TH2 cytokines and pregnancy hormones in pregnant women with a history of recurrent abortion and normal pregnancy

Saber Rokhafrooz[1], Mehri Ghafourian[2,*], Nasrin Saharkhize[3], Sara Iranparast[4]

*1. Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
*2. Fertility, Infertility and Perinatology Research Center, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
*3. Infertility and In Vitro Fertilization Center, Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*4. Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

**Background:** Spontaneous abortion is one of the most common complications of pregnancy. Immunologic factors have been proposed to contribute to the etiology of recurrent abortion. We aimed to measure cytokines in women with a history of recurrent abortion in the first trimester of pregnancy until termination of pregnancy. Moreover, we assessed the relationship between T-helper cytokines and hormones (progesterone and human chorionic gonadotropin [hCG]), involved in normal pregnancy process in the above mentioned groups.
**Methods:** In this case-control study, normal pregnant women (control group, n=30) and those with history of recurrent abortion (case group, n=43) in the first trimester were enrolled. We assessed TH2 cytokines (IL-4, IL-6 and IL-10) and TH1 cytokines (TNFα and INFγ) with enzyme-linked immunosorbent assay. Karyotyping was performed on the women and their spouses to detect chromosomal abnormalities. We also monitored the case group until the end of their pregnancy.
**Results:** The mean concentrations of TH2 cytokines differed significantly in women with a history of recurrent abortion whose pregnancy continued successfully compared with those whose pregnancy failed ($P<0.05$). However, no significant difference was observed between the case and control groups in this regard. The concentration of TH1 cytokines including TNFα and INFγ increased in women with recurrent miscarriage whose pregnancy was unsuccessful compared with normal pregnant women. This difference was significant in TNFα levels ($P<0.05$). We found no correlation between hCG levels and TH1/TH2 trends, while a correlation was observed between progesterone levels and TH2 cytokines ratio.
**Conclusion:** A higher TH2 cytokine level is seen in women with recurrent abortion who have a successful pregnancy, while this trend shifted toward a higher TH1 level in those with unsuccessful pregnancy. Cytokine assessment could be proposed as a marker for predicting success or failure of pregnancy in women with recurrent abortion during the first trimester of pregnancy.

**Keywords:** Recurrent abortion, TH1&TH2 cytokines, hCG, Progesterone

ICIA2018\Veterinary Immunology\Poster\8371
**8371**

# Investigation of the leukogram and tumor necrosis factor-α in native neonatal calves with diarrhea of Sanandaj area, west of Iran

Mahdieh Raeeszadeh[1*], Javad Beheshtipour[2]

*1. Assistant Professor in Pharmacology, Department of Basic Sciences, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran*
*2. DVM Student, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran*

**Background:** Neonatal calf diarrhea is a sickness affecting the newborn calf under 4 weeks of age. Multiple enteric pathogens (e.g., viruses, bacteria, and protozoa) are involved in the development of this disease. Tumor necrosis factor-α (TNF-α) is secreted chiefly by macrophages. TNF-α possesses a wide range of biological activities, including fever induction; direct activation of neutrophils, T cells, and macrophages; direct tumor cell cytotoxicity; cachexia; and induction of other mediators (e.g., IL-1 and IL-6). Hence, the aim of this study was to determine the leukogram and plasma concentration of TNF-α in native neonatal calves with diarrhea of Sanandaj region.
**Methods:** This study was conducted during March to July 2017 in Sanandaj area, west of Iran. All native calves under 30 days of age displaying diarrhea were included in the study. Calves were excluded when showing any other diseases. Finally, the diarrhea group consisted of 10 animals. 10 healthy native calves were considered for the control group. Blood samples were collected from the jugular vein into EDTA tubes. The leukogram and neutrophil/lymphocyte ratio (NLR) were measured. The TNF-α plasma concentrations were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit. Data were analyzed by using an independent t-test.
**Results:** The neutrophilia was the most important finding in the leukogram of the diarrhea group. The NLR was significantly increased in the diarrhea group compared with control group ($P<0.05$). TNF-α was detected at low level (23.51±2.35 pg/ml) in control group. TNF-α level was 176.32±13.58 pg/ml in native neonatal calves with diarrhea. The plasma concentration of TNF-α in the diarrhea group was significantly higher than the control group ($P<0.001$).
**Conclusion:** According to the obtained results, the level of TNF-α and NLR did not increase before the clinical manifestations of diarrhea in native neonatal calves. Further studies are needed to understand this function.

**Keywords:** Tumor necrosis factor-α, Neonatal calf diarrhea, Leukogram

ICIA2018\Vaccine\Poster\8372
**8372**

# The Formulation of Hepatitis B Vaccine in MF59 adjuvant with PPD and Its Immunogenicity Comparison with traditionalHepatitis B Vaccine

Reyhane Mirzaee[1], Fahimeh Nematimonsour[2], Mehdi Mahdavi[3,*]

*1. Department of Biotechnology, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran (IAUPS)*
*2. Department of Biotechnology , Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University , Tehran, Iran (IAUPS)*
*3. Department of Immunology, Pasteur Intitute of Iran, Tehran, Iran*

**Background:** Hepatitis B infection is propagated widely in the world population and the proper approach that could be used to control is application of vaccine. The clinical vaccine in all of the world is a vaccine formulated in Alum adjuvant that has not provided suitable immune response in some immunized cases. Herein, we formulated a new HBs vaccine using MF59 adjuvant with PPD and compared cellular and humoral immune responses versus commercial HBs vaccine.
**Methods:** The HBs Ag was formulated in MF59 adjuvant with PPD and injected s.c. 3 times with two week interval to BALB/C mice. Ten days after last immunization, the levels of cytokines including IL-4, IFN-γ, TNF-α, IL-2 and total antibody were assessed by ELISA method.
**Results**: There was an increase in IL-4 cytokine of Iranian commercial alum vaccine compares with MF59 amounts of IFN-Y cytokine of Iranian Alum and MF59.The study of TNF-a indicates that Iranian commercial vaccine because of its higher inflammation is more exudated than MF59. There was increase in total antibody of Iranian commercial vaccine as compared to vaccine formulated with MF-59.
**Conclusion:** There was significant reduction of IL-4 cytokine and also antibodies shows that vaccine formulated with MF59 can induce cellular immunity as compared to humoral immune response. moreover, less adversary effects of MF59 adjuvant as another beneficiary properties could be considered**.**

**Key word**: HBs- Ag, vaccine, adjuvant, MF59, alum

ICIA2018\Reproductive Immunology\Poster\8395
**8395**

# Evaluation of vascular endothelial growth factor (VEGF) SNP-634C/G and +936C/T polymorphisms in Iranian women suffering from preeclampsia

Seyed Hojjat Hossaini[1], Mehri Ghafourian[2,*], Ata Ghadiri[1], Saber Rokhafrooz[1], Mahin Najafian[3], Ahmad Rouhizadeh[4]

*1. Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
*2. Fertility, Infertility and Perinatology Research Center, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
*3. Department of Obstetrics & Gynecology, Fertility, Infertility and Perinatology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
*4. Cellular and Molecular Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

**Background:** Preeclampsia is a disorder of pregnancy characterized by high blood pressure and proteinuria after 20 weeks of gestation. This disorder is about 6-8% of all pregnancies. Vascular endothelial growth factor (VEGF), an angiogenic factor, plays a significant role in vascular permeability and vascular proliferation. The aim of this study is to assess the various risk factors related to preeclampsia and the VEGF-634C/G & +936C/T polymorphisms in women with preeclampsia.
**Methods:** This study is a case-control study that conducted in two groups of pregnant women with preeclampsia (n=135) and healthy pregnant women (n=135) admitted to hospitals of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. DNA was extracted from the peripheral blood lymphocytes using phenol-chloroform extraction method. The genotypes of the VEGF-634C/G & +936C/T polymorphisms were detected by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). Then, the restriction enzymes (FaqI and NlaIII) were applied to detect +936C/T & -634C/G polymorphisms, respectively. The results were obtained and imaged by 1.5% agarose gel and Gel DOC system. The data were analyzed by SPSS software.
**Results:** In this study the maternal age, gestational age, maternal hemoglobin and maternal BMI were significantly associated with risk of preeclampsia ($P<0.05$), while preeclampsia season was not effective in preeclampsia occurrence. There was no significant difference between the case and control groups regarding VEGF-634C/G & +936C/T polymorphisms ($P>0.05$).
**Conclusion:** Despite the significant relation in various risk factors with preeclampsia, it seems that VEGF-634C/G & +936C/T polymorphisms are not associated with preeclampsia. However, further studies are necessary to more fully assess the different factors associated with preeclampsia.

**Keywords:** Preeclampsia, Polymorphism, VEGF, Pregnancy

ICIA2018\Stem Cells Based Immunotherapy\Poster\8429
**8429**

**Overexpression of miR-34a in the Mesenchymal stem cell by Lentiviral vectors**

Katayuon Bahman Soufiani[1], Ali Akbar Pourfathollah [2], Mahin Nikogoftar Zarif[3], Ehsan Arefian[4]

1. *PhD student, Department of Immunology, Tarbiat Modares University*
2. *Department of Immunology, Tarbiat Modares University*
3. *Iranian Blood Transfusion Organization*
4. *Department of Microbiology, University of Tehran*

**Background**: Nowadays cell therapy is considered as one of the treatments in many diseases, among which mesenchymal stem cells (MSCs) have a distinctive position. Features such as immunogenicity and targeted migration to inflamed tissues, including cancerous regions, have made these cells the best candidate for therapeutic purposes. MicroRNAs are molecules that influence the expression of the gene can be involved in behavioral and evolutionary characteristics of the cells and pathogenesis of many diseases. Significant reduction of miR-34a in many cancers led to the use of this small non-coding molecule as a therapeutic tool in this study. Therefore, in this study, the miR-34a encoding gene was attempted to be integrated using virus packaging in the MSC cell genome.
**Methods**: First, the miR-34a (5p) sequence gene was cloned into the GFP+ lentivirus plasmid. The recombinant plasmid was transfected into HEK-293 cell line by the calcium-phosphate method, along with the encoding plasmids of structural proteins and lentiviral envelopes. The viral supernatant was gathered and in the second stage transduction with the condensed virus was performed on MSCs. Total RNA extraction and cDNA development were performed.
**Results**: The sequence of miR-34a gene into the lentivirus plasmid was confirmed by PCR, enzymatic, colony-PCR and sequencing methods. Real-Time PCR results on cDNA of the transduced MSC cells versus control showed a 34-fold increase in miR-34a expression (p= 0.0000).
**Conclusion**: Using the Cloning and Virus Packaging methods makes it possible to enter the gene in the considered MSC genome and achieve a permanent expression in these cells. Increasing the miR-34a expression in MSCs indicates that these techniques are applicable in modifying MSCs.

**Keywords:** Mesenchymal stem cell, MicroRNA, Virus Packaging

ICIA2018\Veterinary Immunology\Poster\8430
**8430**

**A Passive Surveillance for HP AIVs (H9 & H5s) in Isfahan Birds Garden**

AbdolrezaNabinejad[1] (DVM,Ph.D),Nooshin Askarani[2](MS)

*Isfahan research and education center of agriculture and natural resources Nabinejad@abrii.ac.ir*
*Isfahan research center of teachers, Software group*

**Background**: Isfahan birds garden is located at the Zayandeh rood river and with about 160 specious and 3000 of different birds annually more than 500000 people around the country and tourists visit the birds. Regarding HP AIs (H5N1 and H5N8) outbreak in the poultry farms (industrial and rural) and immigration of immigrant birds to the central part of the Iran, a passive surveillance were carried out, for increasing immunity of the for the visitors and birds.
**Method :**A daily shuttle program for sanitization of garden and gates with widely antiseptic material were recommended, and an active quarantine for a sure immunity, meanwhile educational techniques used for training and refreshing the staffs specially the birds nurses which are at the first line of communication to the birds ,In the laboratory examination a monthly monitoring for NDV and AIV titer well done by HI test ,so 2 ml of wing vein blood in 5-6% of sensitive birds were collected and some swabs of pharynx and stools were prepared in the transporting media for RT-PCR(in suspected birds) molecular detection.
**Result:** The mean ND titer were 5 with CV of 65% and the mean titer of H9N2 were 6 with a CV equal to 98%, but the highest ND titer (9) were related to chickens and pheasant and fowl , the lowest ND titer(2) were related to water fowls such as Ducks, Pelicans , Flamingos ,Gooses . The minimal H9N2 titer were 6 and the maximal value were 10 but no one were positive for In H5N1 and H5N8. The molecular RT-PCR of samples for H5N1 and H5N8 fortunately show negative results...
**Conclusion:** Regarding to the result a monthly vaccination for ND and AI and continues first level of biosecurity were recommended. Also using the training and intelligent board inside and outside the garden with some regular education course were the assessment of this survillence.

**Key words:** AIVs, Birds, Isfahan, Passive, surveillance

ICIA2018\Veterinary Immunology\Poster\8433
**8433**

**Serology of Isfahan Ostrich Chicks for a Complexe Downer Syndrome**

Abdolreza Nabinejad[1] (DVM,Ph.D), Nooshin Askarani[2] (MS)

*Isfahan Research and Education Center of Agriculture and Natural Resources Nabinejad@abrii.ac.ir*
*Isfahan Research Center of Teachers, Software group*

**Background**: Ostrich rearing is developing in Iran and well increased in Isfahan as the first ranking in the country. It was about 20 years ago that a few Ostrich chicks were imported to Iran from South Africa and at the moment we there are more than 5000 breeders just in Isfahan Province with about 7000 chicks for laying or meat production in future. One of the most complicated disease of the ostrich chicks were downing and constipation fallowed by respiratory problems. The clinical signs goes to some management and nutritional difficulties but the contagious form of disease oriented the infectious habitat of the disease meanwhile the more biosecurity shown the list of occurrence.
**Methods:** Sampling of bloods done via jugular and wing vein in the infected chicks aged 2 to 6 months from 5 farms in Isfahan city and its around cities, The samples transported to serology laboratory and the sera were prepared for HI test for ND and AI and also ELISA test kit of symbiotic used for chicken Aadenovirus 1 and Borna virus investigation.
**Results:** Regarding our results Aadenovirus 1 and Borna virus infection were negative comparing to positive and negative controls, but the HI test for ND and AI were positive and valid, in which the CV for ND were 250% with the maximum of 10 ,Minimum were 3 and average of 8 , the CV for AI (H9N2) were 236% with the Maximum titer of 9 ,Minimum titer of 4 and average of 6 , Fortunately no any infection of H5s serotypes were positive According to the clinical and paraclinical results the syndrome were related to per acute ND (enteric form) co working with AI,
**Conclusion:** Therefore vaccination in the breeders (for maternal antibodies) and checks beside disinfecting and biosecurity of the farms were recommended.

**Key words:** Downer syndrome, AI, ND, Ostrich Chicks, Isfahan

ICIA2018\Vaccine\Poster\8450
**8450**

**Is there any relationship between birth season with HBs Ab titer and expression level of TLR2, 3, 4 genes?**

Kousar Smailnejad ganji[1, 2], Mehdi Shahbazi[2], Mousa Mohammadnia Afrouzi[2]

1. *Student research committee, Babol University of medical sciences, Babol, Iran*
2. *Department of Immunology, School of Medicine, Babol University of Medical Sciences, Babol, Iran*

**Background:** Environmental factors such as birth season can play an important role in the development of the immune system and the immune responses to vaccines. The main objective of this study was to determine the association between the birth season with HBs Ab and the expression level of TLR2, 3, 4 genes in response to the hepatitis B vaccine in 3-5 years old children.
**Methods:** In this study, 72 children with the age of 3-5 years old were born in the winter and summer of 2011-2013 (36 in each group) were included. Blood samples were collected, then the HBs Ab titer and TLR2, 3, 4 genes expression levels were analyzed by ELISA method and Real Time PCR, respectively.
**Results:** The results of this study indicated that the average expression of TLR2, 3, 4 genes in the winter-born group was higher than the summer-born group. However, this difference was not statistically significant (TLR2 P-Value = 0.5) (TLR3 P-Value = 0.06) and (TLR4 P-Value = 0.16). The average level of antibody in the summer was greater than the winter. However, this finding was not statistically significant (P-Value = 0.3).
**Conclusion:** There are no relevance between immune responses to HBV vaccine and birth season. However, many studies showed seasonally variable environmental antigens effect on immune response to vaccination. Further research is required to confirm this relevance.

**Keyword:** HBs Ab titer, Hepatitis B vaccine, TLR2, 3, 4 and Birth Season

ICIA2018\Veterinary Immunology\Poster\8456
**8456**

# Infectious Stomatitis CBC in a Lizard Caused by Resistant Pseudomonas Sp.

AbdolrezaNabinejad[1] (DVM,Ph.D), Nooshin Askarani[2] (MS)

1. *Isfahan research and education center of agriculture and natural resources Nabinejad@abrii.ac.ir*
2. *Isfahan research center of teachers, Software group*

**Background**: Reptiles ecosystemic duty are very important , now a days their exhibitions used for human knowledge mean while rearing crocodiles and lizards is going to be a job for export of meat leather. Mouth rot is probably the worst bacterial infection in reptiles and is the cause of many an animal's loss. Bacteria of the groups Pseudomonas, Aeromonas, and Proteus Mycobacterium and salmonellas accumulate in the oral mucous membrane and cause infections, swellings, and a cheesy discharge. Most cases of mouth rot occur after an initial injury to the snout and poor physical condition of the lizard. Early diagnosis of infection is vital for recovery. Current reports goes to an infectious stomatitis of lizard with a 19 ages old, 216.5cm length and 116.5Kg BW.
**Methods:** First of all the animal moved to a hospital and welfare conditions were prepared and the sores of mouth washed using normal saline , the secretion and scratched of mouth mucosa layer with caution sampled and tested for microbiology. The blood sampling of tail vein carried out and CBC and microbial exams.
Results: Regarding to the results the CBC shows some neutrophilia and eosinophilia, the PCV were normal, no bacteria and fungi isolated in blood. The mouth samples showed some G- and some G+ bacteria but no any fungi residues. The differential bacterial cultivation and biochemical test shows Pseudomonas Sp. which were resistant to penicillin and the majority of related beta-lactam antibiotics, Enrofloxacin, Aminoglycosides due to its cell wall and efflux pumps but just resistant to Cephalosoporines and Fosbac..
**Conclusion:** The lizard welfare in artificial .habitat should be prepared or the infectious agents affect them and zoonotic infections and animal death coming.

**Key words**: Lizards, Stomatitis, CBC, Resistant, Pseudomonas

ICIA2018\Vaccine\Poster\8457
**8457**

# Evaluation of the Level of HBs Antibody after Hepatitis B Vaccine among 3-5 years old children in Babol

Kousar Smailnejad ganji[1, 2], Mehdi Shahbazi[2], MousaMohammadnia Afrouzi[2], Mojdeh Ghias Tabari[3]

1. *Student research committee, Babol University of medical sciences, Babol, Iran*
2. *Department of Immunology, School of Medicine, Babol University of Medical Sciences, Babol, Iran*
3. *Department of Biochemistry, School of Medicine, Babol University of Medical Sciences, Babol, Iran*

**Background:** Hepatitis B virus (HBV) infection is an important infectious disease worldwide. Vaccination has known to be the most effective methods for the prevention of transmission and prevalence of HBV. The main objective of this study was to examine the effectiveness of HBV vaccine in children aged 3-5 years old.
**Methods**: The study was conducted in Amirkola Children's hospital during a 6- month period from November 2016 to March 2017. Blood samples were obtained from 120 healthy, 3 to 5-year-old children who had been vaccinated against HBV and HBS antibody concentration was measured by ELISA method. The individuals were divided into two groups according to their anti-HBs titer (responder group who had HBS Ab titer > 10 mIU/ml and non-responder group who had HBS Ab titer ≤ 10 mIU/ ml ). Results were reported as number(n) and percentage (%) by using SPSS version 22 software.
**Results:** 69 of the subjects were boy and 51 of the subjects were girl, respectively. Among the cases, 98(82%) children were responder while, 22(18%) subjects were non-responder.
**Conclusion**: Our results showed that, the antibody response to HBV vaccine was lower than the similar studies. It is suggested, farther studies should be performed to evaluate HBs antibody levels after vaccination especially in high risk children and booster must be administrated, if require.

**Key words:** HBs Ab titer, Vaccination, Children and Babol

ICIA2018\Tolerance and Autoimmunity\Poster\8458
**8458**

# The Efficacy of St. John's wortin the Treatment of Experimental Autoimmune Encephalomyelitis in C57BL/6 Mice

RezaNosratabadi[1],MaryamRastin[2],MohammadKazemiArababadi[1],AkbarAnaeigoudari[3],MahmoudMahmoudi[2]

1. *Department of Immunology, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*
2. *Department of Immunology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
3. *Department of Physiology, School of Medicine, Jiroft University of Medical Sciences, Jiroft, Iran*

**Background**: St. John's wortor *Hypericumperforatum L* (*HP*) is an herbal medicine used in traditional medicine due to antidepressant and anti-inflammatory activities.
**Methods**: Based on these properties, we evaluated the therapeutic efficacy of *Hypericumperforatum extract* (HPE) against experimental autoimmune encephalomyelitis (EAE) an animal model of multiple sclerosis (MS).
**Results**: The clinical score was reduced from 4.56 to 2.89 after treatment with HPE. This change was associated with a decrease in incidence and infiltration of inflammatory cells into the CNS, as well as a decrease in body weight loss. Additionally, treatment with HPE significantly decreased the level of proinflammatory cytokines (IFN-γ, IL-17A and IL-6) and increased anti-inflammatory cytokines (TGF-β, IL-10 and IL-4), in splenocytes from EAE mice. The flow cytometry analysis revealed HPE-treated mice had markedly higher proportions of $CD4^+CD25^+Foxp3^+$ Tregs in the spleen compared to PBS-treated mice ($10.2 \pm 0.27\%$ versus $7.27 \pm 0.24\%$, $p < 0.01$).
**Conclusion**: Collectively, the current study demonstrated HPE could potentially reduce the clinical and pathological complication of EAE.

**Key words**: *Hypericumperforatum*L, Multiple Sclerosis, Experimental Autoimmune Encephalomyelitis (EAE), Myelin Oligodendrocyte Glycoprotein (MOG)

ICIA2018\Veterinary Immunology\Poster\8465
**8465**

# Production of Immunoglobulins Y (IgY) against *Salmonella Typhimurium* in Japanese quail (Coturnix japonica)

Elham Sadat Hosseini Nasab[1], Maryam shakoori[2], Atefeh Esmailnejad[3], Bahman Abdi[4]

1. *Department of Pathobiology School of Veterinary Medicine Shiraz University Shiraz Iran*
2. *Department of Pathobiology School of Veterinary Medicine Shiraz University Shiraz Iran*
3. *Department of Pathobiology School of Veterinary Medicine Shiraz University Shiraz Iran*
4. *Department of Clinical Science Faculty of Veterinary Medicine Shiraz University Shiraz Iran*

**Background:** Salmonellosis is one of the most common infectious diseases all around the world, with *Salmonella enterica*serovars *Typhimurium* (*S.* Typhimurium) and *Salmonella enterica*serovars *Enteritidis* (*S.* Enteritidis) being some of the more common isolates associated with foodborne diseases in humans and animals. Immunoglobulin Y (IgY) is the major serum immunoglobulin in oviparous animals with an overall structure similar to that of mammalian immunoglobulins. As a means of protection to the offsprings, avian transfer their maternal immunoglobulin Y from serum to the egg yolk where it serves as passive immunity of the developing chicks. Chicken egg yolk antibodies have been extensively used for prophylactic, therapeutic detection of microbial contaminants and diagnostic purposes. Oral immunotherapy with specific IgY has been established as an efficient alternative to traditional antibiotic therapy in human and animals. In this study, production and specificity of anti- *Salmonella typhimurium* IgYs in immunized quail eggs were evaluated.
**Methods:** A total of 30 newly hatched *Salmonella* spp.-free female Japanese quails (Coturnixcoturnix japonica) were divided into immunized and control groups. Immunization was carried out with intramuscular injection of formalin and heat inactivated*Salmonella typhimurium* whole bacterial suspensions emulsified with Freund adjuvants. Immunization continued for 3 months to ensure that the antibody had reached a high level. Eggs were collected every week for 100 days. Egg yolk IgY was purified by ammonium sulfate precipitation method. Anti- *S. typhimurium* egg yolk IgY titer and specificity were determined using ELISA and Western blot techniques.
**Results:** *S. typhimurium* specific IgY antibodies were detected in immunized quail eggs and were significantly higher than the control group (p=0.001) which confirmed the immunization procedure. The results also showed higher antibody titer in the group immunized with formalin inactivated immunogen.
**Conclusion:** The study significantly demonstrated the possibility of generating antibody against *Salmonella typhimurium* in quail egg yolks which can be used for immunotherapeutic and immunodiagnostic purposes.
Abstract Text Maximum 2000 Characters around 300 words. (Times New Roman, font size12)

**Keywords:** Salmonella Typhimurium, Immunoglobulin, IgY, Japanese quail

ICIA2018\Tolerance and Autoimmunity\Poster\8475
**8475**

**Longitudinal interferon beta-1a effects in multiple sclerosis**

Parisa Nourbakhsh[1], Ali Ghazavi[1,2], Ali Ganji[1], Mohsen Ebrahimi[4], keyvan Ghasami[4]*, Ghasem Mosayebi[1,3], Sepideh Moradkhani[5]

*1. Department of Microbiology and Immunology, School of Medicine, Arak University of Medical Sciences, Arak, Iran*
*2. Traditional and Complementary Medicine Research Center (TCMRC), Arak University of Medical Sciences, Arak, Iran*
*3. Molecular and Medicine Research Center, Arak University of Medical Sciences, Arak, Iran*
*4. Department of Neurology, School of Medicine, Arak University of Medical Sciences, Arak, Iran*
*5. Department of Immunology, School of Medicine, Kerman University of Medical Sciences,Kerman, Iran*

.(**Background:** Cytokines play an important role in the pathogenesis of multiple sclerosis (MS
Interferon beta (IFN-β) has been at the forefront of MS treatment for many years. The aims of this study were .to determine the levels of Th1/Th2/Th9/Th17 serum cytokines, after 1 year of therapy with interferon beta-1a
.**Methods:** Forty healthy controls and 36 MS patients were enrolled in this longitudinal study
Th1/Th2/Th9/Th17 serum cytokine levels were measured using ELISA kit at inclusion and after 12 months of .interferon beta-1a (CinnoVex) treatment
**Results:** After one year of treatment the recorded significant effects were decreases in IL-17A (917.10±132.05 to 547.83±78.46), IFN-γ (260.80±42.74 to 201.10±51.63) and IL-9 (184.25±12.59 to 142.22±6.73) levels .while no significant change was seen in the IL-5 (165.74±9.65 to 172.96±12.60) level
**Conclusion:** Based on the findings of this study, a reduction in the Th1, Th9 and Th17 serum cytokine levels were seen after 12 months compared with pre-treatment values, which reflects in vitro and animal studies of IFN-β effector mechanisms.

**Keywords:** MS, Interferon beta, Cytokine

ICIA2018\Tolerance and Autoimmunity\Poster\8491
**8491**

**Investigation of the Serum Levels of Anti-tetanus Toxin Antibodies in Patients with Hyperthyroidism**

Mitra Shokri Majelan[1], Hossain Khorramdelazad[1], Mohammad Taghi Rezayati[1], Azam Bagherizadeh[1], Shekoofeh Moghadam[1], Shima Bazaz[1], Fahimeh Mohammadi[1], Abdollah Jafarzadeh[2]

1. *Department of Immunology, Medical School, Rafsanjan University of Medical Sciences, Rafsanjan*
2. *Department of Immunology, Medical School, Kerman University of Medical Sciences, Kerman, Iran*

**Background and Aims:** There are receptors for thyroid hormones such as thyroid stimulating hormone (TSH) on lymphocytes, which indicate the ability of hormones to influence the lymphocyte responses. The aim of this study was to evaluate the serum levels of anti-tetanus toxin antibodies (anti-TTA) in patients with hyperthyroidism.
**Material and Methods:** Totally, 24 patients with hyperthyroidism and 24 age- and gender-matched healthy individuals were enrolled to study. All participants were vaccinated against tetanus according to the national immunization program. The presence of hyperthyroidism confirmed according to the clinical and para-clinical criteria such as TSH levels. The serum samples of participants tested for the levels of anti-TTA by ELISA method. The serum antitoxin concentration 0.1 IU/mL was considered as a protective level of antibody.
**Results:** The seroprotective rate was 100.0% in healthy group and 94.0% in hyperthyroid patients (P=0.52). The mean titer of anti-TTA was 3.28 ± 0.48 IU/ml in healthy group and 4.28 ± 0.50 IU/ml in hyperthyroidism group. There was no significant difference between healthy subjects and hyperthyroid patients regarding the levels of anti-TTA (P=016). In hyperthyroid men, the titer of anti-TTA was higher than hyperthyroid women (5.32 ± 1.42 IU/ml vs 4.01 ± 0.52 IU/ml) but the difference was not significant (P=0.20). Similarly, the titer of anti-TTA in healthy men was higher than healthy women, but the difference was not significant, again (3.74 ± 1.17 IU/ml vs 3.09 ± 0.05 IU/ml, P<0.35).
**Conclusion:** These results showed that hyperthyroidism might has no significant influence on the protective against tetanus. The titer of anti-TTA was not affected by gender of participants.

**Keywords:** Hyperthyroidism, Protection, Tetanus, Anti-toxin antibody.

ICIA2018\Stem Cells Based Immunotherapy\Poster\8496
**8496**

**Banking of mesenchymal stromal cells: a source for autoimmune-disease studies**

Masoumeh Asadi[1], Parvaneh Farzaneh[1], Meysam Ganjibakhsh[1,3], Seyed Abolhassan Shahzadeh Fazeli[1,2], Neda Sadat Gohari[1], Hedieh Rahmati[1], Mehrnaz Izadpanah[1,4] , Shiva Mohammadi[1], Maryam Farghadan[1], Sepideh Ashouri Movassagh [1], Zahra Elyasi Gorji[1]

*1. Human and Animal Cell Bank, Iranian Biological Resource Center (IBRC), ACECR and Tehran, Iran*
*2. Department of Molecular and Cellular Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran*
*3. Department of Anatomy, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran*
*4. School of advanced technologies in medicine, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Technological developments in the stem cell field have increased assurance in the possible benefits of these cells for treating diseases. Mesenchymal stromal cells (MSCs) are using in various autoimmune diseases studies such as diabetes and multiple sclerosis. A proper cell banking procedure can provide a suitable resource of cells for research.
**Methods:** Mesenchymal stromal cells, from different tissues, were isolated by enzymatic digestion. For characterization, specific markers detected by flowcytometry and differentiation ability was evaluated. Multiplex PCR and microbial tests were performed to detect cross-contamination with the cells of other species and microbial contamination, respectively. Short tandem repeat (STR) profiling test was performed to detect cross-contamination with other human cells. Finally, each cell sample was cryopreserved and banked with an identity card.
**Results:** The cells differentiated into mesodermal lineage including osteogenic and adipogenic lineages and also expressed human MSC markers including CD29, CD90, and CD105 whilst lacking in the expression of CD45, CD34. Quality control tests showed no fungal, bacterial, mycoplasma and viral contamination. All of the cell samples authenticated with unique STR profiling and free of cross-contamination with cells of other species.
**Conclusion:** Long-term culture of primary cells increases the risk of microbial contamination, senescence, and cross-contamination. Banking of cells can minimize mentioned risks. Therefore, good cell banking procedure may support the quality of autoimmunity investigations.

**Keywords:** Human mesenchymal stromal cells, Cell banking, Autoimmunity studies

ICIA2018\Tolerance and Autoimmunity\Poster\8499
**8499**

# High Expression of Programmed Death-1 (PD-1) Impairs CD3+ CD8+ T Cells Activity in Patients with Multiple Sclerosis

Atefeh Najmaldini[1], Mohammad Mehdi Mohammadi[2], Ladan Langroudi, Hossein ali Ebrahimi

1. *Department of Immunology, Kerman University of Medical Science, Kerman, Iran*
2. *Neurological Neuroscience Research Center, Kerman, Iran*

**Background:** Patients with multiple sclerosis (MS) have a deficiency of peripheral blood CD8+ T cells which might reduce control of Epstein–Barr virus (EBV) and lead to MS by allowing EBV infected autoreactive B cells to accumulate in the brain. In patients with MS, T cells progressively declined, consistent with T-cell exhaustion.
**Methods:** We used flow cytometry to determine the phenotypes and frequency of CD8+ cells based on the expression of CD8 and CD3 and PD-1 in 30 healthy subjects and 30 patients with MS who had not received corticosteroids or immunomodulatory therapy in previous 3 months. Additionally, Real-time PCR was used to evaluate EBV load in PBMCs.
**Results:** Cytotoxic CD8+ CD3+ T cells are able to remove EBV-infected cells in healthy donors. Our results indicated higher expression of PD-1 on circulating CD3+ CD8+ T cells in MS patients compared to healthy donors. This phenotype is an indication of exhausted T cells.
**Conclusion:** Exhausted CTLs in patients suffering from MS are not able to eradicate virus infected B cells. The results suggest that inefficient immune control of EBV in patients with MS during remission may cause exacerbation of the disease. Future studies on the mechanism of T cell exhaustion may aid to better understanding of the disease and design of effective therapies.

**Keywords:** Multiple sclerosis, Exhausted T Cell, Epstein-Barr Virus, PD-1.

ICIA2018\Reproductive Immunology\Poster\8502
**8502**

# Relative Expression of OX40, OX40L mRNA and OX40L serum levels in women with recurrent spontaneous abortion

Fateme Rahmani[1], Hossein Hadinedoushan[1*], Nasrin Ghasemi[2]

*1. Department of Immunology, Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Science, Yazd, Iran*
*2. Recurrent Abortion Research Centre, Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

**Background:** The aim of this study was to determine the role of OX40 and OX40L in Recurrent spontaneous abortion (RSA). We compared the expression of OX40, OX40L genes in peripheral blood mRNA levels and serum levels of OX40L in the women with RSA history and the control group.
**Methods**: In this case-control study, 40 women with RSA history (case group), and 40 women with no history of abortion (control group) were surveyed. The expression of OX40 mRNA and OX40L mRNA were determined in peripheral blood of the two groups using the quantitative polymerase chain reaction. Also, enzyme-linked immunosorbent assay was used to determine the levels of serum OX40L in the case and the control groups.
**Results:** There were no significant differences in the maternal age of women in two groups (30.1±4.28 years in the case and 30.03±4.23 years in the control group). There was no difference in the levels of OX40 and OX40l mRNA between the case and the control groups (p=0.1 and p=0.6 respectively). Also, there was no significant correlation between the expression of OX40 and OX40L mRNA levels with age and the number of abortions. The correlation between OX40 and OX40L mRNA levels was insignificant. Women with RSA history had significantly higher level of serum OX40L than control group (p=0.03).
**Conclusion:** Our findings showed that the expression of OX40 mRNA and OX40L mRNA were similar between women with RSA history and the control group. The elevation of serum OX40L levels may be considered as risk factor for RSA.

**Key words:** OX40, OX40L, quantitative PCR, RSA

ICIA2018\Stem Cells Based Immunotherapy\Poster\8513
**8513**

# Hanging drop as an optimal method for cancer stem cells enrichment from HT-29 human colorectal cancer cell line in order to use them in cancer immunotherapy

Marzieh Naseri[1], Elmira Gheytanchi [2], Zahra Madjd[1, 2*], Mahmood Bozorgmehr[2*], Jamshid Hajati[3], Roya Ghods[1, 2], Jafar Kiani[1, 2]

*1. Department of Molecular Medicine, Faculty of Advanced Technology, Iran University of Medical Sciences, Tehran, Iran*
*2. Oncopathology Research Center, Iran University of Medical Sciences, Tehran, Iran*
*3. Department of Immunology, School of Medicine, Tehran University of Medical Science, Tehran, Iran*

**Background:** Tumor mass-resident cancer stem cells (CSCs) are suggested to play a key role in recurrence and metastasis of colorectal cancer and its resistance to conventional therapies. On the other hand, immune responses are well known to be the vanguard of natural anti-cancer strategies. However, the inability to target CSCs with current immune approaches may be a significant factor in treatment failures. Dendritic cell (DC) prime with CSC-lysate has demonstrated significant induction of anti-tumor immunity against CSCs both in vivo and in vitro, selectively targeting CSCs. The key step for promoting robust anti-CSC immune responses is their efficient isolation with acceptable purity. Hence, we were about to come up with an optimized and reproducible method for colorectal CSCs enrichment.
**Methods:** In this study, several colon spheres generation methods including hanging drop, culture in serum free media under non-adherent conditions (poly-HEMA and 2% agarose coated plates) and use of external forces were compared; all the methods were carried out at various cell densities. CSC enrichment was verified using flowcytometry analysis for putative molecular markers of colorectal CSCs. In addition, Real time PCR was used to assess the expression of several well-known stemness genes.
**Results:** HT29 colon cancer cells grew best using the hanging drop technique. The percentage of $CD44^+$and $CD133^+$cancer cells among spheroids was higher than that of the adherent cells. Also, the expression of stemness genes including OCT4, SOX2 and KLF-4 was markedly increased in the spheroid cells as compared with that of the control adherent cells.
**Conclusion:** We suggest the hanging drop method as an efficient approach for enrichment of colorectal CSCs to be used in targeted therapeutic modalities. Such suitable isolated CSCs could be utilized for successful generation of lysate-pulsed dendritic cell in future pre-clinical settings.

**Keywords:** Cancer Stem Cell, Dendritic Cell, Colorectal Cancer, Hanging Drop

ICIA2018\Tolerance and Autoimmunity\Poster\8537
8537

**Evaluation of IL-17 Producing Memory Effector CD26 high T Cells in Patients with Psoriasis (Based on PASI Score)**

Behnaz Esmaeili[1, 2], Parvin Mansouri[3], Alipasha Meysamie[4], Maryam Izad[1*]

*1. Immunology Department, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
*2. Immunology, Asthma and Allergy Research Institute (IAARI), Tehran University of Medical Sciences, Tehran, Iran*
*3. Skin and Stem Cell Research Center, Tehran University of Medical Sciences, Tehran, Iran*
*4. Department of Community and Preventive Medicine, Tehran University of Medical Sciences, Tehran, Iran*

**Background :**Psoriasis is a chronic, T cell-mediated inflammatory skin disorder in which Th-17 and its related inflammatory cytokines have a fundamental role in its pathogenesis. Human Th-17 cells express high level of surface CD26. We analyzed the IL-17 producing effector memory T cells (CD26 hi memory T cells) from patients with psoriasis following ex-vivo stimulation with respect to PASI score. Also, to investigate the inflammatory responses that can affect effector T cells differentiation, the level of IL-23, IL-6, TNFα, TGFβ and IL-17 cytokines were monitored as well.
**Methods:** Memory T cells were isolated from 10 patients with psoriasis (6 moderate to severe and 4 patients with mild form) and 10 controls. Ex vivo stimulated IL-17 producing effector (Foxp3+ CD25+ CD26hi) memory T cells were analyzed by flow cytometry. IL-23, IL-6, TNFα, TGFβ and IL-17 cytokine levels were also evaluated by ELISA.
**Results:** Although the IL-17+ effector memory CD26hi T cells reduced in patients compared to controls, it was not significant (P =0.062). However, IL-17+ effector memory CD26hi T cells from patients with mild form showed a significant decrease compared to controls. (p=0.03). We could not find any significant difference regarding IL-23, IL-6, TNFα, TGFβ and IL-17 cytokine levels in plasma and cell culture supernatant samples between patients and controls.
**Conclusion:** Taken together, our results showed that IL-17+ effector memory CD26hi T cells in patients with psoriasis had a decreased trend compared to controls. A significant decrease in IL-17+ effector memory CD26hi T cells only in patients with mild form could be because there are increased number of circulating effector T cells in patients with moderate to severe form.

**Key words:** Psoriasis, CD26, IL-17

ICIA2018\Tolerance and Autoimmunity\Poster\8558
**8558**

# Diminished serum levels of anti-tetanus toxin antibodies in patients with hypothyroidism

Fahimeh Rostamabadi[1], Hossain Khorramdelazad[1], Mohammad Taghi Rezayati[1], Azam Bagherizadeh[1], Shekoofeh Moghadam[1], Shima Bazaz[1], Fahimeh Mohammadi[1], Abdollah Jafarzadeh[2]

1. *Department of Immunology, Medical School, Rafsanjan University of Medical Sciences, Rafsanjan*
2. *Department of Immunology, Medical School, Kerman University of Medical Sciences, Kerman, Iran*

**Background and Aims:** Many disorders including decreased weight of lymphoid organs, decreased count of lymphocytes and suppression of the lymphocytes-related immune response were demonstrated in experimental models of hypothyroidism. The aim of this study was to evaluate the serum levels of anti-tetanus toxin antibodies (anti-TTA) in patients with hypothyroidism.
**Material and Methods:** Totally, 24 patients with hypothyroidism and 24 age- and gender-matched healthy individuals were enrolled to study. All participants were vaccinated against tetanus according to the national immunization program. The presence of hypothyroidism confirmed according to the clinical and para-clinical criteria such as the levels of thyroid hormones. The serum samples of participants tested for the levels of anti-TTA by ELISA method. The serum antitoxin concentration 0.1 IU/mL was considered as a protective level of antibody.
**Results:** The seroprotective rate against tetanus was significantly lower in hypothyroidism patients than healthy group (P=0.02). The mean titer of anti-TTA was in hyperthyroidism group was also lower than healthy group (3.28 ± 0.48 IU/ml vs and 1.67 ± 0.33 IU/ml, P<0.01). There was no significant difference between men and women concerning the serum levels of anti-TAA antibodies neither in hyperthyroid patients nor in healthy subjects.
**Conclusion:** These results showed that hypothyroidism might lead to the lower titer of anti-TTA antibodies and reduce the protection against tetanus. The titer of anti-TTA was not affected by gender of participants.

**Keywords:** Hypothyroidism, Protection, Tetanus, Anti-toxin antibody.

ICIA2018\Tolerance and Autoimmunity\Poster\8558
**8558**

# Diminished serum levels of anti-tetanus toxin antibodies in patients with hypothyroidism

Fahimeh Rostamabadi[1], Hossain Khorramdelazad[1], Mohammad Taghi Rezayati[1], Azam Bagherizadeh[1], Shekoofeh Moghadam[1], Shima Bazaz[1], Fahimeh Mohammadi[1], Abdollah Jafarzadeh[2]

1. *Department of Immunology, Medical School, Rafsanjan University of Medical Sciences, Rafsanjan*
2. *Department of Immunology, Medical School, Kerman University of Medical Sciences, Kerman, Iran*

**Background and Aims:** Many disorders including decreased weight of lymphoid organs, decreased count of lymphocytes and suppression of the lymphocytes-related immune response were demonstrated in experimental models of hypothyroidism. The aim of this study was to evaluate the serum levels of anti-tetanus toxin antibodies (anti-TTA) in patients with hypothyroidism.
**Material and Methods:** Totally, 24 patients with hypothyroidism and 24 age- and gender-matched healthy individuals were enrolled to study. All participants were vaccinated against tetanus according to the national immunization program. The presence of hypothyroidism confirmed according to the clinical and para-clinical criteria such as the levels of thyroid hormones. The serum samples of participants tested for the levels of anti-TTA by ELISA method. The serum antitoxin concentration 0.1 IU/mL was considered as a protective level of antibody.
**Results:** The seroprotective rate against tetanus was significantly lower in hypothyroidism patients than healthy group (P=0.02). The mean titer of anti-TTA was in hyperthyroidism group was also lower than healthy group (3.28 ± 0.48 IU/ml vs and 1.67 ± 0.33 IU/ml, P<0.01). There was no significant difference between men and women concerning the serum levels of anti-TAA antibodies neither in hyperthyroid patients nor in healthy subjects.
**Conclusion:** These results showed that hypothyroidism might lead to the lower titer of anti-TTA antibodies and reduce the protection against tetanus. The titer of anti-TTA was not affected by gender of participants.

**Keywords:** Hypothyroidism, Protection, Tetanus, Anti-toxin antibody.

ICIA2018\Tolerance and Autoimmunity\Poster\8587
**8587**

# Analysis of Th cell Subsets and Regulatory T Cells in Rheumatoid Arthritis Patients with Different Patterns of Response to Treatment

Seyede Rahele Tabatabaei Jafari[1], Niloofar Alvandpour[1], Ahmad Tahamoli Roodsari[2], Zahra Basiri[2], Mehrdad Hajilooi[1], Zohreh Zarei Ghane[1], Ghasem Solgi[1*]

*1. Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.*
*2. Division of Rheumatology, Department of Internal Medicine, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.*

**Background:** Rheumatoid arthritis (RA) as a chronic inflammatory autoimmune disease is characterized by infiltration of activated inflammatory cells particularly, $CD4^+$ T cells into the rheumatoid joints. This study aimed to evaluate the frequencies of Th1, Th2, Th17 and regulatory T cells (Tregs) in peripheral blood samples of RA patients based on the clinical response to treatment.
**Methods:** Forty three RA patients under treatment with disease-modifying antirheumatic drugs (DMARDs) and 15 healthy control subjects were recruited in this case-control study. Patients were subdivided to good-responders (n=23) and poor-responders (n=20) according to the therapeutic response. Frequencies of Th cell subsets as well as Tregs were determined in peripheral blood samples and then compared between different groups of the study.
**Results:** The percentage of Th1 cells in both groups of the patients were found to be significantly higher than controls [good-responders (7.42±4.22%) and poor-responders (7.25±3.75%) *vs*. 2.93±1.57% in the controls, P<0.001]. However, there were no significant differences in the frequencies of Th2 (1.70±٪۰/۹۶*vs.* 1.45±0.73 %, P=0.22), Th17 (0.58±٪۰/۴۷*vs.* 0.51±0.10%, P=0.39) and Treg cells (0.36±٪۰/۱۸*vs.* 0.40±0.19%, P=0.71) in all patients compared to healthy controls.
**Conclusion:** Our results highlighted an increased frequency of Th1 cells in RA patients regardless of good or poor responses to DMARDs in compare with healthy controls.

**Keywords:** Rheumatoid arthritis, Th1, Th2, Th17, Regulatory T cells.

ICIA2018\Vaccine\Poster\8595
**8595**

## Designing and engineering of immunogenic peptides of *Acetinobacter baumannii* OmpA

Kobra Mehdinejadiani[1], Mojgan Bandehpour[2], Mohammad mehdi ranjbar[3],Ali Hashemi[4]*, Seyed Amir Jalali[1], Vahid Yardel[5], Nariman Mossafa[1*]

1. *Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences,Tehran,Iran*
2. *Department of Biotechnology,School of Advanced Technologies in Medicine,Shahid Beheshti University of Medical Sciences, Tehran,Iran*
3. *Department of Immunology,School of Veterinary Medicine , Tehran University of Medical Sciences,Tehran,Iran*
4. *Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences,Tehran,Iran*
5. *Laleh hospital,Tehran,Iran*

**Background**: *Acinetobacter baumannii* is a Gram-negative bacterium that Recently, it has been being known as a leading nosocomial pathogen. As respects, causing infections by this pathogen is high mortality due to inadequate available treatment. An effective vaccine is an unmet medical need. In this study, considering the fact that outer membrane protein A (OmpA) one of the main vaccine candidates, we present T cell and B cell predicted epitopes by sequence-based epitope prediction tools.
**Methods**: At first, OmpA was amplified from *A.baumannii* genomic DNA. Then genomic sequence of OmpA was determined and translated to protein sequence. We submitted the full-length OmpA protein in B cell linear epitope predictors (LBtope, SVMTrip, ABCpred, BepiPred, BcePred, BCPred, IEDB). Predicted linear B cell epitopes were resubmitted for T cell epitope predictions using the MHC-II Binding Predictor in the Immune Epitope Database (IEDB). On the other hand, we investigated tertiary and secondary structures of protein that we could select the best epitopes.
**Results**: Consensus epitopes in different tools with the highest score were selected and engineered. The epitopes contain sequences of F317-332, F122-139, F230-245, F24-50, F197-222.
**Conclusion**: We reason that mentioned peptides could provide protection against *Acinetobacter baumannii* nosocomial infections thereby functioning as potential vaccine candidates.

**Key words**: *Acetinobacter baumannii*, OmpA, Immunogenic peptides, Antigenome technology

ICIA2018\Veterinary Immunology\Poster\8602
**8602**

**Avian Influenza Investigation in Some Aquatic Birds**

AbdolrezaNabinejad[1] (DVM,Ph.D), Nooshin Askarani[2] (MS)

1. *Isfahan research and education center of agriculture and natural resources nabinejad@yahoo.com*
2. *Isfahan research center of teachers, Software group*

**Background**: Influenza type A viruses are of the most significance to public health due to their potential to cause an influenza pandemic. Influenza type A viruses are classified into subtypes according to the combinations of different virus surface proteins hemagglutinin (HA) and neuraminidase (NA). So far there are 18 different hemagglutinin subtypes and 11 different neuraminidase subtypes such H9N2, H5N1 and H5N8 or H7N5 as reported in birds of Iran. Aquatic birds are the primary natural reservoir for most subtypes of influenza A viruses. There are a few of aquatic birds included Swan, Pelican, Sea gull, Ducks, Flamingo and Goose in the Isfahan bird garden ,current report goes to monitoring of these aquatic birds for AIV by serological and molecular tests.
**Methods**: Using a sterile siring about 2 ml of blood of wing vein collected and transfered to lab for AIV titration, and coanal cleft and cloacal swabs were prepared for molecular test.monthly monitoring of 5-6% of sensitive birds were examined randomly; The technical method for serology were HI , ELISA and RT-PCR for molecular and confirmation of suspected titers.
**Result**s: All of the birds showed positive titer for H9N2 but the highest titer were 9 for goose and green head duck. The lowest titre was one and related to pelicans, Flamingos. The sera were negative for H5N1 and H5N8 and H7 but the swabs were checked by RT-PCR technique which confirmed the negative titers
**Conclusion**: This monitoring monthly is carried out and sanitization of garden and gates and with widely antiseptic material carried out daily.

ICIA2018\Veterinary Immunology\Poster\8603
**8603**

# Investigation about Prevalence of Anthrax in the Western Cities of Kermanshah Province, During 2010- 2015

Mehrdad Pooyanmehr[1], Erfan Bahrami[2], Mohammad Reza Khedri[2], Amir Amiri Paryan*[3]

*1. Department of Basic Sciences and Pathobiology, Microbiology section, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran*
*2. Veterinary Student, Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran*
*3. Veterinary Student, Pathobiology & Basic Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran*

**Background:** Zoonotic diseases constitute a public health problem throughout the world. Anthrax is a potentially fatal zoonotic disease and is an important zoonotic disease which is primarily a job-related infection caused by Bacillus Anthracis. The diagnosis of cutaneous anthrax (CA) may be very difficult, particularly in atypical presentations and non-endemic regions. Aim this study, anthrax cases seen between 2010 –2015 (six years) in the western area of Kermanshah province.
**Methods:**Medical records of infected in patients with CA (Clinical, Para clinical information and pathological diagnosis of disease CA such as microbiologic procedures) who had been operated in referral Health Centers Western cities areas of Kermanshah province (Ravansar, Javanrood, Pavehe and Salasebabajany) were collected in the period six years (2010 – 2015). One patient with CA was included in this study.
**Results:**Results indicated that All patient's referent one patient were who diagnosed CA in 4 cities medical center registries, (Ravansar 1, Javanrood 0, Pavehe 0 and Salasebabajany 0 patients) at six years' periodrespectively, per 100,000 (0.001%) person. One patient with a diagnosis of CA was followed up. One patient had a history of animal contact. The clinical presentation of CA was typical in one patient in 2015 (1 case) in Ravansar. Patient was initially misdiagnosed with insect bites or angioedema. Cultures from the lesions were positive for Bacillus Anthracis in one case. Gram stain from the lesions revealed Gram-positive rods in one case. Patient were diagnosed by clinical presentation and a history of contact with sick animals or contaminated animal products.
**Conclusion:** CA is a very contagious and important infectious disease worldwide. Early and accurate diagnosis dramatically affects the prognosis of the disease. The diagnosis of CA may be difficult, especially in atypical presentations and non-endemic areas. Thus, CA should be kept in mind, especially in these situations.

**Keywords:** Bacillus Anthracis, Zoonotic diseases, Western Cities Area, Kermanshah Province

ICIA2018\Tolerance and Autoimmunity\Poster\8604
**8604**

# Investigation of the Frequency of Natural Killer Cells Expressing CD16 bright in Peripheral Blood of Multiple Sclerosis and Neuromyelitis Optica Syndrome Disease Patients by Flow Cytometry

Leila Khani[1], Mir Hadi Jazayeri[2], Mahmood Bozorgmehr[3], Seyed Masood Nabavi[4], Leila Janani[5]

*1. MS student, Department of Immunology, School of Medicine, Iran University of Medical Science, Tehran, Iran*
*2. Assistant Professor, (a) Department of Immunology, School of Medicine, (b) Immunology Research Center Iran University of Medical Science, Tehran, Iran*
*3. Oncopathology Research Center, Iran University of Medical Science, Tehran, Iran*
*4. Associate Professor of Neurology,(MS Fellow, Clinical Epidemiology Expertise Degree)Neurology Group, Department of Regenerative Biomedicine, Cell Science Research Center, Neuroscience and Cognition Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran*
*5. PhD Department of Biostatistics, School of Public Health, Iran University of Medical Science, Tehran, Iran*

**Background:** Multiple Sclerosis (MS) and Neuromyelitis Optica Syndrome Disease (NMOSD) are inflammatory diseases of central nervous system. Despite the similar manifestations of these two diseases, immunopathogenesis and the treatment of these two conditions are totally different. Natural killer cells (NK) are immune cells which play an important role in defending and regulating immune responses. There are two distinct subsets of NK cell based on CD16 and CD56 expression in peripheral blood. $CD16^{bright}$ $CD56^{dim}$ has high cytotoxic activity (90%). NK cells expressing $CD16^{dim}$ $CD56^{bright}$ are usually located in lymph organs (10%), with low cytotoxic activity, in spite of high cytokine production, whereas there is conflicting evidence of NK cells roles in the pathophysiology of MS and NMO. The aim of this study was to compare the frequency of NK cells in peripheral blood of MS and NMO patients.
**Methods:** 15 Naïve MS, 15 relapsing-remitting (RR) MS, 15 NMOSD and 15 healthy subjects were included in our study. The percent of NK cells were evaluated by flow cytometry.
**Results:** The $CD16^{bright}$ median of RR MS, Naïve MS, NMOSD and healthy groups was 7.72, 4.92, 2.13 and 8.10, respectively. There was a significant difference between RR MS and Naïve MS (p=0.029), RR MS and controls (p=0.002), although no significant differences were found between RR MS and NMOSD (p=0.00), Naïve MS and NMOSD (p=0.056), controls and NMOSD (p=0.868), Naïve MS and controls (p=0.187) groups.
**Conclusion:** The present study demonstrated there is a lower frequency of NK cells expressing $CD16^{bright}$ in peripheral blood of Naïve MS and NMO patient comparison to healthy and RR MS subjects. Actually, it seems immunomodulatory drugs prescribed to RR MS patients lead to increases $CD16^{bright}$ frequency. Therefore, more studies are needed to understand the role of different NK cell subsets in pathogenesis and their uses in therapeutic processes.

**Keywords**: Multiple Sclerosis, Neuromyelitis Optica Syndrome Disease, Natural Killer Cell Subsets, Flow Cytometry

ICIA2018\Reproductive Immunology\Poster\8634
**8634**

# Investigation the relationship between C677T polymorphism in MTHFR gene and plasma homocysteine concentration in recurrent fetal miscarriage

Omid Kooshkaki[1], Gholamreza Ananisarab[2], Yalda Zarafshan[3]

1. *Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran*
2. *Department of immunology-School of medicine-Birjand University of medical sciences*
*Birjand University*

**Background:** Recurrent miscarriage is a painful experience for both the physician and the parent. It occurs in 2 to 5 percent of pregnant women. The causes of recurrent abortion are genetic, anatomical, hormonal, and immunological. Recurrent miscarriage etiology is not known in 40 to 50% of cases. Single nucleotide polymorphisms in the coding genes of the enzymes, which regulate metabolic pathways such as methylene tetrahydrofolate reductase (MTHFR), are considered as one of the important contributors to the development of thrombophilia. Thrombosis seems to be a disturbance in circulation between mother and fetus in the pairs of capillaries and ultimately leads to abortion. The aim of this study is to determine the association between C677T polymorphism in MTHFR gene and plasma homocysteine concentration in recurrent fetal miscarriages.
**Methods:** Overall, 100 women were included in the research, the case group comprised of 50 women who had a history of spontaneously recurrent miscarriage with unspecified cause, whereas the controls consisted of 50 women with a history of at least two successful fertility . Methods used in the study included PCR-RFLP with limited effective Hinf-I enzyme in order to investigate polymorphism and ELISA analysis to investigate plasma homocysteine concentration.
**Results:** Based on our results, in the control group, 15 (30%) had heterozygote and 4 (8%) had mutated homozygotes, i.e., a total of 19 subjects (38%) were abnormal. Also, ELISA results showed that, the mean homocysteine level in the case group was higher than that in the control group (P=0.002).
**Conclusion:** Studies in different populations have shown different results for the association between MTHFR polymorphisms and recurrent miscarriages. This relationship observed in our study.

**Keywords:** Polymorphism, MTHFR gene, plasma homocysteine, frequent abortion

ICIA2018\Veterinary Immunology\Poster\8655
**8655**

# Identification of Avian Influenza Virus in Broiler Chickens in Gilan Province

Omid ramezani[1], Jalal shayegh[2], Hossein hosseini[3], Marzie sadeghi[4*]

*1. DVM, Department of Veterinary Clinical Sciences, Shabestar Branch, Islamic Azad University, Shabestar, Iran*
*2. Department of Clinical sciences, Faculty of the Veterinary Medicine, , Shabestar Branch, Islamic Azad University, Shabestar, Iran*
*3. Department of Clinical sciences, Faculty of the Veterinary Medicine, Karaj Branch, Islamic Azad University, Karaj, Iran*
*4. student, Department of Clinical Sciences, Faculty of the Veterinary Medicine, University of Shahrekord, Shahrekord, Iran*

**Background:** Avian Influenzais a contagious and acute respiratory diseases of great importance to public health, which causes many economic losses. Several methods have been proposed for identifying known viruses such as ELISA and HI test but in terms of the speed of RT-PCR detection, it is probably one of the most important methods. The purpose of this study is to identify and detection viruses in Gilan province.
**Methods**: For this purpose, 66 samples of swabs from different cities of Gilan province were collected and analyzed using RT-PCR method.
**Results:** The results showed that 22.73% of the samples were positive, and biosecurity measures should be taken to prevent the spread of the disease.

**Keywords:** broiler chicken, RT-PCR, influenza, Gilan

ICIA2018\Tolerance and Autoimmunity\Poster Discussion\8665
**8665**

# Expression Analysis of PD-1 and Tim-3 Immune Checkpoint Receptors in Vitiligo Patients

Ali Rahimi[1], Hadi Hossein Nattaj[1], Zohreh Hajheidari[2], Abolghasem Ajami[1*], Hossein Asgarian Omran[1, 3*]

*1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
*2. Department of Dermatology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
*3. Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

**Background:** Vitiligo is a skin-related chronic autoimmune disease that degrades melanocytic cells, causing colorless and asymptomatic stains. In recent years, the contribution of immune checkpoint receptors has been addressed in the pathogenesis of multiple autoimmune diseases. In the present study, the expression profile of T-cell immunoglobulin and mucin-domain containing-3 (Tim-3) and programmed cell death-1 (PD-1) checkpoint molecules was investigated on $CD8^+$ T cells of patients with vitiligo as the main pathogenic effector cells in this autoimmune complication.
**Methods:** A total of 30 vitiligo patients and 30 sex- and age-matched healthy controls were included in this study. To determine the frequency of $Tim\text{-}3^+/PD\text{-}1^+/CD8^+$ T cells, PBMCs were stimulated with PHA for 72 h and a three-color flow cytometry method was applied. To measure the cytokines production, PBMCs were stimulated with PMA/ionomycin for 18 h and concentrations of IL-4, IFN-γ and TNF-α were measured in culture supernatants by ELISA. $CD8^+$ T cells were then positively isolated from all participants by magnetic beads separation method and the mRNA expression of PD-1 and Tim-3 molecules was determined by TaqMan based Real-Time PCR.
**Results:** Vitiligo patients have significantly showed more expression of Tim-3 and PD-1 on the surface of their $CD8^+$ T cells than that of normal controls. While, the production levels of TNF-α and IFN-γ were found higher by vitiligo patients than those of controls, IL-4 production was lower in patients with vitiligo. Expression analysis of Tim-3 and PD-1 mRNA confirmed the results obtained from flow cytometry and showed more expression of Tim-3 and PD-1 in $CD8^+$ T cells of vitiligo patients compared to normal individuals.
**Conclusion**: Our results indicate that Tim-3 and PD-1 are involved in immune dysregulation mechanisms of $CD8^+$ T cells in vitiligo. Further studies are needed for better understanding of Tim-3 and PD-1 roles in immunopathogenesis of vitiligo and may introduce useful biomarkers for disease progression and/or immunotherapy.

**Keywords:** Vitiligo, $CD8^+$ T cells, Tim-3, PD-1, Flow cytometry, Real-Time PCR

ICIA2018\Research and Development\Poster\8670
**8670**

# Establishment and introducing of cell line collections to support the immunological investigations in Iran

Parvaneh Farzaneh[1], Seyed Abolhassan Shahzadeh Fazeli[1, 2]

*1. Human and Animal Cell Bank, Iranian Biological Resource Center (IBRC), ACECR, Tehran, Iran*
*2. Department of Molecular and Cellular Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran*

**Background:** Continuous and primary cell cultures are excellent models to study the different biological and pathological aspects of *in vitro* studies. Establishment, collection and distribution of several collections of authenticated contamination free cell samples for various immunological studies can boost research.
**Methods:** To support all topics of immunological investigations we divided the cell collections in two categories. The first one consists of continuous standard cell lines with specific identity which are distributing worldwide by cell banks and the second kinds of required collections were prepared from Iranian native population or laboratory animals. Collections of lymphoblastoid cell lines established by EBV transformation of patients with type one immunodeficiency, also collections of different autoimmune diseases and several types of cancers established by this method. Primary cultures of solid tumor, stromal stem cells of different tissues were two other collections with complete cell characterization and identity.
**Results:** Human and animal cell bank is distributing different cell collections such as cells related to autoimmunity, immunodeficiency, cancer and laboratory animals, which support immunological investigations.
**Conclusion:** Introducing of standard cell line collections may improve and facilitate immunological investigations in Iran.

**Keywords:** Cell line, Collection, Immunology, Investigation

ICIA2018\Tolerance and Autoimmunity\Poster\8677
**8677**

# Evaluation of ATP and HIF-1-alpha in Brain of EAE Mouse Model of Multiple Sclerosis Disease

Seyedeh Mahboobeh Mosavi Mehr, Seyed Alireza Mesbah Namin

*1. Department of Clinical Biochemistry, Faculty of Medical Sciences, TarbiatModares University, Tehran, Iran*

**Background:** Multiple sclerosis (MS) is a chronic autoimmune and inflammatory neurological disease of the central nervous system. Dysfunction of mitochondria of the nervous system to produce enough energy also affect the complete myelination, followed by inflammation in neurons occurred in patients with MS. The aim of this study is evaluation of ATP and hypoxia-inducible factor 1-alpha (HIF1-a) which is considered as the master transcriptional regulator of cellular and developmental response to hypoxia, in brain tissue of mouse model of MS disease.
**Methods:** The C57BL/6 mice were purchased from the Pasture Institute and Hooke kit used for inducing experimental autoimmune encephalomyelitis (EAE), the mouse model of MS disease. High Score of this model (3 to 4) used in this study. The brain tissues of mice were examined for two sets of experiments, one for estimation of HIF1-a using special Elisa kit from Zell Bio GmbH Company and other for measurement of ATP using especial assay kit from Sigma-Aldrich company, after deproteinizing of the samples in 3 group of sham, negative control and EAE mice.
**Results**: Amount of ATP, in contrast to HIF1-a, was significantly decreased in EAE model in comparison with sham and negative control and significant reducing of HIF-1 a, indicating of existence of hypoxia in brain of EAE mice, as expected.
**Conclusion**: Significant attenuation of ATP and existence of hypoxia in brain of EAE mice indicated that the function of mitochondria of the nervous system of EAE impaired to produce of energy affecting of neurogenerative process in this model of MS disease.

**Keywords:** Multiple sclerosis, EAE, ATP, Hypoxia

ICIA2018\Stem Cells Based Immunotherapy\Poster\8687
**8687**

# Optimizing the differentiation of human umbilical cord Wharton's jelly mesenchymal stem cells into perimordial germ-like cells

Samira Ghasemi[1,2], Mohammad Shafiee[1,3], Mohsen Saeedi[1,4], Naser Mobarra[1,5]

1. *Stem Cell Resaerch center,Golestan University of Medical Sciences, Gorgan. Iran.*
2. *Department of Advanced Medical Technologies, Golestan University of Medical Sciences, Gorgan. Iran.*
3. *Department of Medical Genetics, Golestan University of Medical Sciences, Gorgan. Iran.*
4. *Department of Medical Imonology, Golestan University of Medical Sciences, Gorgan. Iran.*
5. *Department of Clinical biochemistry, Golestan University of Medical Sciences, Gorgan. Iran.*

**Background:** Infertility affects 15-25% of couples of reproductive age. An increasing prevalence of infertility is one of the many problems in countries around the world. Assisted reproductive technologies are not successful in some cases. In recent years, the quantity and quality of germs has been reduced. A novel approach to treating infertility is the use of stem cells. In this study, optimizing the differentiation of human umbilical cord Wharton's jelly mesenchymal stem cells into perimordial germ-like cells has been performed.
**Methods:** Human warton's Jelly Mesenchymal Stem Cells (hWJ-MSCs) were prepared. Cell surface markers and their differentiation potential were evaluated. Then the induction of the differentiation of these cells into germ like cells was performed using BMP4 and RA. Expression changes of Oct-4, c-kit, Stella, and Vasa genes were evaluated by Real Time PCR. All data were significant.
**Results:** The morphological changes of hWJ-MSCs, as well as the expression of Oct-4, c-kit, Stella, and Vasa mRNA and protein markers indicate the successful differentiation of these cells into primordial germ like-cells. Also all data was significant. (P value <0.05).
**Conclusion:** Our results showed that the differentiation of human umbilical cord Wharton's jelly mesenchymal stem cells into perimordial germ-like cells well done with a slight variation in existing differentiation protocols. It can be used to treatment of infertility problems caused by impairment in the structure or function of germ cells.

**Key words:** differentiation, PG- like Cell, hWJ-MSC

ICIA2018\Reproductive Immunology\Poster\8691
**8691**

# The effect of active form of 1,25VitD3 on Treg /Th17 axis (percentages, gene expressions and cytokines level) in patients with Unexplained Recurrent Spontaneous Abortion (URSA)

Elham Abdollahi[1, 2], Houshang Rafat panah[3, 4], Seyed Abdolrahim Rezaee[4], Maryam Rastin[5]

*1. Immunology Research Center, Department of Medical Immunology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*2. Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran.*
*3. Research Center for HIV/AIDS, HTLV1 and Viral Hepatitis, Iranian Academic for Education, Culture and Research (ACECR), Mashhad Branch, Mashhad, Iran.*
*4. Inflammation/Inflammatory Diseases Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
*5. Immunology Research Center, Bu-Ali institute , Mashhad University of Medical Sciences, Mashhad, Iran*

**Background:** Unexplain Recurrent Spontaneous abortion (URSA), defined as three or more consecutive pregnancy losses before the 20th week of gestation, occurs in 1%–5% of women of reproductive age. CD4+CD25+ CD127- FoxP3+ Treg cells constitute a minority of the CD4+ T-cell population in peripheral blood. T helper 17 (TH17, $CD3^+$ $CD8^-$ $IL\text{-}17^+$) cells involve in pathogenesis of chronic inflammatory diseases as well as URSA. Treg/Th17 ratio increases in patients with URSA compared to healthy pregnant women. Vitamin D3 has been shown to inhibit Th17 cell responses and to induce differentiation of Tregs and/or expansion of fork head box protein 3 (FOXP3) regulatory T cells. Considering the imbalance of Th17/Treg in RPL patients, and the effects of Vitamin D3 on these two cell subsets. The aim of this study was to evaluate the effect of vitamin D on the alternation of Treg/Th17 ratio in patients with URSA.
**Methods:** 20 patients with URSA were sampled for 10 ml whole blood to isolate peripheral blood mononuclear cells (PBMCs) using ficoll-hypaque density gradient centrifugation. Isolated cells were cultured in the presence of 50 nM 1,25VitD3. T reg and Th17 cells were analyzed by flowcytometry after and before treatment with 1,25VitD3.
**Results:** TGF-B gene expression and IL-10 gene expression also increased in patients with URPL (P value<0.05) after treatment with vitD (P value<0.05). Significant increase in Il-10 level also observed in patients. The results indicated that vitamin D increase Treg/Th17 ratio. There was a significant difference between the percentage of $CD4^+CD25^{bright}$ $CD127^-$ T cells before and after treatment with vitamin D (cells (0.59% vs1.24% P<. 05).
**Conclusion:** This study showed that the role of vitamin D3 seems to be to provide an anti-inflammatory condition which is in favor of pregnancy maintenance. We can conclude this metabolite can exert as a *supplementary therapeutic* in patients with URSA.

**Key words:** URSA, T regulatory cells, Th17 cells, 1,25VitD3

ICIA2018\Tolerance and Autoimmunity\Poster\8724
**8724**

# Expression of miR-326 Relapsing-remitting Multiple Sclerosis Responders and Nonresponders to Interferon Beta Therapy

Mahtab Fattahi[1], Nahid Eskandari*[2], Fattah Sotoudehnejad[1], Vahid Shaygannejad[3]

1. *Department of biology, science and research branch, Islamic Azad University*
2. *Department of Immunology, School of Isfahan Medical, Isfahan University of Medical Sciences*
3. *Department of Neurology, Isfahan Neurosciences Research Center, Isfahan University of Medical Sciences*

**Background:** Multiple sclerosis (MS) is a chronic, inflammatory and demyelinating disease that affects the central nervous system. Th17 cells are the main mediators of inflammatory process in autoimmunity disease including MS. miRNAs are likely involved in most biological processes, and play important roles in the immune system function. Dysregulated expression of miRNAs is associated with pathological conditions, including autoimmune diseases. Interferon beta (IFN-β) represents one of the most commonly administered drugs for the treatment of the RR-MS patients. The purpose of this study was to evaluate miR-326 expression in responders and nonresponders to interferon beta treatment.
**Methods:** A total of 70 patients including responders and nonresponders to INF*B* (35) were enrolled. We analyzed the expression level of miR-326 using peripheral blood from RR-MS patients at 12 months after starting with IFN-β therapy. Real-time qPCR was performed to analyze miR-326 expression.
**Results:** These results indicated miR-326 expression particularly reduced in responders compared to nonresponders, although this was not statistically significant.
**Conclusions:** Overall, miR-326 expression levels may act as a marker of the biological effects of IFN-β therapy.

**Keywords**: Interferon-beta, Multiple sclerosis, miR-326

ICIA2018\Reproductive Immunology\Poster\9737
**9737**

**Antisperm antibodies in the serum of unmarried woman (virgin): a case study**

Ali Al-fahham[1]

*Department of Basic Science Faculty of Nursing University of Kufa*

**Background:** The study of antisperm antibodies (ASA) etiology is very important in the diagnosis and treatment of infertility. Studies of presence of antisperm antibodies in the bodies of unmarried women (virgins) are very rare, so that this article aims at studying, in more details, the possible causes and interpretations behind the development of antisperm antibodies in virgins.
**Methods:** This descriptive case study included 35-years old single women with positive ASA. Description and clinical history of the patients were assessed by special questionnaire provided for this purpose. The concentration of serum ASA was estimated by using Enzyme-Linked Immunosorbent Assay (ELISA). Complete blood count was done by using HumaCount System. Total serum IgG and IgM was calculated by Radial immunodiffusion assay. The isolated bacteria were identified according to microscopically, biochemical tests and VITEK 2 system. All laboratory investigations and diagnostic procedures were done in the hospital between 1st to 15th December 2017.
**Results:** The mean serum antisperm antibody concentrations is (62.1 IU/ml), it is considered as positive titer. Complete blood count is normal, except for slight increase in WBC count and percentage of basophils, monocytes and lymphocytes. This study recorded high concentrations of serum total IgG and IgM levels (1870 IU/ml and 255 IU/ml respectively). The UTI was confirmed by counting total bacterial concentration (167220 CFU/ml) in the urine, and diagnosis of suspected causes showed the following species: *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus haemolyticus* and *Proteus mirabilis.*
**Conclusion:** There is two suggested mechanisms to explain ASA in virgins: (1) antigen cross-reactivity between sperm and bacterial antigens to which antibodies can react; (2) induction of the immune system by antigens of sperm ingested into the gastrointestinal tract with contaminated food and drink.

**Keywords:** Antisperm, Antibodies, virgins, Infertility, Case-Study

ICIA2018\Reproductive Immunology\Poster\9740
**9740**

# Evaluation of the expression of virus related Toll like receptors in placenta from hydatidiform mole patients

Mahboobeh Zolala[1], Mohhamad Mahdi Mohhamadi[1], Shahriyar Dabiri[2], Maryam Iranpoor[2], Manzoomeh Shamsi Meymandi[3], Fatemeh Raeisi Nejad[4]

1. *Department of Immunology School of Medical University of Kerman*
2. *Department of Pathology School of Medical University of Kerman*
3. *Department of Physiology School of Medical University of Kerman*
4. *Department of Immunology School of Medical University of Jiroft*

**Background:** Mole hydatidiform is an abnormal pregnancy which is characterized by over proliferation of trophoblasts and vesicular edema in placental villi with or without embryo. The basic etiology of gestational trophoblastic neoplasia is unknown. During trophoblastic disease a considerable over proliferation in placenta trophoblasts in seen which in some cases cause malignancy. In this study, we examined viral TLRs (TLR3,7,8,9) in HM placenta samples using IHC technique to possibly find a correlation between viral infection and HM. in this study, The expression levels of viral TLRs were evaluated in normal placenta and compared with HM ones.
**Method:** In a case/control study, 39 samples of HM and 43 samples of control obtained from archive of pathology laboratory of target hospital. Certain viral TLRs were monitored using IHC assays. The obtained data were statistically analyzed using Mann Whitney U method.
**Results:** The differences in TLR3 and TLR9 expression levels between HM and control samples were statistically significant (P value = 0.0001 and 0.03, respectively). In case of TLR7 and TLR8 no significant difference were observed.
**Conclusion:** Results of this study elucidated the increased expression of TLR3 and TLR9 markers in HM samples comparing control ones. We concluded that HM may be related with dsRNA and CpG DNA virus infections. As TLR9 response is observed during both viral and bacterial infections, it seems that a bacterial infection may be also involved in generation of HM. The expression levels of TLR7 and TLR8 markers in HM samples were same as control ones. So, it could be concluded that ssRNA virus are not involved in HM generation.

**Key words:** mole hydatidiform, Toll like receptor, immunohistochemistry, placenta

ICIA2018\Reproductive Immunology\Poster\9756
**9756**

# Pro-inflammatory Th17 type and regulatory T cell cytokines in women with unexplained recurrent spontaneous abortion

Narges Roomandeh[1, 2], AboTaleb Saremi[2], Javad Arasteh[3], Fatemeh Pak[4], Majid Mirmohammadkhani[5], Parviz Kokhaei[4, 6], Ahad Zare[2, 7]

*1. Student Research Committee and Department of Immunology, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran*
*2. Sarem fertility and infertility research center (SAFIR) and Sarem Cell Research Center (SCRC), Sarem Women's Hospital, Tehran, Iran*
*3. Department of Biology, Faculty of Basic Sciences, Islamic Azad University Central Tehran Branch, Tehran, Iran*
*4. Cancer Research Center and Department of Immunology, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran*
*5. Research Center for Social Determinants of Health, Semnan University of Medical Sciences, Semnan, Iran*
*6. Immune and Gene Therapy Lab, Cancer Centre Karolinska, Karolinska University Hospital, Stockholm, Sweden*
*7. Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Elevated evidences showed that unexplained recurrent spontaneous abortion (URSA) is associated with inflammatory responses and breakage of immunological autotolerance.Th17 cells have an important role in autoimmune disease and loss of tolerance to the fetus. Regulatory T cells play an important role in the maintenance of tolerance during pregnancy. Therefore the balance between Th17 and Treg cells may elucidate the pathophysiology of URSA. In this study we aimed to investigate the serum concentration of regulatory and inflammatory cytokines in both normal and URSA females.
**Methods:** Forty-six women with URSA and 28 non-pregnant control women with at least one successful pregnancy were included in this study. Serum was obtained from both groups and stored at -70$^{oC}$. The serum concentration of IL-17, IL-21, IL-22, IL-10 and TGF-β were quantitatively determined by ELISA.
**Results:** The levels of IL-17, IL-21 and IL-22 in sera were significantly higher in URSA women compared with these in normal controls ($P<0.001$, $P=0.01$ and $P< 0.001$ respectively). Also, TGF-β serum concentration as a Treg cytokine, was significantly lower in URSA patients than that in normal women ($p=0.02$). However, the difference in serum IL-10 level between URSA women and control group was not statistically significant ($P=0.49$).
**Conclusion:** Our results suggest that according to higher serum levels of IL-17, IL-21 and IL-22 and lower level of TGF-β in cases compared with healthy controls, enhancement in Th17 associated cytokines levels and reduction in Treg type cytokine may be one of the factors that indicate increased Th17 cell function and reduced Treg activity in URSA.

**Key words**: IL-21, IL-22, Lymphocyte therapy, URSA

ICIA2018\Tolerance and Autoimmunity\Poster\9773
**9773**

# The Relationship between Serum Level of IL-33 and IL-35 with Amount of Thyroid Auto-antibodies in Pregnant Women with Multiple Sclerosis

Mahdavi R[1], Jamali M[2], Rostami Rad M[3]

1. *Department of Immunology, Birjand University of Medical Sciences, Brigand, Iran*
2. *Department of Immunology, Medical School, Kerman University of Medical Sciences, Kerman, Iran*
3. *Department of Microbiology, Medical School, Tehran University of Medical Sciences, Tehran, Iran*

**Background:** Multiple sclerosis (MS) is an autoimmune-mediated demyelinating disease of the central nervous system (CNS). Interleukine-35 (IL-35) has been described as an immune suppressive cytokine, also IL-33 is a cytokine with both pro- and anti-inflammatory effects. These two cytokines have been implicated in the immune pathogenesis of MS. Thyroid auto-antibody hormones also change during pregnancy and postpartum but these changes may be affected by MS disease. The aim of this study was to determine the association between serum levels of IL-33 and IL-35 with amount of thyroid hormones during early pregnancy and at six months postpartum period in pregnant MS women compared to healty pregnant women.
**Methods**: In case-control study, we analyzed 55 pregnant MS women and 55 healthy pregnant women as a control group from January 2016 to December 2017 in the Afzalipoor Hospital, Kerman (a city located in the southeast of Iran). The serum levels of IL-33 and IL-35 were measured by enzymes linked immunosorbent assay (ELISA) and level of thyroid auto-antibody hormones (anti-TG and anti-TPO) were assessed by electrochemiluminescence (ECL) method.
**Results**: The serum levels of IL-33 and IL-35 in pregnant MS women *during* the *first trimester* were significantly higher compared to control group ($P<0.001$) and pregnant women with MS presented with significantly lower anti-TG and anti-TPO in the beginning of pregnancy ($P<0.01$)
**Conclusions**: We conclude that elevated levels of IL-33 and IL-35 at the beginning of pregnancy may be associated with decreased thyroid auto-antibodies in the first trimester and association between these cytokines with the levels of thyroid hormones at six months postpartum period were reversed.

**Keywords**: Interleukine-33, Interleukine-35, Multiple sclerosis, Thyroid hormone, Pregnancy

ICIA2018\Research and Development\Poster\9786
**9786**
**Compression of different methods for the rapid establishment of B lymphoblastoid cell lines**

Masoumeh Asadi[1], Parvaneh Farzaneh[1], Mehrnaz Izadpanah[1,4], Samaneh Mahmood Aghdam[1], Shiva Mohammadi[1], Zahra Elyasi Gorji[1], Seyed Abolhassan Shahzadeh Fazeli[1,2], Mansoureh Farhangniya[1]

*1. Human and Animal Cell Bank, Iranian Biological Resource Center (IBRC), ACECR and Tehran, Iran*
*2. Department of Molecular and Cellular Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran*
*3. Department of Anatomy, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran*
*4. School of advanced technologies in medicine, Tehran University of Medical Sciences, Tehran, Iran*

**Background**: Recent studies have been reported that Epstein-Barr virus (EBV) immortalization is a Gold Standard method to keep high-molecular-weight DNA in long-term. Therefore, banking of EBV-derived cells has been providing an appropriate reference material for immunologic diseases studying. Since a variety of methods exist for the immortalization of B-lymphocytes by EBV, in this study four different methods were compared to the success rate of immortalization.
**Method**: Blood samples were collected under sterile conditions and thirty samples were considered for each method. Group A: Peripheral Blood Mononuclear Cells (PBMCs) were isolated and then infected with EBV. Group B: PBMC pellets were frozen using 90% FBS, 10% DMSO for two months. Then, the cells were thawed and infected. Group C: whole blood was frozen and then, the frozen blood was thawed and infected. Group D: whole blood was washed and directly infected.
**Result**: Lymphoblastoid cell lines (LCL) were simply generated by fourth methods. All the established the cell lines showed typical rosette morphology while single cells were also seen in the all groups. The success rate of the generated LCL in each group was 86%, 93%, 60%, and 70%, respectively.
**Conclusion**: Our results showed a success rate of the group B was highest. Furthermore, by this method can save time and cost but this method is not applicable for the low volume of blood. Also, it is suggested using D method for blood volume less than 1 ml.

**Keywords**: Lymphoblastoid cell lines, EBV, Success rate

ICIA2018\Veterinary Immunology\Poster\9808
**9808**

# Evaluating the effects of low molecular weight sodium alginate as an immunostimulator, also as a novel prebiotic combined with Bactocell® (a probiotic) on humoral and mucosal immune responses of Asian seabass (*Latescalcalifer*) juveniles

GhasemAshouri[1, 3*], NasrollahMahboobiSoofiani[1], Seyed Hossein Hoseinifar[2], Seyed Amir Hossein Jalali[1, 3], Amir Mani [1], Hien Van Doan [4]

*1. Department of Fisheries, Faculty of Natural Resources, Isfahan University of Technology, Isfahan 84156–83111, Iran*
*2. Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.*
*3. Department of Research Institute for Biotechnology and Bioengineering, Isfahan University of Technology, Isfahan 84156–83111, Iran*
*4. Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200 Thailand*

**Background:** Using immunostimulants as bio-friendly and environmentally safe agents is an alternative approach to combat diseases in aquaculture. Therefore, the present study was performed to evaluate the potential effect of individual or combined administration of dietary low molecular weight sodium alginate (LMWSA) and Bactocell® on haematological, humoral and skin mucosal immune responses of *L.calcarifer* juveniles.
**Methods:** Fish (12.0 ± 0.2 g ) were fed experimental diets as follows: Control (diet 1, basal diet), 5 g $kg^{-1}$ LMWSA (diet 2), 10 g $kg^{-1}$ LMWSA (diet 3), 0.9 × $10^7$ CFU $g^{-1}$Bactocell®(diet 4), 5 g $kg^{-1}$ LMWSA + 0.9 × $10^7$ CFU $g^{-1}$Bactocell®(Diet 5), and 10 g $kg^{-1}$ LMWSA + 0.9 × $10^7$ CFU $g^{-1}$Bactocell® (Diet 6). At the end of the trial, blood samples from the caudal vein and skin mucus were collected for evaluation of immunological parameters.
**Results:** Results indicated a significant ($P< 0.05$) increase in innate immune parameters including serum lysozyme, bactericidal, hemolytic and respiratory burst activities as well as mucosal immune responses including lysozyme and bactericidal activities when diet was supplemented with immunostimulants. Moreover, the combined effects of LMWSA with Bactocell® resulted in more pronounced immunological responses compared to the control and singular administration. Red and white blood cell counts significantly increased with either singular or combined administration of LMWSA and Bactocell®compared with the control group ($P< 0.05$).
**Conclusion:** These results indicated that combined administration of LMWSA and Bactocell® can be considered as a beneficial feed additive and immunostimulant in *L. calcarifer* juveniles.

**Keywords:** Asian sea bass, Bactocell®, Sodium alginate, Mucosal immunity, Innate immunity, Haematological parameters

ICIA2018\Vaccine\Poster\9816
**9816**

# Immunogenicity of Oma87 in murine model against *Acinetobacter baumannii* infections

Raziyeh Abdolhamidi[1], Iraj Rasooli[2]

1. *M.Sc. Student, Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran*
2. *Professor, Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran*

**Background:** *Acinetobacter baumannii* is an emerging gram negative coccobacillus causing nosocomial infections such as pneumonia, meningitis and bacteremia in intensive care units. *A.baumannii* infections are becoming a serious health problem with the rapid advent of drugresistant strains, resulting in urgent need to develop strategies based on non-antibiotic. The purpose of this study is to identify if an outer membrane protein with molecular weight of about 87 kDa (oma87) has the potentials to be an efficient vaccine candidate to combat *A.baumannii* infection.
**Methods**: Oma87 which has a molecule length of 841 amino acids is conserved in more than 95% of *A.baumannii* strains. Gene encoding an outer membrane protein (ABAYE1583) was identified and was then cloned and expressed in *E.coli*. A recombinant protein with molecular weight of 87 kDa was detected and analyzed with SDS-PAGE. The purified protein was used as an antigen in mice groups.
**Results:** The antigen elicited antibodies against *A.baumannii*.
**Conclusion:** In conclusion, Oma87 is a highly safe and protected protein that can serve as a valuable antigen for developing an effective vaccine or antibody preparation to control *A. baumannii* infections.

**Keywords:** *A.baumannii*, Immunogenicity, Vaccine, OMA87

ICIA2018\Stem Cells Based Immunotherapy\Poster\9835
**9835**

# Wharton's Jelly-Derived Mesenchymal Stem Cell's Conditioned Media Increase Neutrophils Viability and Function

Mohammad Mahmoudi[1], Mahsa Taghavi[2], Seyed Mahmoud Hashemi[1, 3], Nima Rezaei[2, 4, 5]

*1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*2. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
*3. Department of Applied Cell Sciences, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*4. Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Dr. Gharib St, Keshavarz Blvd, Tehran, Iran*
*5. Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Sheffield, UK*

**Background:** Mesenchymal stem cells (MSCs) have immunomodulatory properties on a variety of leukocyte subsets through direct interaction or releasing soluble factors. They have mostly been defined as immunosuppressive, but recent studies have shown that they have supportive effects on the functions of some immune cells such as Neutrophils, which play an important role in the acute inflammatory response and in the clearance of bacterial infections.
**Methods:** We investigated the effects of Wharton's jelly-Derived Mesenchymal stem cell's conditioned media on the viability and function of neutrophils. Neutrophils were derived from healthy donors and cultured with or without WJ-MSC's conditioned media to evaluate neutrophil's viability, apoptosis and respiratory burst. The treatment of neutrophils with WJ-MSC' conditioned media resulted in increased viability, decreased apoptosis and enhanced the respiratory burst.
**Conclusions:** Thus, we conclude that the use of WJ-MSC's conditioned media may be a promising therapy for increasing immunity by promoting the anti-microbial activity of Neutrophils.

**Keywords:** Mesenchymal Stem Cell, Neutrophil, Conditioned Media, Viability

ICIA2018\Reproductive Immunology\Poster\9863
**9863**

**Association between rs1049174 NKG2D gene polymorphism and unexplained recurrent spontaneous abortion (RSA) in Iranian women**

*1. Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences,Yazd,Iran.*
*2. department of immunology, shahid sadoughi university of medical science , international campus, yazd, iran*
*3. Department of Genetics and Molecular Biology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
*4. Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
*5. Recurrent Abortion Research Center, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
*6. Research Center for Food Hygiene and Safety, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
*7. Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Science, Isfahan, Iran*
*8. Zoonotic Diseases Research Center, School of public health, Sahid Sadoughi University of Medical Sciences, Yazd, Iran*

**Background:** According to previous investigations, maternal immune responses may affect the fetus, and cause recurrent pregnancy loss or infertility. Natural killer (NK) cells are the main immune cell population of decidua. They function as an important part of the uterine immune system and play a significant role in outcome of pregnancy. NK cells express inhibitory and activating receptors that regulate their function. NKG2D is one of the best known activating receptor of NK cells which recognizes its ligand on abnormal or stressed cells and activates NK cells to kill them. Objective: We have studied the polymorphism of NKG2D gene to determine alleles and genotypes distribution in Iranian women and whether it could be associated with risk of RSA.
**Methods:** The genetic polymorphism of NKG2D gene was studied by PCR-RFLP in 140 women with RSA and 175 control women with at least one successful pregnancy and without any known pregnancy loss. Statistical analysis was done after study assessment and data extraction.
**Results:** A significant difference was observed between patients and controls in regard with GG-genotype frequency of the NKG2D gene. The distribution of genotypes frequencies was also proved to be significantly different between the cases and controls ($p<0.05$).
**Conclusion:** This study demonstrated a significant association between NKG2D gene polymorphism (rs1049174 G/C) and risk of RSA in Iranian women.

**Keywords:** RsanKg2dpolymorphismrpl

ICIA2018\Reproductive Immunology\Poster\9864
**9864**

**Survey of CTLA-4 gene polymorphism in women with recurrent pregnancy loss**

Vaziri Nezamdoust F, Hadinedoushan H, Ghasemi N.

*1. Department of Immunology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
*2. Recurrent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

**Background:** Recurrent pregnancy loss (RPL) is one of the most important pregnancy problems. Epidemiologic studies have revealed that 1-2% of women experience recurrent pregnancy loss. An aberrant regulation of immunological, metabolic, vascular and endocrine processes leads to RPL. Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) expresses on active T cells and causes of inhabitation and toleration of active T cells. CTLA-4 gene expression in fibroblast cells of the placenta throughout pregnancy and during pregnancy is increased and causes to tolerance during pregnancy.
**Methods:** This study was carried out on two groups of women who were referred to the Yazd Reproductive Sciences Institute.150 women with a history of at least 2 RPL as the case group and 150 women who didn't have a history of abortion with at least one healthy child as the control group. Five milliliter peripheral blood was obtained from all samples and then DNA was extracted. A SNP rs 3087243 of CTLA-4 gene was analyzed using the RFLP-PCR method.
**Results:** There was no difference between the two groups regarding age of women (26.7±3.5 vs. 28.6±3.6 yr). In the case group, the genotype frequencies of rs 3087243 polymorphisms were AG (46%), AA (24%), and GG (30%), and in the control group, they were AG (59.7%), AA (23.3%), and GG (17.3%). There was a significant difference between the genotypes of AA, AG, and GG in two groups (p=0.022).
**Conclusion:** Our result indicates that the rs 3087243 AG genotype may contribute to RPL development in Iranian women.

**Keywords:** Recurrent pregnancy loss, Single nucleotide polymorphisms, CTLA-4 gene.

ICIA2018\Stem Cells Based Immunotherapy\Poster\9868
**9868**

# The level of CD133 expression in colorectal cancer cell lines

Zahra Asadzadeh[1, 2], Elham Safarzadeh[1, 2], Behzad Mansoori[1], Behzad Baradaran[1, 2]*

1. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Faculty of Medicine, Department of Immunology, Tabriz University of Medical Sciences, Tabriz, Iran*

**Introduction:** Colorectal cancer is one of the most common malignant tumors worldwide. Colorectal cancer cells have previously been demonstrated to contain a subpopulation of CD133+ tumor cells that have the ability to initiate tumor growth and are thus referred to colorectal cancer stem cells. In this study, we have proposed to evaluate the expression of CD133 in colorectal cancer cell lines.
**Methods:** Colorectal cancer cell lines HT29, HCT116 and SW480 were cultured in RPMI-1640 medium. Total RNA was isolated from cultured colorectal cancer cells via Trizol reagent (Ambion, TX, USA) according to the manufacturer protocol. Then cDNA was synthesis by the cDNA synthesis kit (Biofact, Korea) and CD133 expression levels were measured using Quantitative reverse transcription PCR (RT-qPCR).
**Results:** we measured CD133 expression levels in three colorectal cell lines. RT-PCR analysis showed the levels of CD133 expression in HT-29, HCT-116, and SW-480 cell lines which were 98.30±0.20, 69.62±1.51, and 0.23±0.06 respectively.
**Conclusion:** In conclusion, the CD133 was found to be expressed in high levels in HT-29 cell line of colorectal cancer. The results showed the HT-29 cell line might be a good option for researchers to analysis on cancer stem cell biology and therapy in colorectal cancer.

**Keywords:** CD133 expression, colorectal cancer, Quantitative reverse transcription PCR

ICIA2018\Research and Development\Poster\9874
**9874**

**Association of major depressive disorder with Lipid peroxidation (LPO)**

Abdolghader Taene [1], Ghazaleh Khalili Tanha[2], Gholamreza Anani Sarab [3]

*1. Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran*
*2. MSc of cellular and molecular biology, Cellular and Molecular Research Center, Birjand university of medical science, Birjand, Iran*
*3. Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran*

**Background:** Major Depression Disorder (MDD) is one of the most common psychiatric disorders and has been ranked by some estimates as the fourth leading cause of disease burden worldwide. Increasing numbers of studies indicate that oxidative stress play an essential role in pathophysiology and some symptom of MDD. One of the most important damaging effects of free radicals is the onset of lipid peroxidation which leads to destruction of cell membranes and LPO is an indicator for lipid peroxidation.
**Methods:** Blood samples from forty MDD patients, which 20 patients with treatment and 20 without treatment in first episode, and 20 healthy volunteers as control were collected. Patients diagnosed with MDD according to the Diagnostic and Statistical Manual of Mental Disorders- V (DSM-V). Samples were collected from Department of psychiatry Emam Reza Hospital, in Birjand. The level of Serum LPO was measured by lipid peroxidation kit (Navandsalamat, Iran) according to the manufacturer's instructions. The differences in mean LPO between groups were analyzed.
**Results:** The result revealed a notable rising in the average of serum MDA in without treatment patient as compared to control group (P=0.001), this level showed no difference with treatment group (P=0.15). Also the results showed an increase of BDI (Beck Depression Inventory) score (36.42 ± 10.90) in without treatment patients in compare to treatment group (24.47 ± 15.64) and control group (9.56 ± 8.87) (P<0.005).
**Conclusion:** In the present study, we demonstrated oxidative stress in patients with MDD, which are reflected by increased levels of MDA as a marker of oxidative stress.

**Keywords:** Major depressive disorder, Lipid peroxidation (LPO), oxidative stress

ICIA2018\Veterinary Immunology\Poster\9881
**9881**

# Evaluation of an Indirect ELISA Using a Tachyzoite Surface Antigen SAG1 for Diagnosis of *Toxoplasma gondii*Infection in Cats

Farzaneh Hosseini[1], Morteza Hosseininejad[1]

*1. Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran*
*2. DVM Student of Veterinary Medicine, Shahrekord University, Shahrekord, Iran*
Corresponding author's e-mail: hosseini-m@vet.sku.ac.ir

**Background**: Toxoplasma gondii infection is very common in cats throughout the world. Most cats are sub-clinically infected and potentially fatal clinical disease occurs in some of them. The aim of this study is to develop an indirect enzyme linked immunosorbent assay (ELISA) test using an affinity purified tachyzoites surface antigen (SAG1) to detect *T.gondii*infection in cats.
**Methods:** Six sero-negative kittens were used in this study; four kittens received $10^4$ T.gondii tachyzoites of NED strain (type III) and the remaining two were used as uninfected controls. Serum samples were collected within 41 days and were evaluated for anti- T. gondii antibodies using indirect fluorescent antibody test (IFAT) and ELISA method.
**Results**: IgG antibodies were detectable at least from eight days after tachyzoites inoculation and an increasing pattern in both serum ELISA indices (SIns) and IFAT titers were detected. SIns were significantly different in sera of cats presenting different IFAT titers. In order to evaluate the performance of ELISA to detect anti-T. gondii antibodies of naturally infected cats, serum samples were also collected from household and stray cats and evaluated in the same way. IFAT was regarded as the standard test and sensitivity and specify of the ELISA to detect the infection in naturally infected kittens were analyzed using two-graph receiver operating characteristic (TG-ROC) analysis. An area under curve (AUC) of 0.996 revealed the test as a highly accurate test with relative sensitivity and specificity of 100 and 96% for a cut-off value of 0.10 for SIn.
**Conclusion:** This study develops an ELISA test for the serological diagnosis of T. gondii infection in cats and can be used for serological evaluation of the infection with relatively high sensitivity and specify.

**Key words:** *Toxoplasma gondii*, surface antigen 1 (SAG1), P30, enzyme linked immunosorbent assay (ELISA), cats.

ICIA2018\Vaccine\Poster\9901
**9901**

# Immunogenic role of ABAYE2132 recombinant protein against *A.baumannii*

Zeinab Mahmoodi[1], Iraj Rasooli[2]

1. *M.Sc. Student, Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran*
2. *Corresponding author, Department of Biology, Shahed University, Tehran, Iran*

**Background***: Acinetobacter baumannii*, an aerobic Gram-negative, non-flagellated opportunistic coccobacillus is one of the most common and highly antibiotic resistant pathogens. It is associated with high morbidity and mortality rates. Adherence of microorganisms to host cells is an initial and important step in the pathogenesis of *Acinetobacter baumannii*. This has led to investigations development of vaccine to prevent or treat *A.baumannii* infections.
**Methods:** In the present study, a fimbrial protein known as ABAYE2132 which was predicted as vaccine candidate by in silico analysis and was found to be conserved among the *A.baummannii* strains, was cloned and expressed in *E.coli* BL21(2566).The protein was purified by Ni-NTA chromatography. Immunization with ABAYE2132 generated high antibody titers at >64000 serum dilutions. The mice were immunized with recombinant protein and were challenged with *A. baumannii*. The number of bacterial cells in the immunized mice was determined 24h after the challenge.
**Results and conclusion:** These results indicate that ABAYE2132 vaccination could be a vaccine development strategy to prevent *A.baumannii* infection.

**Keywords:** *A.baumannii*, Pili, Adhesion, Recombinant protein, Vaccine

ICIA2018\Veterinary Immunology\Poster\9916
**9916**

**Molecular Detection of Wolbachiapipientis in Dirofilaria infected dogs using HRM Real-Time PCR**

FatemeManshori[1], FatemeJalousian*[1], Seyed Hossein Hosseini[1], Minoo Soltani[2]

1. *Department of parasitology, Faculty of veterinary medicine, University of Tehran*
2. *Department* of mycology, Faculty of veterinary medicine, University of Tehran

**Background:** Dirofilariasis, one of the most important vector-borne zoonotic infections. This worm causes a severe and fatal disease called heartworm disease in dog; it also creates pulmonary nodules in humans. *Dirofilariaimmitis*, harbour intracellular bacteria of the genus *Wolbachiapipientis* (Rickettsiaies).These bacteria play an important role in the pathogenesis and immune response to filarial infection.
**Objective:** *The main objective of* the *present study* is to investigate the occult infection of dirofilariasis in dogs based on the detection of *Wolbachia*.
**Methods:** In the present study 138 blood samples from stray dogs from Gilan, Mazandaran and Ghazvin provinces was collected. A sample which had microfiler in peripheral blood was confirmed as *Dirofilariaimmitis* positive sample based on morphological and molecular character of microfiler. In this sample, FTSZ gene of *Wolbachiapipientis* was amplified by specific primers and PCR product was sequenced. This sample was introduced as Gold standard and HRM-Real TIME PCR was optimized based on the melting temperature of the Gold standard sample. Other samples was assayed by HRM Real- TIME PCR.
**Results:** Melting temperature was determined 58.5˚c for FTSZ gene of *Wolbachia.* The results showed that in Gilan province 77.3% of samples were positive, in Mazandaran 57.6% and in Ghazvin 28.3% of samples were positive. However using parasitology method (modified knott) it was shown 45.5% infection in Gilan, 15.2% infection in Mazandaran and 2.5% in Ghazvin.
**Conclusion:** The high percentage of infection based on wolbachiadetection showed the cases of occult infection of dirofilariasis in dogs. This resultssuggest that alternative diagnostic tests should be applied to determine the occult infections. In addition to, the presence or absence of these bacteria in association with microfilaria is of importance in the management and control of dirofilariasis as a zoonotic disease.

**Keywords**: *Dirofilariaimmitis*, *Wolbachiapipientis*, HRM-Real Time PCR, Iran

ICIA2018\Veterinary Immunology\Poster\9935
**9935**

# Serological Evaluation of Experimental Toxoplasma gondii Infection in Cats by Using Immunoblotting Based on an Affinity Purified Surface Antigen

Mohammadreza Alijani*[1], MortezaHosseininejad[2*], Farzaneh Hosseini[2]

*1. DVM Student of Veterinary Medicine, Shahrekord University, Shahrekord, Iran*
*2. Professor, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran*
Corresponding author's e-mail: mohammadrezaalijani1992 @gmail.com

**Background**: Toxoplasma gondii is an apicomplexan parasite that infects human and almost all warm-blooded animals. The life cycle of the parasite includes an asexual reproduction in intermediate hosts (Mammals and birds) and a sexual reproduction in definitive hosts. Cats are both the intermediate and the definitive host for T. gondii. The aim of this study was to investigate anti-T. gondii antibodies in cats by using an immunoblot method based on a major surface antigen; SAG1.
**Methods**: Six sero-negative kittens were infected intraperitoneal by Rh strain of T. gondii. Serum samples were collected and evaluated for the presence of IgG antibodies by using two techniques; immunoblotting and indirect fluorescent antibody test (IFAT).SAG1 based immunoblotting was able to detect anti-T. gondii antibodies at least eight days after infection.
**Results:** The results obtained from immunoblotting shows that it is comparable with the standard IFAT test so that infection is detectable in early stages of the disease when serum samples are evaluated with this immunoblotting method.
**Conclusion:** According results of this study shows that Immunoblotting using affinity purified T. gondii SAG1 antigen is a sensitive diagnostic test and is capable to diagnose early Toxoplasma gondii infection.

**Keywords**: Cats; Immunoblot; P30; SAG1; Toxoplasma gondii.

ICIA2018\Reproductive Immunology\Poster\9940
**9940**

# *Aloe vera*-induced modulation of nitric oxide signaling pathway and androgenesis in the testis rat leydig cells

Samira Asgharzade [1]*, Mahmoud Rafieian kopaei [2], Amin Mirzaeian [1]

*1. Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran*
*2. Medical Plant Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran*

**Background:** Nitric oxide (NO), a product of inducible nitric oxide synthase (iNOS), contributes in germ cell apoptosis. This study was aimed to evaluate the effects of *Aloe vera* gel (AVG) on male Wistar rat reproductive organ, serum NO level, and expression of iNOS gene in leydig cells.
**Methods:** Adult male Wistar rats (n=36) were used for experiments in three groups. The experimental groups were orally administered with the AVG extract solution once-daily as follow: 150 mg.kg-1; group A, 300 mg.kg-1; group B, and only normal saline; group C (control group). They were mated with untreated females and the reproductive and chemical parameters were assessed for each group, including semen quality, serum testosterone, sperm fertility, gonad and body weight, serum NO concentration (by the griess method), and iNOS gene expression (using RT-PCR).
**Results:** The testes weight, serum testosterone, as well as sperm count and fertility of the AVG treated groups were significantly reduced when compared to the control ($P$<0.001). Concentration of serum NO was significantly increased (37.1±4.63 µM) in the administrated group with higher AVG concentration, compared to the control group ($P$<0.001; 10.19±0.87 µM); however, iNOS mRNA expression was increased in the treated animals ($P$<0.001).
**Conclusion:** iNOS may play a functional role in spermatogenesis via apoptosis, reducing sperm count, but further studies are needed to illustrate the mechanisms by which AVG exerts its negative effects on spermatogenesis and sperm quality.

**Keywords:** Nitric oxide, *Aloe vera*, rat, leydig cells

ICIA2018\Vaccine\Poster\10024
**10024**

# Design and in silico analysis of a new antigen, based on heat stable toxin, to be encorporated in candidate vaccines against enterotoxigenic *Escherichia coli* (ETEC)

Abbas Hajizade[1]*, Ali Hatef Salmanian[2], Jafar Amani[3], Firouz Ebrahimi[1], Ayoob Arpanaei[4], Yousof Tarverdizade[1]

1. *Biology Research Centre, Faculty of Basic Sciences, Imam Hossein University, Tehran, Iran*
2. *Plant biotechnology department, National Institute for Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran*
3. *Applied Microbiology Research Centre, Baqiyatallah University of Medical Sciences, Tehran, Iran*
4. *Department of Industrial and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran*

**Background:** As one of the main bacterial leading causes of morbidity and mortality in children under 5 years of age, especially in developing countries, enterotoxigenic *Escherichia coli* (ETEC) are of the main causative agents of infectious diarrhea that play a significant role in public health. Indeed, the bacteria are responsible for the majority of traveler's diarrhea. So, it is necessary to develop an efficient vaccine against the pathogen. Pathogenic strains of ETEC produce two different enterotoxins: heat-labile and/or heat-stable toxins along with many colonization factors. All pathogenic strains of ETEC express at least one of these toxins. There are many proposed candidate vaccines against ETEC based on LT toxin. However, few candidate vaccines are introduced against STa. Progresses in bioinformatics have led to the development of many in silico tools which can help us to design and evaluate the immunogenicity of the vaccine candidates.
**Methods:** Here, in this study we designed a new antigen based on a mutated form of STa toxin. The physico-chemical parameters and the secondary and tertiary structures of the antigen were calculated. Prediction of the antigenic B-cell and T-cell epitopes were performed by appropriate softwares. The protein was then reverse-translated to DNA and the resulted DNA was codon optimized according to the codon preference of *E. coli* Bl21(DE3) host cells.
**Results:** The results showed that the structure of the designed antigen has a proper 3D structure in which the main epitopes are exposed and the domains are separated. There are potent B- and T-cell epitopes in the protein. Physicochemical properties of the protein as well as the structure of mRNA showed that the designed gene can be translated in *E. coli* host cells.
**Conclusion:** In conclusion, the designed antigen structurally and immunologically has almost all factors to be incorporated in candidate vaccines.

**Keywords:** Heat stable toxin (STa), enterotoxigenic *Escherichia coli* (ETEC), Candidate vaccine , Immunoinformatics

ICIA2018\Reproductive Immunology\Poster\10027
**10027**

# Relation of Gene's expression Profile of IL-17 and IL-23 in Cumulus cells and their Follicular Fluid Concentrations in Infertile Patients at the Risk of Ovarian Hyperstimulation Syndrome (OHSS)

Leila Motamedzadeh [1, 3], Hossein Hadinedoushan [2], Ehsan Farashahi Yazd [3], Farzaneh Fesahat [3], Mohammad Mahdi Mohammadi [1, 4]

1. *Department of Immunology & Allergy, Kerman University of Medical Sciences, Kerman, Iran*
2. *Department of Immunology, Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Science, Yazd, Iran*
3. *Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Science, Yazd, Iran*
4. *Physiology Research Center, Kerman University of Medical Sciences, Kerman, Iran*

**Background:** Ovarian hyperstimulation syndrome (OHSS) is one of major complications during assisted reproductive technology treatment. This study aims to determine the concentrations of interleukin IL-17 and IL-23 in follicular fluid (FF) as well as their mRNA levels in cumulus cells of infertile women at the risk of OHSS.

**Methods:** The cumulus cells of oocytes from 80 infertile women were divided into two groups of case (n=40) with an average age 29.6±1.4 years and control (n=40) with an average age 29.2±1.1 according to the patients' infertility etiologies. The control group was the infertile patients with male factor infertility etiologies while; infertile women at the risk of OHSS were defined as case group in this study. IL-17 and IL-23 concentrations in FF were determined. mRNA expression levels of IL-17 and IL-23 of samples were measured using real time polymerase chain reaction method in all subjects.

**Results:** The concentrations obtained from FF demonstrated the higher levels of IL-17 in case group (P=0.04), while, it showed no remarkable difference in IL-23 (P=0.3). The mRNA levels of IL-17 and IL-23 showed no significant differences between two groups. There was a positive significant correlation between FF concentrations of IL-23 and the oocytes maturation rates in case group (P=0.01). The numbers of follicles, MII oocytes, immature oocytes, fertilized oocytes and formed embryos were significantly higher in case group.

**Conclusion:** Our finding showed the mRNA expression of IL-17 and IL-23 were similar in two groups, and IL-17 may be considered as risk factor for OHSS.

**Key Words:** OHSS, IL-23, IL-17

ICIA2018\Reproductive Immunology\Poster\10033
**10033**

**Evaluation of Regulatory B cell in Blood of Women with Recurrent Spontaneous Abortion (RSA)**

Farzad Nazari[1], Davar Amani[1], Esmaeil Mortaz[1]

*Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background**: The main function of regulatory B cells (Breg) is producing of IL-10 and this cytokine control immune balance during early human pregnancy, so Breg cells emerge as important players in pregnancy. The function of Breg in immune-mediated reproductive disorders (e.g. Recurrent Spontaneous Abortion (RSA)) is unknown. The aim of this study was to evaluate the Breg cells in RSA patients.
**Methods:** Fresh blood of 10 RSA patients and 12 Control were harvested and then the surface expression of $CD19^{+}$, $CD24^{hi}$, and $CD38^{hi}$ on B cells were measured by flowcytometry (FACS calibour), and about 10,000 events were measured in all cases.
**Results:** Significant upregulation of B cells with $CD19^{+}$ $CD24^{hi}$ $CD38^{hi}$ phenotype (Breg cells) was seen in normal pregnant (mean: 7.83%) whereas downregulation of markers was seen in these cells in women suffered from RSA (mean: 0.9%) at the first trimester. Statistical analysis is going.
**Conclusion:** Our data show that decrease in Breg cells in early gestation might have important role in recurrent spontaneous abortion via regulation of immune response.

**Keywords:** Regulatory B cell, IL-10, Abortion, Reproductive immunology, RSA

ICIA2018\Reproductive Immunology\Poster\10034
**10034**

# Evaluation of regulatory B cell in blood in women with recurrent spontaneous abortion (RSA) and Normal Pregnant Individual

Farzad Nazari[1], Davar Amani[1], Esmaeil Mortaz[1]

*Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

**Background**: Regulatory B lymphocytes are important players of immune response by producing of IL-10 during human normal pregnancy, whereas the function of this cell during Recurrent Spontaneous Abortion (RSA) is unknown. The goal of this investigation was to determine Breg cells percent in RSA patients in comparison of normal pregnant individuals.
**Methods:** Fresh blood of 10 RSA patients and 10 Control (non-pregnant women) was harvested and then the surface expression of $CD19^{+}$, $CD24^{hi}$ and $CD38^{hi}$ on B cells were measured by flow cytometry (FACS calibour), and about 10,000 events were measured in all cases.
**Results:** The percent of Breg cells (4.18%) in non-pregnant women was significantly higher than RSA patients (0.9%). $P<0.05$
**Conclusion:** The result of this study show that Breg cells might have important role in RSA.

**Keywords:** Regulatory B cell, IL-10, Abortion, Reproductive immunology, RSA

ICIA2018\Veterinary Immunology\Poster\10045
**10045**

# Evaluation of the production level of the polyclonal antibody against Listeria monocytogenes in animal models

Mansoor Khakpoor[1], Yasin Bagheri[2*], AlirezaBarati

1. *Department of Pathobiology, Tabriz Branch, Islamic Azad University, Tabriz, Iran.*
2. *Young Researchers And Elite club, Tabriz Branch Islamic Azad university, Tabriz, Iran*

**Background:** Listeria monocytogenes is a short basillary Gram-positive, spore-free and intracellular parasitism which transmitted through contaminated vegetables, milk, cheese and meat to human or animal. Immunoglobulin Y (IgY) is a great and cheap source for polyclonal antibodies that obtained from egg yolk of immunized lying hens. These antibodies can be an appropriate replacement for other antibodies that produce by laboratory animals. Recent years, IgY is applied to preventing, diagnosis and treatment of disease in human medicine researches.
**Methods:** In current study, 12 laying hens were divided into 2 groups: Control group (2 hens) and Treatments (10 hens). Treatments immunized with Listeria monocytogenes antigens in 3 steps.
**Results:** Finally, by measuring total protein and yolk globulins of collected eggs were proved that there was no significant difference in levels of globulins obtained in 9 and 10 weeks after first injection while they showed a significant difference compared to the control group ($p<0.05$).
**Conclusion:** The amount of globulin and total protein in the eleventh week had significant reduction and showed no significant difference with controls. Accordingly, it could be concluded that hens' immunization increased yolk globulin levels in the ninth and tenth weeks after injection.

**Key words:** polyclonal antibodies, Listeria monocytogenes, egg yolk

ICIA2018\Vaccine\Poster\11063
**11063**

# Induction of Immune Responses by DNA Vaccines Formulated With Dendrimer and Poly (Methyl Methacrylate) (PMMA) Nano-Adjuvants in BALB/c Mice Infected With Leishmania major

Fatemeh Tabatabaie[1], Nasim Samarghmarghandi[2], Somayeh Zarrati[3], Fatemeh Maleki[4], Mehdi ShafieeArdestani[5], Taher Elmi[1], Sayed Hussain Mosawi[6]

*1. Department of Parasitology and mycology, School of Medicine, Iran University of Medical sciences, Tehran, Iran*
*2. Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
*3. Department of Microbiology, Science & research Branch, Islamic Azad University, Tehran, Iran*
*4. Department of Parasitology, Faculty of Para Medical Sciences, Iran University of Medical Science, Tehran, Iran*
*5. Department of Radiopharmacy, Faculty of Pharmacy, Tehran University of Medical sciences, Tehran, Iran*
*6. Department of Biology and Microbiology, Faculty of Medical Technology, Khatam AL Nabieen University, Kabul, Afghanistan*

**Background**: Leishmaniasis is a parasitic disease induced by a protozoan from the genus Leishmania. No effective vaccine has yet been developed against the disease.
**Methods**: After the plasmid construction and preparation of adjuvants, three intramuscular injections of the nano-vaccin (100 µg) and the recombinant TSA protein (20 µg) were subcutaneously performed. Eventually, the challenged animals were infected with the parasites ($1*10^6$ promastigotes). After the last injections of the nano-vaccines, the responses of their antibody subclasses and cytokines were assessed via ELISA method before and after the challenge.
**Results**: This study revealed that the new nano-vaccines were strong and effective in inducing specific antibody and cellular responses and reducing the parasite burden in the spleen compared to the control groups of Leishmania major-infected BALB/c mice.
**Conclusion:** Based on the results, we can suggest that the formulated vaccines are suitable candidates for further studies in the field of leishmaniasis control.

**Keywords**: PMMA, Dendrimer, Leishmaniasis, TSA

ICIA2018\Veterinary Immunology\Poster\11088
**11088**

# Immunogenicity of the attenuated line of Leishmania infantum in dog

Zeynab Fallah[1], Hamid Daneshvar[2], Hossin Kamiabi[3]

1. *Department of Parasitology, Medical School of Kerman University, Kerman*
2. *Department of Immunology, Medical School of Kerman University, Kerman*
3. *Department of Parasitology, Medical School of Kerman University, Kerman*

**Background:** *Leishmaniainfantum (L. infantum)*, a causative agent of visceral leishmaniasis (VL), is endemic in Iran and several Middle East (1). Control interventions involving immunization of dogs to prevent infection could be beneficial as dogs are both the natural host and the main reservoir of L. infantum (2). Control of VL in dogs can lead to a reduced prevalence of disease in associated human populations (3).
**Methods:** Promastigotes of L. infantum JPCM5 (MCAN/ES/98/LIM-877) were cultivated in HOMEM with 10% FCS in the present of gentamicin. The attenuated line of parasite was used at a concentration of $10^9$ cells /mL in PBS. Serum was prepared from the blood samples taken derived from the peripheral veins of the dogs. Serum Leishmania-specific IgG was measured using ELISA.
**Results:** All dogs presented increased levels of IgG antibodies over the 12-month period of observation.
**Conclusion:** The attenuated line of L. infantum was immunogen and induced anti leishmanial-IgG.

**Keywords:** Attenuated line of Leishmania infantum, dog, immunogenicity

ICIA2018\Immunology of chemical victims\11171
**11171**

**Evaluation of serum levels of Pro-inflammatory cytokines )TNF-α, IL1-β (in Sulfur Mustard(SM) exposed patients with long term pulmonary complications**

Ashraf vafa , Tooba Ghazanfari1, Naghizadeh MM

*1. Immunoregulation Recearch Center, Shahed University, Tehran, Iran*

**Background**: Exposure to mustard gas leads to acute and chronic toxic effects on the pulmonary system. The results of various studies have been suggested the role of Pro-inflammatory cytokines including IL1-β and TNF-α in the pathogenesis of Pulmonary inflammatory diseases. IL-β and TNF-α are recognized as a key mediator of systemic inflammatory reactions and numerous changes including altered cell signaling, migration, changing on cytokine production and fever.
**Aim**: The aim of this study is the evaluation of serum levels of IL-1β and TNFα in SM-exposed patients with long term pulmonary complications.
**Materials and Methods**: In this study, 65 SM-exposed patients as exposed group and 69 unexposed people as control group were studied. The both exposed and control groups were homogeneous in terms of gender, age and body mass index. Pulmonary function test was performed by spirometry. The serum levels of IL-1β, TNF-α were measured by ELISA method.
**Results**: The serum levels of TNF-α was high in SM-exposed group compared to control group. But There were no significant differences between the serum levels of IL-1β in SM exposed group and the control group.
**Conclusion**: Due to increased serum levels of TNF-α in exposed group compared to the control group, It may play an important role in pulmonary inflammatory complications. It is a proof of TNFα effects on pulmonary system as a mediator of inflammatory reactions. Although there were no significant differences in the serum levels of IL-1β in SM-exposed group compared to the control group, It doesn't rollout the role of IL-1β in pathogenesis of long term pulmonary complications, because it may have local changes in pulmonary system and long term complications. Therefore, locally assessment of IL-1β would be valuable.

**Keywords**: Mustard Gas; TNFα; IL-1β; pulmonary complications ;

ICIA2018\Immunohematology \12215
**12215**

**Long-term effects of sulfur mustard on viability of peripheral blood mononuclear cells**

Ensieh Sadat Mirsharif [1], Tooba Ghazanfari [1*]

*1. Immunoregulation Research center, Shahed University, Tehran, Iran*

**Background**: Sulfur mustard (SM) or (bis-[2-chloroethyl] sulfide) is a chemical warfare agent that attacks mainly skin, eye and lungs. Although Medical effects of exposure to SM in humans include ocular, dermal, respiratory, reproductive and developmental toxicity, gastrointestinal injuries, hematological, and cancer, but the effects of SM-exposure on body are not restricted to these known injuries. The aim of this study is examine the long-term effects of SM on viability of peripheral blood mononuclear cells in SM victims of the Iraq-Iran war (1980–1988).
**Matherials and methods**: During a case -control study,110 SM-exposed individuals and 109 unexposed as control group were recruited. PBMC were purified from whole blood samples of participants using Ficol-Paque gradient. Cells were suspended in culture medium RPMI 1640, 10% FBS and cultured in the absence and presence of PHA in 96-well plate (1* 106 cells/ml) at 37 °C and 5% CO2 for 48 h. Cell viability was assessed with MTT assay. Stimulation index was calculated and compared between groups.
**Results**: The Stimulation index (Percentage of surviving cell) of PBMC in SM-exposed group and control groups were 111.83± 20.71 and 114.62 ±22.68, respectively. There were no significant differences in exposed group compared with controls. There was no significant difference in the viability of PBMC in SM-exposed group compared to their matched control subjects.
**Conclusion**: The results of this study indicate that long-term complications of mustard gas may not be associated with viability of the blood cells.

**Key words**: Sulfur mustard, long-term effect, peripheral blood cells, viability

ICIA2018\Innate immunity and inflammation\12216
**12216**

**Serum level of IL-17A and long term pulmonary complications (27 years after sulfur mustard exposure)**

Ali Mohammad Mohseni Majd[1*], Tooba Ghazanfari[1], Mohammad Mehdi Naghizadeh[1]

*1. Immunoregulation research center of shahed University, Tehran, Iran*

**Background:** Interleukin 17A (IL-17A) is the one of key cytokines produced by human activated Th17 and is the most important cytokine in family of Th17 cytokines about lung immunity. This cytokine as the pro-inflammatory cytokine was described recently and particularly as an important parameter for chronic inflammatory diseases. Growing up of IL-17A level of serum has been associated with chronic inflammatory of lung complications and it can be effective on sulfur mustard (SM)-lung as distinct disease.
**Methods:** We collected serum samples from 112 healthy individuals and 109 SM-injured patients with prolonged pulmonary complications and were classified based on Auscultation, Pulmonary assessment, pulmonary symptoms and respiratory consequence severity. Serum level of IL-17A was investigated by ELISA method.
**Results:** The serum level of IL-17A in SM-exposed group and non-exposed group showed no significant difference. Our results also show there are no significant auscultation comparison between SM-exposed group and control group. Furthermore, the results have not showed any significant comparison in cough and hemoptysis groups between SM-exposed group and non-exposed group.
**Conclusion:** Generally, IL-17A may not be a certain agent in pathogenesis of lung complications but could intensify the effects of disease and make it worse; also the mutation on STAT3 gene can cause to decrease of IL-17A level. This toxic gas could affect on genes expression of different pathways in long term. STAT3 gene deficiency can cause to decrease of TH-17 cells number by non-differentiation in origin, subsequently the level of IL-17A as a main cytokine of TH-17 will be in low level.

**Keywords:** Interleukin 17A, Th17, lung, inflammation, chronic disease.

ICIA2018\Immunoparasitology \12217
**12217**

# Effects of hydroalcoholic extract of *Glycyrrhiza glabra* and glycyrrhizic acid on nitric oxide production in peritoneal macrophages of *Leishmania major* infected BALB/c mice

Shima Sheikhi[1], Ali Khamesipour[2], Tayebeh Radjabian[3], Tooba Ghazanfari[1]

*1. Immunoregulation Research Center, Shahed University, Tehran, Iran*
*2. Center for Research and Training in Skin Diseases and Leprosy, Tehran University of Medical Sciences, Tehran, Iran*
*3. Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran*

**Background:** Cutaneous leishmaniasis is a neglected disease caused by different species of *Leishmania*. The macrophage linage is the main cells for the growth of *Leishmania* and nitric oxide(NO) production by them is one of the ways to destroy microorganisms. There are some natural drugs, including *Glycyrrhiza glabra* extract and its components, which showed to act as immunomodulator and enhance macrophage activation. The aim of this study was to explore the effect of hydroalcholic extract of *G. glabra* and its main component glycyrrhizic acid on macrophage NO production in *Leishmania major* infected BALB/c mice**.**
**Methods:** 35 *Leishmania major* infected BALB/c mice were randomly divided into 5 groups; the groups of mice were treated intraperitoneally for one month as following; 200 or 600 mg/kg of *G. glabra* root extract, its component glycyrrhizic acid (200mg/kg), *Glucantime* (160 mg/kg) or phosphate buffered saline as a negative control. The mice were scarified and the peritoneal macrophages were removed and the nitric oxide level was measured using Griess test.
**Results:** The mean production of nitric oxide in macrophages of groups received 200 or 600 mg/kg of the extracts, glycyrrhizic acid, *Glucantime* and negative control was11.7 , 9.7, 5.1, 6.9 and 3.6nmol/ml, respectively. The highest amount of nitric oxide was produced in the groups that received the extract, and it was statistically significant in comparison with the negative control, or glycyrrhizic acid or *Glucantime* treated groups.
**Conclusion:** The hydroalcoholic root extract of *G. glabra* induced macrophage activation, which resulted in an increase in nitric oxide production in macrophages of *Leishmania major* infected BALB/c mice.

**Keyword:** Nitric Oxide, Macrophage activation, *Leishmania major*, *Glycyrrhiza glabra*

ICIA2018\Immunology of chemical victims\12218
**12218**

# Evaluation of IL-2 and IL-10 level in peripheral blood mononuclear cells of sulfur mustard exposed Victims

Nafiseh Zand, Tooba Ghazanfari[1]

*1. Immunoregulation Research Center,Shahed university,Tehran,Iran*

**Background:** Sulfur mustard (SM) is a chemical agent which has short and long term toxicity against many organs. The respiratory tract is one of the main targets, and is the most disabling long term complication of SM. IL-10 is an anti-inflammatory cytokine that is important in the regulation of immune response and increased in chronic inflammation and IL-2 has a critical role in regulating both cellular and humoral chronic inflammatory responses. These cytokines play a main role in the pathogenesis of chronic airway inflammation in asthma and chronic obstructive pulmonary disease. The aim of this study is to evaluate the level of IL-2 and IL-10 in PBMC of sulfur mustard exposed Victims.
**Methods:** 100 SM exposed patients and 109 age and sex matched control subject were included in case-control study. Their PBMC were isolated from whole blood by using ficol-paque gradient. The cells were allowed to proliferate and secrete cytokines through cultured in 37°C for 24h with 5 % CO2 with and without PHA as a mitogen stimulator. The levels of IL-2 and IL-10 of supernatant of culture were measured by ELISA methods.
**Results: The** baseline of IL-2 and IL-10 in exposed group were significantly higher than control group ($P < 0.05$). But PHA-induced IL-2 and IL-10 level were not significantly different between control and exposed group ($P > 0.05$).
**Conclusion:** Based on the results of present study, Mustard gas affects the production of these cytokines in the exposed Victims even after years of exposure. So, this dis-regulation of immune system may has important role on the respiratory and immune systems at SM exposed people in long term.

**Keywords:** Sulfur mustard, IL-2, IL-10, chronic inflammation

ICIA2018\ Psychoneuroimmunology and immunoendocrinology \12219
**12219**

# The effect of instability stress and exogenous oxytocin on the number of the natural killer cells in peripheral blood and spleen in male rats

Zahra Omidi[1], Mohammad Reza VaezMahdavi[2], Mohammad Vojgani[3], Marzieh Eghtedar doost[1], Nayere Askari[4], Tooba Ghazafari[1]

1. *Immunoregulation Research Center , Shahed University, Tehran. Iran*
2. *Department of physiology, Faculty of Medical Sciences, Shahed University, Tehran. Iran*
3. *Department of Immunology, Faculty of Medical Sciences, Tehran University, Tehran. Iran*
4. *Department of physiology, Faculty of Basic Sciences ,Shahid Bahonar University, Kerman. Iran*

**Background**: Natural killer cells are innate immune lymphocytes that they play a major role in defense against tumors and viral infections. Many factors such as social stress act on the immune system. This type of stress is an important factor in the etiology of psychopathological disorders, including depression and anxiety. Oxytocin is a 9-amino acid hormones synthesize in the central nervous system and some of the peripheral tissue and plays a main role in response to stress, and its injection reduces depression and stress-induced anxiety. The aim of this study was the evaluation of instability stress effect and oxytocin on the number of NK cells in rats exposed to this stress.
**Methods**: WISTAR rats were subjected to instability stress for 21 days, hence their cage-mate were changed every three days. From the 11th day, a group of rats received 20 microliter and the other group received 40 microliter 1 mg/ml oxytocin and the control group received normal saline by intranasal rout. At the end of study, the animals were anesthesia and then were killed. Blood sample and spleen tissue were obtained and the number of NK cells was counted by flow cytometry by CD3-CD161 + markers.
**Results**: The number of NK cells in the peripheral blood and spleen was 2.67% and 4.12% (received low dose) and 3.80% and 4.69% (received high dose) and the normal saline group were 5.20% and 4.48% respectively. Statistically, there was no significant difference in the NK cells count in between groups that receiving oxytocin with different doses and with control group.
**Conclusion**: Instability stress had not effect on the number of NK cells in the spleen and blood, and also the doses of 20 and 40 µL of 1mg/ml oxytocin did not a significant effect on the count of NK cells.

**Keywords**: Natural killer cell, Instability stress, Oxytocin

ICIA2018\Mucosal and Gastrointestinal Immunology\Oral\12224
**12224**

# Study the correlation of serum IL-6 with stages of tumor in patient suffered from colorectal cancer

Hossein Ali Khazaei[1], Alireza Khazaei[2], Naeges Sarlogzaei[3], Ehsan Khazaei[4], Amin Khazaei[5], Bahman Khazaei[6], Amin Roustami[6], Fariba Hejazynia[7] , Sina Nabi Zadeh[6]

1. *Clinical Immunology Research Center, Department of Immunology and Hematology, Zahedan University of Medical Sciences, Zahedan-Iran*
2. *Associated prof in Surgery at Faculty of dentistry. Zahedan University of Medical Sciences. Zahedan-Iran*
3. *Associated prof in Social medicine Faculty of Medicine, Zahedan University of Medical Sciences. Zahedan, Iran*
4. *Zahedan University of Medical Sciences, Zahedan-Iran*
5. *Dentist*
6. *MD*
7. *Ali Ebneh Abi Taleb Hospital of Zahedan University of Medical Sciences, Zahedan-Iran*

**Background:** Colorectal cancer is the second leading cancer in the five most common cancer in men and women. Cytokine including IL-6 is involved in cancer and various autoimmune diseases. Increased levels of IL-6 in serum and tumor tissue of patients with colorectal cancer, ovarian, breast, and prostate cancers have been reported. The aim of this study was to evaluate serum levels of IL-6 with stages of tumor in patients suffered from colorectal cancer.

**Materials and Methods:** This cross-sectional study was carried out on 40 adult patients with colorectal cancer referred to a hospital clinic in Zahedan Ali Ebnh Abi Talib. Serum levels of interleukin-6 in these patients was assessed using ELISA method. Results obtained were analyzed by using ANOVA and Tukey tests.

**Results:** The mean serum levels of IL-6 was obtained as 0.86± 2.012 pg/ml that was varied from at least 0.86 to up to 4.1 pg/ml. Based on tumor size in patients with colorectal cancer, the results showed in patients with tumor size of less than 20 mm, the concentrating of IL-6 was 1.36 ± 0.42 pg/ml and in patients with more than tumor size of 20 mm was obtained as 2.2 ± 0.86 pg/ml. There was significant difference between the two averages as confirmed by t-test. In other words, the levels of IL-6 in patients with large tumor size is significantly higher. Significant differences was observed based on the levels of IL-6 and pathologic condition of tumor. Comparison of the two stages according to Tukey test showed that the serum levels of IL-6 level was similar in stage I and II and had lowest amount of IL-6. Stage IV had significantly higher levels of IL-6 and the levels of IL-6 in stages III had significantly higher than in stage I and II and lower than in stage IV. In other words, by increasing the level of staging, the amount of interleukin-6 was significantly increased (P value = 0.000).
**Conclusion:** The results of this study showed that between the amount of serum levels of interleukin-6 and tumor stage and tumor size were significant correlation. Our findings emphasize the importance and immunological role of inflammatory cytokines, particularly interleukin-6 in this disease.

**Keywords:** IL-6, stages of tumor and colorectal cancer

# Index

## A

A. A. Amirzargar 989
A. Aghaie 638
Abass Rezaei 610, 612
Abazar Roustazadeh 160
Abbas Ahmadi 907
Abbas Ahmadi Vasmehjani 635
Abbas Alamian 274
Abbas Azadmehr 157, 478, 480, 482, 552, 611, 675, 677, 795, 856, 857
Abbas Bahador 365
Abbas Fayezi 576
Abbas Gerami 554
Abbas Ghaderi 28, 29, 76, 171, 294, 298, 618, 619, 628, 641, 645, 648, 649, 685
Abbas Hajifathali 532, 533
Abbas Hajizade 1050
Abbasian A 608
Abbasi Ardeshir 307, 911, 931
Abbas Jamshidian 550
Abbas Lotfi 986
Abbas Mirshafiey 371, 397, 427, 428, 440, 715, 865
Abbas Mirshafieyd 362, 756
Abbas Rezaei 34, 181, 214, 229, 252, 256, 475, 492, 493, 584, 672, 953, 956
Abbas Sahebghadam Lotfi 548
Abbas Shirdel 660
Abdolghader Taene 211, 810, 811, 881, 1044
Abdolhakim Hosseini 316
Abdolhussein Shakurnia 616, 621, 762, 766, 771
Abdolkarim Pajoumand 809
Abdolkarim Sheikhi 994
Abdollah Ardebili 778
Abdollah jafarzadeh 519
Abdollah Jafarzadeh 102, 372, 432, 639, 673, 909, 936, 938, 1012, 1018, 1019
AbdollahJafarzadeh 994
Abdollah Jafarzadeha 362, 756
Abdollah Salimi 662
Abdolrahim Nikzamir 184, 459
Abdolrahim Rezaei 376
Abdol Rasoul Talei 618, 645
Abdol-Rasoul Talei 298
Abdolreza Esmaeilzadeh 63, 984
AbdolReza Fazel 300, 663
Abdolreza Nabinejad 1005
AbdolrezaNabinejad 1004, 1007, 1022
Abdolreza Sotoodeh Jahromi 751, 888
Abdossaleh Zar 751
Abolfazl Yari 563, 564, 567
Abolghasem Ajami 526, 647, 820, 896, 1027
Abolghasem Allahyari 660
AboTaleb Saremi 1035
Aboulfazl Rahmani 795
Abtahi Froushani 333
Abtahi Froushani Seyyed Meysam 911, 931
A.Dalimi 580
A.Dehghan 819
Adeleh Adelipour 256
Adeleh Mahdizadeh 138
Adel Fekri 825, 971
Adel Mohammadzadeh 24, 248
Adibe Rahmani 551
Afsaneh Aghaie 88
Afsaneh Jahangirian 241
Afsaneh Jamali 101
Afsaneh Sharifian 801
Afshin Amari 831, 961
Afshin Derakhshani 593, 594, 596
AfshinDerakhshani 598

AfshinMoniri 327
Afshin Moradi 134, 407, 443, 460
Afshin Namdar 259
Afshin Nikkhoo 193, 926, 927, 928, 930
Afshin Ostovar 23
Afshin Tahmasebi 372
Aghdas Mirjalili 145
Agheel Tabarmolahasan 829
Aghil Bahramian 778
Ahad Mokhtarzadeh 464, 729
Ahad Zare 24, 25, 1035
A. Heravian 798
ahereh Taheri 415
Ahmad Asoodeh 143
Ahmad Bagheri 551
Ahmad Gharehbaghian 738
Ahmad Hosseini 31
Ahmad Karkhah 442, 702, 990
Ahmad Majd 25, 278, 281
Ahmad Oryan 415
Ahmad Rahimi 37
Ahmadreza Jamshidi 130
Ahmad Reza Jamshidi 94, 126, 127
Ahmad-Reza Mahmoudi 40
Ahmad Rouhizadeh 1002
Ahmad Tahamoli Roodsari 1020
Ahmad Tajemiri 739
Ahmad Zavaran Hosseini 237, 665
Ahmed Al-Humadi 712
Ahmed Sedegh 238
Ajami A 100
ajediKhanian, M. 724
Akbar Anaeigoudari 1009
Akbar Dorgalaleh 86, 740
Akbar Ghorbani 58
Akbar Safaei 28
Akram Abouie Mehrizi 262, 541, 967
AkramAbouie Mehrizi 965
Akram A. Mehrizi 263, 978
Akram Davoodi 600
Akram Firouzi Amandi 165
Akram Jamali 37
Alaleh Rezalotfi 35
Ali Aghebati Maleki 51
Ali Ahmad Bayat 56, 172, 319
Aliakbar Amirzargar 257, 258, 963, 964
Ali Akbar Amirzargar 127, 259, 654
Ali Akbar Delbandi 224, 352, 388, 502, 503, 504, 734
Aliakbar Haddad Mashadrizeh 314, 453, 455
Aliakbar Haddad-Mashadrizeh 709
Aliakbar Movassaghpour 532
Ali Akbar Nasiri 545
Ali Akbar Pourfathollah 88, 742, 1003
Ali Akbar Pourfatollah 741
Ali Akbar Rahim Rahimi 451
Ali Akbar Saboor Yaraghi 447
Ali Akbar Velayati 393, 394
Ali Al-fahham 1033
Ali Amiri 389
Ali Anisian 246
Ali Anissian 173, 293
Ali Arabkhazali 910
Ali Ariafar 32, 292, 648
Ali asadirad 64
Ali Asadirad 185, 512
Ali Asadi Rad 443
Ali Asghar Tehrani 196
Ali Dabbagh 169
Ali Eshaghi 455
Ali farahi 811
Ali Farahi 810
Ali Farazmand 428
Ali Fattahi Bafghi 145
Ali Fouladvand 565

Ali Ganji 111, 1011
Ali Ghajari 569
Ali Ghazavi 111, 1011
Ali Gorgin Karaji 579, 581, 582, 585, 586, 600
Ali Grgin karaji 22
Ali Hashemi 268, 778, 779, 1021
Ali Hatef Salmanian 261, 975, 977, 1050
Ali H. Salmanian 978
Ali jalili 282, 919
Ali Jalili 463, 629, 630, 640, 920
Alijan Tabarraei 160
Ali Khamesipour 542, 1060
Ali Khodadadi 153, 201, 614
Ali Mahdavinezhad 345
Ali Masjedi 193, 926, 927, 928, 930
Ali Mohamadi 679
Ali Mohammad Behroozikhah 274
Ali Mohammadi 30, 42, 288, 474, 687, 689
Ali Mohammad Mohseni Majd1 1059
Alimohammad Varzi 85
Ali Mosayebian 19
Ali mostafaie 355
Ali Mostafaie 439, 551, 739, 900
Ali Mostafaie3, Kamran Mansouri 865
Ali Movahedi 138
Ali Niazi 227
Ali Nohekhan 482
Alipasha Meysamie 1017
Ali Pourmohammad 391
Ali Rahimi 526, 1027
Ali Ramezani 114
Ali Rastegari 193, 927, 928, 930
Ali ReazaFarahani 274
Alireza Abdollahi 295
Alireza Ahmadi 992
Alireza Andalib 181, 610, 612, 672
AlirezaAndalib 302
alireza asef 910
Alireza Azargoon 389
AlirezaBarati 1054
Alireza bari 660
Alireza Biglari 51, 63
Alireza Esmaeili 399
AlirezaEsmaeili 125
Alireza Ghanavatinejad 220, 221
Alireza Ghavami 289
Alireza Izadi 368
Alireza Khabbazi 830
Alireza Khazaei 1063
Alireza Khoshdel 986
Ali Reza Khosravi 108
Alireza Mardomi 291
Alireza Mehdizadeh 726
Alireza Mirzaei 646
Alireza Najafi 799, 812
Alireza Pasdar 138
Alireza Rafiee 390
Alireza Rafiei 324, 398, 844, 854
Alireza Razavi 293, 300, 663, 843
Ali Reza Razavi 447
Alireza Refeie 650
Alireza Rezaei Manesh 587
Alireza Rezaiemanesh 127, 128, 129, 405, 827, 828
Alireza Rezvani 31
Alireza salami 820
Alireza Shekohifar 688
AlirezaShoae-Hassani 65
Alireza Tahamtan 114
Alireza Vakilian 936, 938
Ali Reza Zali 244
Alireza Zamani 228, 488, 521, 522, 604, 746, 761, 853, 863, 966
AlirezaZamani 973
Ali R Khoshdel 757

Ali Saeedi Boroujeni 201
Ali Salmani 970
Ali Soleimanzadeh 946, 947, 948, 949, 952
Ali Vahid Dastjerdi 972
Ali Zekri 666
Allahyari Abualqasem 782
Alvanegh Mahmood Tavallaei 58
Amani Saeede 240, 996
Ameinh Hosseini 646
Amin Farhang 980
Amin Ghanivash 908
Amin Isanejad 754
Amin kamrani 299, 944
Amin Khazaei 1063
Amin Lafzi 744, 968
Amin Mirzaeian 651, 1049
Amin Oveisi 303
Amin Reza Nikpoor 785
Amin Roustami 1063
Amin Safa 909
Amin Seyfaddiny 121
Amin Shafizad 606
Amin soltani 852
Amin Soltani 605, 607
Amirabbas Salmani 18
AmirabbasSalmani 565
Amir Ali Hamidieh 65, 235
Amir Amiri Paryan 776, 839, 871, 1023
Amir asghari 935
Amir Avan 138
Amir Baghbanzadeh 464
Amir Ghaemi 304
Amir-hassan Zarnani 92
Amir Hassan Zarnani 176, 214, 220, 221, 293, 470, 471, 492, 496
Amir-Hassan Zarnani 40, 173, 493, 497
Amirhossein Alimohammadian 323, 691
Amir Hossein Faghfouri 726
Amir Hossein Jahangiri 330
Amir Mani 1038
Amir Mizbani 144
Amir Moghadam Ahmadi 986
Amir Mohammad Alborzi 380
Amir Mohammad Malvandi 93
Amir Rahimi 708
AmirReza AghebatKhayr 121
AmirReza Dehghanian 649
Amir Reza Heidari 249
Amir Sadeghi 918
Amir Salek Farrokhi 223, 400, 496, 497, 505, 655
Amir Shakouri 177, 879
Amirtaher Eftekharrosadat 655
Amir Tukmechi 767
A. Mohammadi 1 103
Amouzegar A 100
Amrolah Mostafazade 348, 512
Amrollah Mostafazadeh 858
AmrollahMostafazadeh 238, 611
Anahita Abbasi 535
Anahita Najafalizadeh 229, 610, 612
And Abdol mohamad Rostami 68
Anders Malmström 136
Andisheh Niakan 29
Andreas Kurz 115
Anne Puel 74
Anoshirvan Kazemnejad 60
Antimo D'Aniello 212
Antoine Haddad 796
Antonie Zwiers 136
AnwarFathollahi 239
A. Radmanesh 798
Aram Mohammadi 204, 206, 486
Aram Mohammmadi 202, 479
Arash Arashkia 66, 472, 542

Arash Kalantari 588, 590, 800
Arash Khojasteh 205
Arash Pourgholaminejad 147
Arash Samoudi 567
Arbabi Mohsen 773
Arda Kiani 325
ArdaKiani 328
Ardalan Asadollah pour 32
Ardeshir abbasi 353, 884
Ardeshir Hesampour 509
Aref Hosseinian Amiri 398
Arezki Chekaoui 167
Arezo Dabiri 974
Arezo Firoozeh 48
Arezo Gohari Shabgah 385
Arezoo Gowhari Shabgah 892
Arezoo Naderi 166
Arezou Abbasi 820
ArezouDabiri 723
Arghavan Asghari 826
A.R.Khoshdel 981
A.R. Khosravia 777
Arron Munggela Foma 259
Arsalan Jalili A 444
Aryaeian Naheed 404, 880
Arya Sohrabi 196
Asadollah Baesi 621, 762, 766, 771
Asadollah Mohamadi 660
Asadollah Mohammadi 377, 384, 789, 808
Asal Golchin 178
Asgarian-Omran H 100
Asghar Abdoli 382, 803, 807
Asghar Afshari 146, 272
Asghar Aghamohammadi 73, 337, 339, 340, 341
AsgharAghaMohammadi 72
Asghari M 135
A.Shahmohammadi 819
A. Sharifzadeha 777
Ashkan Rasouli 802
Ashraf vafa 1057
Ashraf Vafa 749
Asl iranifam Neda 996
Asma khanzade 935
Asrin Safari 117
Asyeh Javanian 576
Atabati Hadi 782
Ata Ghadiri 153, 616, 831, 834, 1002
Atefe Alirezaee 665
Atefe Ghamar Talepoor 163
Atefeh Abedini 325, 328
Atefeh Esmailnejad 555, 1010
Atefeh Najmaldini 1014
Atefeh Sadeghi Shermeh 388
Atefeh Yaftiyan 330
Atefe Mehrmanesh 585
Atena Mozafari 975, 977
Atieh Ehsani 203
Atiye Mellati 821
Atoosa Gitiara 443
Atousa Homafar 626
Atri Ghods 645
Ava Nasrolahi 351
Ayoob Arpanaei 1050
Ayssouda Hossein zade 363
Azad bahrampour 933
Azad Bahrampour 534, 877
Azam Bagherizadeh 936, 938, 1012, 1018, 1019
Azam bolhassani 929
Azam Bolhassani 765, 985
Azam heidari 566
Azam Jamshidian 518
AzamMoradi Zarmehri 976
Azam Rahimi 529
Azam Rahimpour 530

Azam Roohi 603
Azar Baradarn 141
A. Zavaran Hosseini 476
Azin Aghamajidi 611
Azita Talebzadeh Bonakdar 259
Azizi F 100

## B

Babak Abdolkarrimi 85
Babak Afkari 822
Babak Beikzadeh 273
Babak Ghalehbaghi 189
Babak Khodadadi 588, 590, 800
Babak Negahdari 56
Babak Vazirianzade 153
Bahador Bagheri 158, 558
Baharak Qa'edi 480, 482
Bahare Fazeli 808
Bahareh Abd Nikfarjam 426
Bahareh Fazeli 379
Bahareh Golkaran 544
Bahareh Hasani Sabzevar 314, 709
Bahareh Kardideh 401, 402
Bahareh Keshavarzi 40
Bahareh Mohebi 185
Bahareh Pakpour 937
Bahareh Razeghi Haghighi 286
Bahareh Rostami 929
Bahare Niknam 243, 515, 516
Bahare Shokoohian 231, 313
Bahman Abdi 1010
Bahman akbari 452
Bahman Khalesi 274
Bahman Khazaei 1063
Bahman Mansouri Miotlagh 866
Bahram Kazemi 234
Bahram Nikkhoo 629, 784
Bahram Yavari 573
Bakhshi Joibari Fahimeh 148
Bakhtiari Zahra 996
Baratali Mashkani 378, 788
Bassam Badra 796
Batol Zamani 403
Batoul Pourgheysari 615
BayazidGhaderi 629
B. Baradaran 103
BehnamKalali 238
Behnam Sanei 141
Behnaz Esmaeili 1017
Behnaz khani 956
Behnoosh Tayebi 25
Behnoush Baradaran 813
Behrooz Ilkhanizadeh 24
Behrooz Vaziri 230, 420
Behrouz Gharesi fard 498, 955
Behrouz Gharesi Fard 217, 499, 957
Behrouz Jalali Ghassam 455, 709
Behrouz Nikbin 94
Behzad baradaran 729
Behzad Baradaran 30, 36, 42, 128, 129, 405, 464, 474, 669, 674, 676, 677, 679, 686, 687, 689, 827, 828, 1043
BehzadBaradaran 306
Behzad Baradaran1 684
Behzad Einollahi 528
BehzadEinollahi 970
Behzad Ghareyazie 230
Behzad Hatami 443
Behzad mansoori 30
Behzad Mansoori 42, 288, 474, 677, 679, 687, 689, 1043
Behzad Nazel Khosroshahi 992
Beikzadeh L 804
Bijan Bambai 227
Bijan Khademi 76, 294, 641
Bijan Pirnia 381, 485
Bijan Sedighi Moghaddam 617

Bogoljub Ciric 68
B. Sedighimoghadam 638

## C

ChafiaTouil-Boukoffa 167
Charbonnier LM 26
chardouli R 100
Chatila TA 26
Christopher A McCulloch 207
Cynthia Aslan 692, 693
Cyrus Jalili 402

## D

Danial Ghasemi 993
Dariush Shanehbandi 288, 687, 689
Davar Amani 64, 180, 190, 200, 348, 349, 512, 656, 745, 748, 751, 918, 1052, 1053
Davood Bashash 671
Davood Jafari 259, 417
Davood Javanmard 809
Davood Rostamzadeh 31
Davoud Ghaffarian 376, 782
Delavari Mahdi 773
Delirezh Nowruz 307
Diba karimi 979
Dimitrios Balomenos 410
Djanette Hakem 167
Doroudchi M 77
Doroudchi, M 724
Dr. Abdolrahim Rezaee 791
Dr. Ali Reza Moradi 381
Dr.Ali Shirifi Zarchi 52
Dr. Houshang Rafatpanah 786
Dr Kambiz Pirnia 485
Dr. Kambiz Pirnia 381
Dr.Kaveh Kavousi 52
Dr.Maryam Darayee 703
Dr. Mehran Bakhshesh 806
Dr. Mohammad Mehdi Ranjbar 806
Dr. Rasool Roshan 381
Dr. Reza Boostani 786
Dr. Reza mansouri 392
D. Shanehbandi 103

## E

Ebadi Maryam 773
Ebarahim Rahimi 635
Ebrahim Eftekhar 891
Ebrahim Mazloomi 24
Ebrahim Mirzadeghan 40
Ebrahim Sakhinia 822
Ebrahim Taghizadeh 166
Ebrahim Zabihi 611, 620
EbtekarMasume 748
E. Davarpanah 267
Eelco D Keuning 247
Effat Farzanehnia 226
Ehsan Arefian 701, 1003
Ehsan Farashahi Yazd 942, 1051
Ehsan Khazaei 1063
Ehsan Nazemalhosseini Mojarad 467
Ehsan Nazemol hosseini Mojarad 187
Ehsan Nazemolhosseini Mojarad 466
Ehsanollah Ghaznavi Rad 770
Ehsan Sohrabi 733
Ehsan Soltaninejad 257, 963
Ehsan Zare Mehrjardi 735
Ehssan Shariat bahadori 142
Eisa Salehi 97
Elahe Aleebrahim Dehkordy 605, 607
Elahe Alikhani 235
Elahe Amani 117
Elahe Amirinezhad Fard 439, 900
Elahe Davarpanah 420
Elaheh Amani 591
Elaheh azizi 466
Elaheh Safari 232

Elaheh Talebi Ghane 746
Elahe Izadi 823
Elahe Mansoori 891
Elahe Safari 433, 835
Elham Abbasi 867
Elham Abdollahi 1031
Elham Aghamohamadi 460, 918
Elham Aghamohammadi 184, 459
Elham Ahmadi 861
Elham Alipour fayez 388
ElhamAmirchaghmaghi 736
Elham Atabati 826
Elham Bagbani 42
Elham baghbani 729
Elham Baghbani 464, 686, 687, 689
Elham Bagherinia 581, 582, 585
Elham Boustanipour 666
Elham Gholami 265, 420
Elham Harati 843
Elham Jafari 489
ElhamKashafi Jahromi 728
Elham Khalaf Adeli 742
Elham Mahabadi Ashtiyani 522
ElhamMahabadi-Ashtiyani 973
Elham Mahlooji 20
Elham Moases Ghafari 425
Elham Mohammadi 539
Elham Mohit 321, 454
ElhamNadimi 670
Elham Rahimi 834
Elham Rajaei 831
Elham Razani 671
Elham Roshandel 532, 533
Elham Sadat Hosseini Nasab 555, 1010
Elham Safarzadeh 42
Elham Safarzadeh1 1043
Elham Shokati 433
Elham Zangane 237
Elmira Gheytanchi 1016
Elmira shiuokhi 347, 733
Elnaz Asghari 731
Elnaz Farahani 246
Emad kordbacheh 980
Emami AH 608
Ensieh Lotfali 569
Ensieh Sadat Mirsharif 97, 1058
Erfan babaei 282, 919
Erfan Bahrami 776, 839, 871, 1023
Erfan Sadeghi 82, 836
Erna Domsgen 60
Esfahanian F 100
Eskandarian Maryam 218, 219
Eskandar Kamali-Sarvestani 68
Eskandaryan M 716
Esmaeil Allahmoradi 99, 290
EsmaeilAsli 274
Esmaeil Ebrahimie 227
Esmaeil Jarrahi 456
Esmaeil mortaz 910
Esmaeil Mortaz 119, 120, 296, 325, 328, 332, 342, 343, 388, 393, 394, 511, 1052, 1053
EsmaeilMortaz 327
Esmaeil Saneei Moghaddam 740
Esmaiil Mortaz 64
Ezzati Mehmandust Olya Mohammad 773

## F

Fabiano Oliveira 265
Faeze Fakour.k 791
Faezeh Abbasi Rad 338, 387, 956
Faezeh Asghari 684
Faezeh Maghsood 609
Faeze Khodadadi 658, 659
Fahime Aeine negin 149
Fahimeh Arabpour 606

Fahimeh Dadkhah 727
Fahimeh Khadem 387, 956
Fahimeh Lavi 253, 514
Fahimeh Mohammadi 1012, 1018, 1019
Fahimeh Nematimonsour 1001
Fahimeh Ranjbar Kermani 794
Fahimeh Rostamabadi 1018, 1019
Fanuel Songwe 279
Farah Bokharaei Salim 799, 812
Farahmand M 804
FarahnazBodaghkhan 289
Farahnaz Ghahremanfard 631, 637
farahnaz motamedi 987
Farahzad Jabbari Azad 18, 565
Faramarz Souri 860
Fardin Ahmadkhani 367
Fardin Fathi 662
Farhad Abolnezhadian 280
Farhad Babaie 128, 129, 405, 827, 828
Farhad Gharibdoost 80, 126
Farhad Jadidi Niaragh 193, 926, 927, 930
Farhad Jadidi-Niaragh 165, 928
FarhadJadidi-Niaragh 43
Farhad Rezaei 371
Farhad Riazi Rad 390
Farhad Salari 22, 579, 585, 586, 600, 692
FarhadSalari 581, 582
Farhad Seif 189, 587, 635, 832
Farhad Shahsavar 389
Fariba Asgharpour 868
Fariba ghorbanzadeh 785
Fariba Hejazynia 1063
Fariba Keramat 105, 761, 774
Fariba Mirjalili 735
Fariba Ramezanali 736
Fariba Rashid 468
Fariba sohrabi 739
Fariborz Bahrami 390
Fariborz Mokarian 229, 610, 612
Fariborz Zandieh 281
Farideh Talebi 137, 252, 254, 408, 409
Faride Jowkar 297, 642
Faride Sadeghkhani 701
Farid Semsarha 583
Farid Soleimani Mohammadi 784
Farimah Masoumi 254
Farin Malekifard 855, 946, 947, 948, 949, 952
FarnazFahimi 178
Farnaz Hajizadeh 940
Farnaz Malekifard 418, 419
Farnaz Zahedifard 143
Farrin Babaei Balderlou 361
Farrokh Ghahremaninejad 278
Farshad Foroughi 257, 258, 963
Farshid Fathi 82, 412, 836
Farshid Mossallaie 993
Farshid noorbakhsh 257
Farshid Noorbakhsh 96, 137, 252, 254, 339, 408, 409
Farshid Saadat 55
Farshid Yeganeh 206, 239, 246, 416
Farzad Nazari 1052, 1053
Farzad Yazdani 295
Farzaneh Chahardahcherik 380
Farzaneh Dasturian 725
Farzaneh Farshdari 321, 454
Farzaneh Fesahat 942, 1051
Farzaneh Ghaderi Shabankareh 171
Farzaneh Ghorbani 209
Farzaneh Hosseini 1045, 1048
Farzaneh Peighambarzadeh 229
Farzaneh Rostami 453
Farzaneh Saffari 673
Farzaneh Tafvizi 922
Farzaneh Tofighi Zavareh 427

Farzane Ordoni aval 675, 677
Farzin Javid 166
Fateme akbari mehni 592
Fateme Babaha 73
Fatemeh Adjaminejad 114
Fatemeh Ahmadi 751
Fatemeh Ahmadi Motamayel 345
Fatemeh Atyabi 193, 927, 928, 930
Fatemeh Ayoobi 102, 986
Fatemeh Boroun 568
Fatemeh B. Rassouli 658, 659, 872, 873, 876, 885
Fatemeh Dadgar Pakdel 643
Fatemeh Doroudian 417
Fatemeh Ezzatifar 324
Fatemeh Faraji 176
Fatemeh Ghaemimanesh 56, 172, 319
Fatemeh Goudarzi 773
Fatemeh Hadi 312, 699, 783
FatemehHadi 50
Fatemeh Hajari Taheri 472
FatemehHajari Taheri 66
Fatemeh Hajighasemi 705, 706, 707
Fatemeh Hajmanochehri 795
Fatemeh Hasheminia 453
Fatemeh Heidarnejad 444
Fatemeh Hemati 575
Fatemeh Hesam Pour 499
Fatemeh Hosseini 680
Fatemeh Izadi Jahromi 649
Fatemeh Kazemi Lomedasht 475, 561, 698
Fatemeh khadir 415
Fatemeh Khaki Khatibi 890
Fatemeh Maleki 1055
Fatemeh Mami 246
Fatemeh Mardani 133, 251, 253, 399
Fatemeh Mirzaeyan 651
Fatemeh Mohammadizadeh 939
Fatemeh Motevalli 985
Fatemeh Nasri 499
Fatemeh Noparast 554
Fatemeh Pak 631, 637, 1035
Fatemeh Pourgholi 437
Fatemeh Raeesi Nejad 489
Fatemeh Raeisi Nejad 1034
Fatemeh Ranjbar 572
FatemehRanjbar 571
Fatemeh Razavi 325
Fatemeh Rezaei 222, 500, 960
Fatemeh Rezayat 584, 953
Fatemeh Sadeghifar 411
Fatemeh Saffari 673
Fatemeh Samiezadeh Mashadi 314
Fatemeh Sarlati 206
Fatemeh Sefid 706, 707
FatemehSefid 705
Fatemeh Shafiee 615
Fatemeh Shahhosseini 176
Fatemeh shojaei jeshvaghani 261
Fatemeh Tabatabaie 1055
FatemehTazari 562
Fatemeh Vafashoar 823
Fatemeh Yari 231, 313
Fatemeh Yarian 449, 450
FatemehYarian 185
FatemehYazdiyan 59
FatemeJalousian 1047
FatemeManshori 1047
Fateme Mousavi 278
Fateme pak 460
Fateme Rahmani 1015
fateme ranjbar 601
Fateme ranjbar 592
Fateme Sadat Dashti 869
Fateme Sadri Ardalani 310

Fateme Shaabanpour Aghamaleki 682, 683
FatemeShaabanpour Aghamaleki 53, 652
FatemeZare 320
Fatma Zohra Mazari 167
Fattah Sotoodehnejad Nematalahi 449
Fattah Sotoudehnejad 1032
Fazeli MS 608
Fazel Shokri 39, 43, 114, 175, 221, 266, 310, 447, 448, 471, 603
Fereshteh Abdolmaleki 226
Fereshteh Alsahebfosoul 79, 182, 520, 727
Fereshteh Alsahebfosul 974
Fereshteh Bahmani 403
Fereshteh Mehdipour 298, 618, 641, 645
Fereshteh Pourabdolhossein 442
Fereshteh Rasa 891
Fereshte Salami 341
Ferial Taleghani 481
FGhaffarifar 580
F. Gorzin 989
Firoozeh Khamoushi 586, 600
Firouz Ebrahimi 275, 1050
Firoz Ebrahimi 550
Foroogh Nejatollahi 61, 670
Forough Golsaz Shirazi 448, 603
Forough Golsaz-Shirazi 39, 43, 175
Forouzan Yousefi 514
Forouz Keshani 480
F. Rahmani 989
Frank A. Redegeld 296
Frank Momburg 174
Friborz Hovanloo 751
F. Vafashoar 131

## G

Garshasb Rigi 697, 720
Georges Snounou 978
Gert Folkerts 120
Geshvad Nasiri Pirbazari 976
Ghada Kchour 772, 796
GhasemAshouri 1038
Ghasemi N 1042
Ghasem Janbabaei 290
Ghasem Mosayebi 111, 1011
Ghasem Solgi 35, 105, 345, 488, 774
Ghasem Solgi1 1020
Ghazaleh Bordbari 241
Ghazaleh Khalili Tanha 211, 1044
Ghazal Fatemi-Nasab 114
Ghazanfari T 847, 848, 874, 875
Gholamalikardar 232
Gholamali Kardar 278
Gholam Ali Kardar 20, 25, 279
Gholamhossein Hasanshahi 322, 763
Gholamhossein Hassanshahi 490
Gholam Hossien HasanShahi 121
Gholamreza Ahmadian 720
Gholamreza Ananisarab 1025
Gholamreza Anani Sarab 87, 89, 156, 688, 881, 883, 1044
Gholamreza AnaniSarab 211
Gholamreza Asadikaram 368
Gholamreza Azizi 212, 337, 341
Gholamreza Habibi 146, 272
Gholamreza Hatam 421
Gholamreza Javadi 319, 468
Gholamreza Nikbakht Brojeni 276
Gholamreza Nikbakht Brujeni 270
Gholamreza Pourmand 256
Gilda Parsamanesh 348, 512
Giti Farsi 757, 986
Golbahar Hosseini 979
Golnaz Babamiri 591
Golnaz Rashidi 153, 614
Golshan Sheikhansaria 943
Gordon A. Ferns 138

## H


Habbib Dastmalchi Saee 903
Habibollah Mirzaei 114
Habib Onsori 669, 674
Habib Soheyli 770
Hadi Atabati 376
Hadi Esmaili Govarchin Galee 851
Hadi Ghazanfari 365
Hadi Hassannia 680
HadiHassannia 43
Hadi Hossein Nataj 398
Hadi Hossein-Nataj 19, 45, 182
Hadi Hossein Nattaj 305, 526, 657, 1027
Hadi Mirzaeei 545
Hadi Mohagheghian Yaghoubi 352
HadiMoheb Alian 575
Hadinedoushan H 1042
Hadi Poormoghim 823
Hadiseh Farahani 417
Hadiseh Rezazadeh 45
Hadis khamevar 370
Hadi smaili Govarchin Ghalee 906
Hadis Moradjodaki 108
Hadi Zeidabadi 892
Hafezeh Davari 138
Haideh Namdari 429, 859
HajarAbbasi 70
Hajar Safari 620
Hajar Shali1 356
Hajar Yaghoobi 545
Hakim Azizi 545
Haleh Sarrafnia 801
Halimeh Hassanzadeh 411
Hamed Bagheri 925
Hamed Bashiri 988
Hamed Farhadi Kohan 761
Hamed Fouladseresht 721, 722
Hamed mohammadi 129
Hamed Mohammadi 36, 128, 405, 827, 828
Hamid Abtahi 111
Hamid Ahanchian 18
Hamid Aria 106, 366, 768, 769
Hamid Asadzadeh Afgdaei 134
Hamid Asadzadeh Aghdaee 349
Hamid Asadzadeh Aghdaei 67, 185, 186, 188, 407, 443, 465, 468, 801
Hamid Asadzadeh Aghdayi 348, 512
Hamid Badali 367
Hamid Daneshvar 149, 1056
Hamide danesh 592
Hamideh Rahimi 142, 896
Hamide Kouhpeikar 873, 876
Hamid Eshaghi 72
Hamid Farahmand 556
Hamid Farhangi 976
Hamid M. Niknam 417, 542, 543
Hamid Mohamad Jafari 324
Hamidreza Abdolsamadi 345
Hamid Reza A. Naghash 731
Hamid Reza Bidkhori 385, 411
Hamidreza Hakkakifard 997
Hamidreza Jamaati 325
Hamidreza mohajerani 921
Hamid Reza Mohammadimotlagh 401
Hamid-Reza Mohammadi-Motlagh 865
Hamid reza Mortazavi 22
Hamidreza Nouri 462
Hamid Reza Nouri 442, 620, 702, 704, 868, 990
Hamid Reza Rashidinejad 909
Hamid Reza Sadeghipour 843
Hamidreza Safari 156
Hamid Reza Taheri Nakhost 381
Hamid R. Golkar 403
Hamid Salimi 380

Hamid Shamsi 653
Hamzeh Rezaei 345
Haniehheydarlou 216
Hanieh Nikkhah 862, 903, 904
Hanie jafardokht bonjar 592
Hanie Mahaki 746
Haniye Ghasiyari 180
Hasan Borji 575
Hasan Namdar Ahmadabad 501
Hashemi, A 914
Hashemi Seyyed Mahmoud 911
Hashem Marawne 733
Hassan Abolhassani 341
Hassan Mahmoudi 761
Hassan Malekinejad 855
Hassan Nikoueinejad 528, 970
Hassan Rokni-Zadeh 51
Hatam GholamReza 148
Hatami H 874, 875
Hedayatollah shirzad 607
Hedayatollah Shirzad 183, 458, 852
HedayatollahShirzad 615
Hedieh Rahmati 717, 1013
Hediyeh Ghalikhani 486
Hediyeh Gholampour Sardashti 89
Hediyeh Rafighdoust 449, 450
Helia Azimi 797
Helya Sadat Mortazavi 809
Hematabadi M 100
Hemati N 536
Hengameh Akhavan 202
Henrike Veninga 136
HesamBabaei Khameneh 611
Hesam Karimi 540
Hien Van Doan 1038
Hiva Mohammadizad 112, 113
Hiva Sharebiani 48
H. Jafarinejad 798
H. Jafarinezhad 638
H. Noormehr 476
Hobbenaghi Rahim 240
Hoda Kavosi 126
Hoda Rahmani 268
Hodjatallah Rabbani 56, 172
Hodjattallah Rabbani 319
Homa Davoodi 622, 678
Homa Mohseni Kouchesfahani 529
Homa Sadri 570
Hoorieh Soleimanjahi 101, 540, 813
Hosain AqaHosaini 57
Hosein Ghambarian 64
Hosein Ghanbarian 334, 445
Hosein Honari 925
Hosein Khan Ahmad 672
Hosein Zolfagharian 276
Hosna salmani 590
Hossain Khoramdelazad 102
Hossain Khorramdelazad 639, 936, 938, 1012, 1018, 1019
Hossein Aazami 189
Hossein Akbari 528, 970
Hossein ali Ebrahimi 1014
Hossein Ali Ghaffaripouur 910
Hossein Ali Khazaei 1063
Hossein Asgarian Omran 290, 305, 398, 526, 654, 657, 896, 1027
Hossein Asgarian-Omran 19, 45
Hossein Batooli 20
Hossein Dalili 60
Hossein Fallahi 50, 312, 699, 783
Hossein Ghaedamini 921
Hossein Ghanbarian 250, 624, 983
Hossein Golmoghadam 383
Hossein Golmoghaddam 897
Hossein Goudarzi 778, 779

Hossein Hadinedoushan 869, 942, 1015, 1051
HosseinHadinedoushan 320
Hossein Hafezi 82
Hossein Hodjati 106, 366, 768, 769
Hossein hosseini 1026
Hosseini F 557
Hossein Javdani 810
Hossein Javdani Javdani 811
Hossein Khanahmad 181, 475
Hossein Khan-Ahmad 34
Hossein Khorramdelazad 490
Hossein Mahmoudvand 422, 423
Hossein Modirrousta 146, 272
Hossein Montazerghaem 725
Hossein Mortazavi 144
Hossein Motedayyen 82, 152, 200, 214, 492, 493, 715
Hossein Nikkhah 351
Hosseinpur Z 847, 848, 874, 875
Hossein Rahavi 363
Hossein Rahimi 660
Hossein Vazini 992
Hossein Yousofi Darani 141
Hossien Hafezi 412, 836
Hossien Mortazavi 318
Hossien Moteddayyen 412, 836
Hossien Rezvan 414
Hossin Kamiabi 1056
Houda Belguendouz 167
Houri Edalat 58
Housein Malekafzali Ardakani 318
Housein Malekafzali Ardekani 144
Houshang Rafat panah 1031
Houshang Rafatpanah 377, 378, 379, 384, 385, 659, 660, 788, 789, 808, 872, 873, 876, 885, 902
HoushangRafatpanah 658
Houshang Rafat Panah 376
H. Poormoghim 131
Hussam Al-Humadi 123, 712
Hyeryon Lee 143

## I

Ian M Adcock 119
Ian M. Adcock 120, 327, 393, 394
Ibrahim Toktaz 697
Ilnaz Rahimmanesh 181, 672
Iman Farahani 111
Iman Naamipouran 961
Ingrid Muller 318
Iradj Maleki 647
Iraj Rasooli 1039, 1046
Iraj Salehi 488, 521
IrajSalehi 973
Izad M 100

## J

Jafar Ai 529
Jafar Amani 261, 978, 1050
Jafar kiani 331
Jafar Kiani 1016
Jafar Mahmoudian 173
Jafar Majidi 107, 129, 827
Jafar Majidi. PhD 107
Jafar Salimian 350
Jahangiri A.H 716
Jahangiri Amir Hossein 218, 219
Jalal Abdolalizadeh 177, 879
Jalal Khoshnoodi 39, 43, 175, 266, 448, 603
Jalal shayegh 1026
JalehBarar 178
Jaleh Zolghadr 498
Jalil Adabi 117, 591
Jalil Kuhpayehzadeh 823
Jalil Mehrzad 93
Jalil Tavakkol Afshar 785
Jalil Tavakol Afshari 18, 892
JalilTavakol Afshari 565

Jamali M 732, 1036
Jamal Mohammadi Ayenehdeh 243, 515, 516
Jamileh Norouzi 350
Jamshid Hadjati 47, 309
JamshidHadjati 668
Jamshid Hajati 1016
Jamshidian Tehrani Narges 536
Jamshidi Fatemeh 404, 880
Jamshid Yazdani 792, 793
Jasper J. Koning 136
Javad Absari 528
JavadAbsari 970
Javad Akhtari 844
Javad Alizadeh 767
Javad Arasteh 1035
Javad Beheshtipour 553, 1000
Javad Dadgar Pakdel 643
Javad Fathi 980
Javad Hosseini 908
Javad Masoumi 936
JavadMohammadi 172
Javad Shokri Shirvani 462, 917
Javid Morad Abbassi 282
JavidSadeghi 178
Jean Villard 531
Jesus G Valenzuela 265
J. G. Valenzuela 267
Johan Garssen 120, 327, 393, 394
Joke M. M. den Haan 136
Joo Hwan No 143

## K

Kalantari-Khandani B 673
Kalantar Kurosh 148
Kambiz Bagheri 90
KambizYousofzadeh Eshkevari 326
Kamran Mansouri 739
Kamran Mousavi Hosseini 794
Kamran Rostami 180, 460
Karima Lahmar-Belguendouz 167
Karimi Ali 996
Karimi MH 536
Karin Mink 238
Kasra Esmaeilnia 146
Katayoon Bidad 94
Katayuon Bahman Soufiani 1003
Kaveh Baghaei 67, 134, 185, 348, 407, 443, 461, 512, 617, 998
Kaveh Baghai 64
Kaveh Khazaeel 958, 959
KavehTari 640
Kayhan Azadmanesh 174
Kazem Ahmadi 109, 233, 434, 878
Kazem AhmadI 373
Kazem Mashayekhi 349
Kazem Nemati 722
Kazem Parivar 365
KeivanGohari Moghadam 97
Keivan Shams 992
Kenji Chamoto, Tasuku Honjo 41
Kenneth Lundstrom 542
keramati, M 914
Khadijeh Koushki 349
Khaje-Bishak Y 135
Khatereh Kafshdouzan 558
Khatereh Noormohammadi 361
Khodakaram Jahanbin 961
Khodamorad Jamshidi 646
Khoshfetrat Ali Baradar 240
Khosrow Ebrahim 751
Kiarash Ghazvini 48, 815
Kimia Taghavi 328
Kimia Zandeih 172
Kobra Entezami 433
Kobra Mehdinejadiani 268, 316, 1021

Kokhaei P 608
Kosar Abouhamzeh 212
Kosar Salehitorabi 596
Kousar Smailnejad ganji 1006, 1008
Kousar Smailneja ganji 547, 895
Koushansineh sepehr 300, 663
Kurosh kalantar 383
Kurosh Kalantar 116, 155, 421, 993

## L

Ladan Langroudi 33, 1014
Laleh Babaeekhou 262, 541
Laleh Mavaddatiyan 905
Laleh Sharifi 371, 440
Latif Gachkar 809
Leila Bidar L 955
Leila Eini 513
Leila Farhadi 907, 908
Leila Hadadi 654
Leila Hatami Giklou Jajan 230
Leila Janani 1024
Leila Karimi 288
Leila Khani 1024
Leila Mobasheri 87
Leila Moradi 72
Leila Motamedzadeh 942, 1051
Leila Nejatbakhsh Samimi 283
Leila Nematollahi 321
Leila Parvaneh L 72
Leila Shakerian 60, 408
Leili Aghebati Maleki 944
Leili Aghebati-Maleki 165, 943, 969
Lennart Hammarstrom 60
Lisa G M van Baarsen 247
Lotfollah Behroo 589

## M

M.2, Karimi 77
Maede Hamidi 616
Maedeh Moradi 694
M.A.Fahmidekar 981
Mahboobeh Nazari 173
Mahboobeh Razmkhah 294
Mahboobeh Zolala 1034
Mahboobe Razmkhah 628
Mahboubeh Nikookalam 870
Mahbubeh Zolala 489
Mahdaneh Roshani 778, 779
Mahdavi R 732, 1036
Mahdi Abdollahi 156
Mahdi Alahgholi Hajibehzad 488, 521, 522, 966, 973
Mahdi Alahgholi-Hajibehzad 853
Mahdi AlahgholiHajibehzad 746
Mahdi Asmar 781
Mahdi Barati 158
Mahdi Behdani 176, 475
Mahdi Dehghanmanshadi 320
Mahdieh Amiri 562
Mahdieh Azizi 416
Mahdieh khazaee 133, 251
Mahdieh Khazaee 399
Mahdieh Molanouri Shamsi 101
Mahdieh Naghavi Alhosseini 622, 678
Mahdieh Raeeszadeh 553, 1000
Mahdi Fasihi-ramandi 233
Mahdi Fasihi Ramandi 548
Mahdi Fasihi-Ramandi 109, 373
Mahdi Ghasemi artiyan 759
Mahdi Ghasemiartiyan 758
Mahdi Ghatrehsamani 607, 852
Mahdi Goudarzvand 212
Mahdi Habibi-Anbouhi 176
Mahdi Kholoujini 992
Mahdi Mahmoudi 80, 94, 126, 127, 130, 397
Mahdi Pouramir 620
Mahdi Rohani 609

Mahdi Samani 484
Mahdi Shabani 310
Mahdi Shekarabi 224
Mahdi Taghaddosi 824, 825, 991
Mahdi Taghadosi 401, 402, 817, 818, 971
Mahdi Tarhini 772, 796
Mahdi Vojdanian 127, 130
Mahdiye Saadi 442, 990
Mahiar Jeloudari Mamaghani 273
Mahin Najafian 1002
Mahin Nikogoftar 235, 896
Mahin Nikogoftar Zarif 1003
Mahla jafari 592
Mahlajafari 601
Mahla Jafari 571
MahlaJafari 572
Mahmood Bakhtiyari 169
Mahmood Bozorgmehr 220, 221, 234, 513, 1016, 1024
Mahmood Jeddi Tehrani 266, 310, 471, 603
Mahmood Jeddi-Tehrani 39, 43, 175
Mahmood Saffari 200, 715
Mahmood Sheikhfatolahi 986
Mahmoud Hassani 66, 472
Mahmoudi Mahdi 404, 880
Mahmoud Jeddi Tehrani 448
Mahmoud Khaksary Mahabady 958, 959
Mahmoud Khayatian 725
Mahmoud Mahmoudi 251
Mahmoud Mahmoudi 125, 133, 249, 253, 399, 514, 1009
Mahmoud Mokbel 772
Mahmoud Rafieian kopaei 605, 1049
Mahmoud Vahidi 234
Mahnaz Ghaebi 494
Mahnaz Sourani 754
Mahnoosh Foroughi 169
Mahsa Alem 159, 430, 901
Mahsa Eshkevar Vakili 993
Mahsa Hajivalili 437
Mahsa Jafari 392
Mahsa Javadian 692, 693, 694, 695
Mahsa Mahdian 345
Mahsa Mohammadzadeh 56
Mahsa najafzadeh 599
Mahsa Najafzadeh 596, 597
Mahsa Rahgoshay 763
Mahsa Rahimzadeh 606, 725, 891
Mahsa Rezaeepoor 79, 520
Mahsa Saeedi Niasar 801, 923
Mahsa Safari 864
Mahsa Sobhani 228
Mahsa Taeba 362, 756
Mahsa Taghavi 1040
Mahsa Tanha 92
Mahshid Mehdizadeh 533
Mahshoid Mohammadipour 231, 313
Mahsima Shabani 339
Mahtab Fattahi 1032
Mahtab Gholamzadeh 802
Mahtab Maghsudlu 794
Mahyar Malekzadeh 648, 649
Mahyar Nourian 465
Mahyat Jafari 261, 975, 977
MaisamGhaedi 292
Majgan Bagherzadeh 547, 895
Majid Ahmadi 215, 216, 494, 495, 945, 954
Majid Changi-Ashtiani 51
Majid Faridgohar 528, 970
Majid Ghayour-Mobarhan 138
Majid Khoshmirsafa 13, 189, 283, 388, 587, 635, 832
Majid Marjani 120
MajidMarjani 327
Majid Mirmohammadkhani 1035
Majid Mirsadrai 575
Majid Momeni Moghaddam 411

Majid Mossahebi Mohammadi 300, 663
Majid Naderi 86
Majid Sirati Sabet 184, 459
Majid Tebianian 122
Majid Validi 702
Malek Hossein Asadi 623
Malihe Aghasizade 138
Maliheh Moradzadeh 160, 286, 346, 633, 634, 728
Malika Terrahi 167
Mana Momeni 576
Mandana Sattari 200, 201, 202, 203, 204, 205, 206, 479, 481, 483, 484, 486
Manijeh Khanmohammadi 40
Mani Ramzi 531
Manizhe Faghih 190, 918
Manizheh Habibpour 86
Mansoor Khakpoor 1054
Mansoureh Farhangnia 730
Mansoureh Farhangniya 1037
Mansour Khakpur 744, 968
Mansour Salehi 727
Manzoomeh Shamsi Meymandi 1034
Maral Nazarifar2 583
Marco Maccarana 136
Marjan Mansourian 79, 520
Marjan Nassiri Asl 426
Marjan Zamani 786
Markus Gerhard 238
Martin Skerhut 238
Martje Erkelens 247
Maryam Abedi 297
Maryam Abolghazi 417
Maryam Abolhasani 173
Maryam Ahmadzadeh 321, 454
Maryam Ajami 741
Maryam Akhtari 130
Maryam Akrami Somea Bozorg 41
Maryam Alinejad Dizaj 342, 343
Maryam Ameri Torzani 262
Maryam Asadi 869
Maryam Azarafza 647
Maryam Azimi Mohamadabadi 524
Maryam.Azizpour.m 546
Maryam Bahrami 843
Maryam Bayat 157, 431
Maryam Behfar 65
Maryam Darayee 708
Maryam Davarpanah 18
Maryam Diansaei 166
Maryam Ekhtelat 834
Maryam Erfanmanesh 984
Maryam Fakhimi 32, 628, 642
Maryam Fakhmi 297
Maryam Farghadan 1013
Maryam Ghahremani Piraghaj 334, 445
Maryam Ghane 450
Maryam Golara 266
Maryam Habibzadeh 462
Maryam Heidari 67, 134, 407, 415, 461, 998
Maryam Hemmatzade 827
Maryam Hemmat zadeh 36
Maryam Hemmatzadeh 129
Maryam Hosseini 840
Maryam Iranpoor 1034
Maryam Izad 429, 524, 859, 1017
Maryam Kafizadeh 763
Maryam Karami 621
Maryam Kargar 447
Maryam karimi 784
Maryam keykhaee 799, 812
Maryam Khabareh 358
Maryam Kheirandish 241
Maryam khoshkhooi 565
Maryam Khoshkhooi 18

Maryam Khosravi 380
Maryam Mahdiyar 635
Maryam Mahjoub 587
Maryam Mehri 482
Maryam Mhammad Ganji 302
Maryam Moazamigoodarzi 111
Maryam Mobini 398, 603
Maryam Moghaddam Matin 411
Maryam Mohammad-Ganji 34
Maryam Mohammadian 843
Maryam mollaee alamooti 987
Maryam Moogooei 363
Maryam Moossavi 593, 594
Maryam Moradi 576, 988
Maryam Moradi Chaleshtori 690
Maryam Moslehi Baharanchi 422, 423
Maryam Naseroleslami 309
Maryam Nemati 102, 432, 909
Maryam Nezafat Firizi 501
Maryam Nikoonezhad 533
Maryam Nourizadeh 60, 185, 617
Maryam Rastin 125, 133, 249, 251, 253, 399, 1009, 1031
Maryam Roozbehkia 397
Maryam Rostamtabar 704
Maryam Sahebari 125
Maryam sahlolbei 331
Maryam Salimi 444
Maryam Shabani 168, 441
Maryam Shahhoseini 736
Maryam shakoori 1010
Maryam Sharifshoushtari 20, 25
Maryam Sharif Shoushtari 281
Maryam Sohrabi 853
Maryam Tajabadi Ebrahimi 937
Maryam Tavakoli 220, 221
Maryam Vaezjalali 797
Maryam Valizadeh 741
MaryamYousefi 173
Maryam Zare 957
Maryan Skandaryan 330
Marzie Askari 488
Marzieh Ebrahimi 35
Marzieh Eghtedar doost 1062
Marzieh Heidarzadeh Arani 20
Marzieh Mansouri 531
Marzieh Mohammadian Manesh 559
Marzieh Naseri 1016
Marzieh Nejati Fard 480
Marzieh Norouzian 606
Marzieh Rezaei 619
Marzie Motaghy 121, 322
Marzie Norouzian 641
Marzie sadeghi 1026
Marzie Zilaee 280
Marziyeh Mohammadi Kordkhayli 432
Marziyeh Mohammadi-Kordkhayli 102
Marziyeh mohammadi kordkheyli 519
Masha Hoseini 957
Masomeh Tehrani 621, 766
Masoomeh Asadifard 87
Masoomeh Baghaei 193, 927, 928, 930
Masoome Keykha 447
Masoud Etemadifar 79, 520, 727
Masoud Ghorbani 230
Masoud haghshenas 447
Masoud Moghimi 510
Masoud Movahedi 281
Masoud Movassaghi 119
Masoud Nafari 456
Masoud Reza Seyfi Abad Shapouri 271
Masoud Shahdroodian 976
Masoud Shirmohammadi 694, 695
Masoud Soleimani 509
MasoudSoleimani 33

Masouma Mowahedi 83
Masoumeh Asadi 717, 730, 1013, 1037
Masoumeh Douraghi 266, 371
Masoumeh Ebtekar 63
Masoumeh Ebtekara 540
Masoumeh Mazandarani 622
Masoumeh Rajabibazl 530
Masoumeh Tavakoli Yaraki 646
Massood Hosseinzadeh 625
Massoud AH 26
Massoud Saidijam 761
Masume Ebtekar 656, 745
Masumeh Ahanjan 775
Masumeh Avari 790
Matin Aghalar 37
Mazarzadeh 819
Mazdak Ganjalikhani hakemi 520
Mazdak Ganjalikhani-hakemi 79, 181, 475
Mazdak Ganjali khani-Hakemi 34
Mazdak Ganjalikhani Hakemi 229, 252, 302, 338, 340, 387, 974
Mazdak Ganjalikhani-Hakemi 73
MazdakGhanjalikhan Hakemi 74
M.Badri 580
Mehdi Aleahmad 221
Mehdi Aleahmad 220
Mehdi Alikhani 456
Mehdi Barati 631, 637
Mehdi Behdani 472
Mehdi Dadashpour 165
Mehdi Ebrahimiyan 369
Mehdi Fouladi 178
Mehdi Ghahary 109
Mehdi Golchin 539
Mehdi Kadivar 609
Mehdi Kalani 106, 366, 768, 769
Mehdi KazemporDizaji 327
Mehdi Kazempour Dizaji 328
Mehdi Mahdavi 544, 632, 870, 995, 1001
Mehdi Mahmoodi 757, 986
Mehdi Malek Jamshidi 427, 428
Mehdi Mirsaeidi 119
Mehdi Montazer 28
Mehdi Raie 82, 412
Mehdi Ramazanpour 325
Mehdi ShafieeArdestani 1055
Mehdi shahbazi 917
Mehdi Shahbazi 112, 113, 547, 700, 787, 895, 1006, 1008
Mehdi Shamsara 235
Mehdi Shekarabi 189, 388, 587
Mehdi Shkarabi 832
Mehdi soltanzadeh Yamchi 917
Mehdi Talebi 532
Mehdi Tourani 462
Mehdi Yousefi 147, 209, 215, 216, 396, 427, 428, 437, 494, 495, 523, 821, 840, 940, 943, 944, 945, 954, 969
Mehdi Yusefi 107, 192
Mehdi Yusefi. PhD 107
Mehran Akbari 314
Mehran Falahpour 883
Mehran Gholamin 242
Mehrangiz Dezfoulian 923
Mehran Habibi-Rezaei 887
Mehran Moharreri 378, 788
Mehrdad Gholami 371
Mehrdad Hajilooi 105, 345, 774, 1020
Mehrdad Hashemi 56, 172
Mehrdad Iranshahi 658, 659, 872, 873, 876, 885
Mehrdad Payande 739
Mehrdad Payandeh 355
Mehrdad Pooyanmehr 776, 839, 871, 1023
mehrdad ravanshad 987
Mehri Barabadi 258, 963
MehriBarabadi 257

Mehri Ghafourian 762, 771, 831, 961, 999, 1002
Mehri Hajiaghayi 426
Mehri Shahir 332, 511
Mehrnaz Izadpanah 730, 1013, 1037
Mehrnoosh Doroudchi 106, 163, 366, 383, 436, 721, 722, 768, 769, 897, 957
Mehrnoosh Maalhagh 888
Mehrnoosh pashaei 631, 637
Mehrnoosh Ramezani 40
Mehrnoosh Zagheh 966
Mehrzad Ghari 319
Meinoosh Shabani 809
Meraj Tabatabaei 40
Meysam Abtahi Foroushani 653, 775
Meysam Amidfar 723
Meysam Ganjibakhsh 717, 1013
M. Faranoosh 638
M.H 77
M. Hedayat 989
Mihan Pourabdollah Toutkaboni 328
Milad Ameri 661, 916
Milad Asadi 42, 288, 692
Milad Gholami 81
Milad Hemati 575
Milad Moloudizargari 296
MiladMoloudizargari 327
Milad sabzevary-ghahfarokhi 183
Mina Alimohammadi 650
Mina Biria 203
Mina Ghorbanpour 257
Mina Mirzaee 995
Mina Motallebnejad 868
Mina Rahmanpour 105
Mina Rezaei 49
Minoo Adib 664
Minoo Mohamadi 937
Minoo Shaddel 653
Minoo Soltani 1047
Mir Davood Omrani 78
Mir Hadi Jazayeri 1024
Mir Saeed Yekaninejad 257, 259, 279, 963, 964
Mitra Sabetghadam Moghadam 350
Mitra Salehi 187
Mitra Shokri Majelan 1012
M. Javadian 103
M. J. Mahmoudi 989
M.Khojasteh 819
M.Mahmoodi 819, 981
M. Nadji 131
Moazzeni Seyyed Mohammad 218, 219
Moazzeni S. M 716
Moein Farshchian 411
Mohaddase Jarrahi 752, 753
MohaddesehGhezelcheh Khamsiyan 303
Mohadesehsadat Hosseini 546
Mohadeseh Toghyani Khorasgani 718
Mohamad Ali asadi 383
Mohamad Ali Assarehzadegan 823
Mohamad Ali Ibrahim zade 854
Mohamad Ali Ibrahim Zade 844
Mohamad Amin Pourhoseingholi 460
Mohamad Reza Fazlollahi 25
Mohamad reza Haghshenas 297, 642
Mohamadreza Khosravi Farsani 754
Mohammad Abbasi 604
Mohammad Abdi 908
Mohammad Ali Haghighati 563, 564, 567
Mohammad Ali-Hasanzadeh 955
Mohammad Ali Judaki 266, 448
Mohammad Ali Mansournia 794
Mohammad Ali Mazloomi 265
Mohammad Alimoradi 739
Mohammad Ali Sahraian 525
Mohammad Ali Shokrgozar 176

Mohammad Amin Behmanesh 958
Mohammad amin Boshagh 920
Mohammad Amin Boshagh 463
Mohammad Amin Pourhosseingholi 180
Mohammad Amir Alizade Tabrizi 478
Mohammad Ayubi Rad 757
Mohammad Azizi 274
Mohammad DavoodzadehGholami 232
Mohammad Ebrahim Ghaffari 761
Mohammad Fayyad Kazan 772, 796
Mohammad Fereidouni 577, 578, 593, 594, 596, 597, 598, 599, 826
Mohammad Ghasemi 153, 280
Mohammad Gholamian Hamadan 746
Mohammad Hadi Sadeghian 658, 659, 873, 876, 976
Mohammad Haghighi 488
Mohammad Hasan Bemanian 554
Mohammad Hasan Motedayen 276
Mohammad Hossein Baradaran 356
Mohammad Hossein Dabbaghmanesh 649
Mohammad Hossein Ghahremani 173
Mohammad Hossein Ghorbani 188
Mohammad Hossein karimi 955
Mohammad Hossein Karimi 535
Mohammad Hossein Kazemi 266, 532
Mohammad Hossein Modaresi 66
Mohammad Hossein Nicknam 94, 127
Mohammad Hossein Niknam 964
Mohammad Hossein Nikoo 722
MohammadHossein Sanei 181
Mohammad Hossein Somi 655
Mohammadhossein Yazdi 632
Mohammad Hossein Yazdi 870
Mohammadi MM 673
Mohammadi Rahim 240
Mohammadi, S.A 914
Mohammad Jafari 352, 734, 799, 812, 992
Mohammad Jahantigh 550
Mohammad Javad Abasszadeh Afshar 489
Mohammad Javad Ashraf 294
Mohammad Javad Fattahi 649
Mohammad JavadGharavi 570
Mohammad Javad Motamedi 975, 977
Mohammad Javad Nasiri 391
Mohammad Javad Zibaeenezhad 163, 722
Mohammad Kamali 322
Mohammad Kamalinejad 843
Mohammad Kazemi 727
Mohammad Kazemi Arababadi 145
Mohammad KazemiArababadi 1009
Mohammad Keramatipour 643
Mohammad Khojasteh 861
Mohammad Khosravi 568, 951, 958, 959
Mohammad Mahdi Eftekharian 210, 228
Mohammad Mahdi Hayatbakhsh 892
Mohammad Mahdi Mohammadi 259, 489, 651, 942, 1051
Mohammad Mahdy Ranjbar 276
Mohammad Mahmoudi 1040
Mohammad Majidi 444
Mohammad Masoud Shoushtarian 829
Mohammad Mehdi ADIBZADEH SERESHGI 373
Mohammad Mehdi Akbarin 660
Mohammad Mehdi Amiri 39, 43, 175, 310, 603
Mohammad Mehdi Koshayar 660
Mohammad Mehdi Mohammadi 909, 1014
Mohammad Mehdi Naghizadeh 1059
Mohammad mehdi ranjbar 1021
Mohammad Mehdi Soltan Dallal 371, 440
Mohammad mirzakhani 917
Mohammad Moafi 414
Mohammad Mofateh 577
Mohammad Nemati 568
Mohammad Nouri 451
Mohammad Rafiee 604, 897

Mohammadraman Molodi 907
Mohammad Rashno 153, 961
Mohammad Rastegar Moghadam 314
Mohammad Reza Abbaszadegan 242
Mohammadreza Alijani 150, 1048
Mohammad Reza Atashzar 635
Mohammad Reza Bolouri 388
Mohammad-Reza Bolouri 40
Mohammad Reza Fazlollahi 279
Mohammad Reza Gharaati 456
Mohammad Reza Hafezi Ahmadi 889
Mohammad Reza Haghshenas 294
Mohammadreza Hajizadeh 986
Mohammad Reza Hedayati Moghaddam 385
Mohammadreza Jalali 444
Mohammad Reza Keramati 976
Mohammad Reza khedri 871
Mohammad Reza Khedri 776, 839, 1023
Mohammad Reza Mirzaei 623
Mohammad Reza Mirzaeic 362, 756
Mohammad Reza Mofateh 596
Mohammad RezaMofatteh 598
Mohammad Reza Mokhber Dezfouli 273
Mohammad Reza Mozdianfard 20
Mohammad Reza Nejadmoghaddam 173
MohammadrezaSharifi 302
Mohammad Reza Shokri 220
Mohammad-Reza Shokri 221
Mohammadreza Talebi Ardakani 479
MohammadrezaTohidkia 178
Mohammad Reza Tohidkia 451
Mohammad Reza VaezMahdavi 1062
Mohammad Reza Yazdani 436
Mohammad reza Zali 67, 134, 185
Mohammad Reza Zali 70, 180, 184, 188, 349, 407, 443, 459, 460, 461, 465, 801, 918, 922, 923, 998
Mohammad R Hajizadeh 757
Mohammad Rostami Nejad 180, 184, 190, 349, 459, 460, 922
Mohammad Rostami-Nejad 918
Mohammadsadegh Naghizadeh 599
Mohammad Sadegh Naghizadeh 577, 594, 883
Mohammad sadegh Soltani Zangbar 498
Mohammad Salehi 369
Mohammad Shafiee 1030
Mohammad Shahbazzadeh 864
Mohammad Shahrooei 51, 576
Mohammad Shojaei 888
Mohammad Taghikhani 655
Mohammad Taghi Rezayati 936, 1012, 1018, 1019
Mohammad-Taghi Rezayati 102, 639, 938
Mohammad Taher Afshounpour 101
Mohammad Taheri 78, 210, 554
Mohammad Taher Tahoori 400
Mohammad Tajfard 138
Mohammad Varahram 120, 393, 394
Mohammad Varhram 327
Mohammad Vodjgani 80, 126, 643
Mohammad Vojgani 1062
Mohammad Yousef Alikhani 604, 761
Mohammad Zamanian 757
mohammad zare mehrjerdi 559
Mohammad Ziaee 85
Mohhamad Mahdi Mohhamadi 1034
Mohmmad Hosein Karimi 537
Mohmmad Sadegh Falahatkar 795
Mohsen Abolhassani 66, 230, 472
Mohsen Akhiani 820
Mohsen Arabpour 618
Mohsen Badalzadeh 25
Mohsen Ebrahimi 1011
Mohsen Farhang 633, 634
Mohsenfarhang zargar 286
Mohsen Hamidpour 738

Mohseni Mahboobeh 219
Mohsen Jafarian dehkordi 972
Mohsen Khalili 898
Mohsen Masjedi 154, 182, 715
Mohsen Molaei 600
Mohsen Nafar 964
Mohsen Najimi 550
Mohsen Naseri 156, 444
Mohsen Rastegari Pouyani 865
Mohsen Rokni 447, 976
Mohsen Saeedi 1030
Mohsen Salesi 755
Mohsen Shahmoradi 615
Mohsen Tadayyon 385
Mohsen Tehrani 45, 290, 647, 657, 820
Mohtaram Nasrollahi 781
Mojdeh Ghias Tabari 547, 895, 1008
Mojde Kazemi 909
Mojgan Astaneh 305
Mojgan Bagherzade 787
Mojgan Bagherzadeh 112, 113, 917
Mojgan bandehpour 450
Mojgan Bandehpour 234, 268, 690, 1021
Mojgan Ghaedi 363
Mojgan Mohammadi 18, 565, 892
Mojgan Mortazavi 256
Mojgan Naghitorabi 834
Mojgan Sanjari 489
Mojgun Mokhtari 182
Mojtaba Bagheri 882
Mojtaba Farahbakhsh 802
Mojtaba Mirzaei 275, 550
Mojtaba Nofeli 925
Mojtaba Salamatian 850, 894
Mojtaba Shahnazi 856, 857
Mojtaba Shohan 458
Momeni Mohamad 962
Mona Abbasi kalabe 455
Mona Abbasi kalabeh 453
Mona Amani 835
Mona Hemmati Chegeni 85
Mona Moshiri 371, 440
Mona Sadeghalvad 401
Mona Salahshoor dahr 449
Mona Salahshoor Dahr 450
Mona Salimi 143
Monireh Golpour 650
Monireh Jahantigh 69
Monireh Kazemimanesh 174
Monireh Mohsenzadegan 189
Morad Rahimi 551
Mortaza Ghasemi 521
Mortaz , Esmaeil 996
Mortaz Esmaeil 240
Morteza Abkar 197, 198, 548
Morteza Bagheri 726
Morteza Djamali 20
Morteza Fallahpour 283
Morteza Hosseininejad 557, 1045
MortezaHosseininejad 1048
Morteza Hosseinzadeh 963, 964
MortezaHosseinzadeh 257
Morteza Jafarinia 898
Morteza Mahmoudi 173
Morteza Oladnabi 675, 677
Morteza Sagharjoghi Farahani 590, 800
MortezaSagharjoghi Farahani 588
Morteza Salimian 738
Morteza Samadi 83, 125, 298
Morteza Shamsizadeh 740
Mosayeb Rostamian 417, 543
Moslem Ahmadi 310, 955
Moslem Kulivandi 982
Mostafa Aalipour 563, 564, 567

Mostafa Ejtehadifar 111
Mostafa Haji-Fatahaliha 840
Mostafa Haji molahoseini 169
Mostafa Haji Mollahoseini 799, 812
Mostafa Haji Molla Hoseini 21, 239, 416
Mostafa Haji MollaHoseini 246
Mostafa Hosseini 448
Mostafa Jarahian 174
Mostafa Manian 138
Mostafa Moin 20, 25, 281
Motahareh Bahadori 39
Motahareh Ehsani 547, 700, 787, 895
Motahhareh Ghasemi 695
Mousa Mohammadnia afrouzi 787
Mousa Mohammadnia Afrouzi 917, 1006
Mousa Mohammadnia-Afrouzi 112, 113, 547, 895
MousaMohammadnia Afrouzi 1008
Mousa Mohammadnia afrrouzi 700
Mousa Tavassoli 418, 419
Mozafar Mohammadi 61
Mozhdeh Namvarpour 122
M.R.Hajizade 981
M.Rohban 981
M.R.Shafipoor 981
Mrs.Zahra Sasanipour1 703
M.S.Fazaeli 981
M. Soltani 103
M. Soltania 777
M.Vakili 819

## N

Nadereh Naderi 606, 725, 891
Nadereh Taee 422, 423
Nader Tajik 176, 214, 243, 256, 492, 493, 502, 503, 504, 515, 516
Nadia Manavi 709
Naeges Sarlogzaei 1063
Nafise Esmael 672
Nafiseh Esmaeil 229, 338, 387, 610, 612, 852, 956
Nafiseh Tabasi 133, 251, 253, 399
NafisehTabasi 125
Nafiseh Zand 1061
Naghizadeh MM 749, 847, 874, 1057
Naghmeh Sattarahmady 197, 198
Naghmeh Shirafkan 679
NaghmehShirafkan 306
Naghmeh Zamani 922
Nahid aboutaleb 860
Nahideh Afzale Ahangaran 69, 899, 903
Nahid Ekandari 412
Nahid Eskandari 152, 154, 336, 520, 672, 727, 974, 1032
NahidEskandari 138, 636
NahidGhaedAmini 138
NahidJivad, HoseinTeymoori 326
Nahid Rezaei 252
Naiyer Hosseinahli 687, 689
Najaf Allahyari Fard 227
Najibeh Shekari 684, 692, 693, 694, 695
Najmefrozannia 747
NajmeShahinfard 605
Nakisa Zarrabi Ahrabi 443
Namdar Ahmadabad Hasan 148
Narges Amirjamshidi 611
Narges Arkian Boroujeni 977
NargesArkian Boroujeni 975
Narges Roomandeh 1035
Narges Rostami 193, 926, 927, 928, 930
Narges Valizadeh 379, 385, 815
Nariman Mosaffa 40, 186, 268, 715
Nariman Mossafa 1021
Narjes Hasan Fakhrabadi 275, 550
Narjes Soleimanifar 94
Naseh Sigari 919
Naser Mobarra 1030
NaserSamadi 178

NaserShagerdi Esmaeli 626
Nasimmohammad kheshtchin 309
Nasim Rahmani Kukia 884
Nasim Samarghandi 664
Nasim Samarghmarghandi 1055
Nasiri Z 848
Nasrin Ghasemi 1015
Nasrin Hosseinahli 689
Nasrin Iranshahi 818, 824, 825, 971, 991
Nasrin Iranshai 817
Nasrin Masoudzadeh 144
Nasrin Moazzen 18
Nasrin Nourollahzadeh 775
Nasrin Saharkhize 999
Nasrin Zare 636
Nasrollah Erfani 294, 297, 642
NasrollahMahboobiSoofiani 1038
Nasser Gholijani 116
Nassim Kheshtchin 47, 668
Nassim rahmani 227
Nastaran Mohseni 561, 698
Nastaran Sadat Savar 542
Navid Dashti 126
Navid D. D jadid 978
Navid D. Djadid 263
Navid Dinparast Djadid 967
NavidDinparast Djadid 965
Navideh Haghnavaz 684
Navideh Mirakhani 947
Navid Mijani 845
Navid Shomali 693
NavidShomali 306, 679
Nayere Askari 1062
Nazanin Amiri 188
Nazanin Atieh Kazemi sefat 315
Nazanin Fathi 153, 614
Nazanin Gholampour Farogi 455
Nazanin Gholampour Faroji 314, 453, 709
NazaninJafarpour 669
Nazanin mojtabavi 47
Nazanin Mojtabavi 293, 823
Neda Ahmadi afshar 418, 419
Neda Dezfuli 394
Neda Heidari 70, 409
Neda Mahavar 577, 598, 599, 826
Neda Mohsenpour 51
Neda Mokhberian 624
NedaMousaviNiri 309
Neda Sadat Gohari 1013
Neda Shajari 676
Negara Azarpira 625
Negar Behnamfar 163
Negar seyed 265
Negar Seyed 420
Negar Vanaki 130
Negar Varae 510
Negin Maleki 323, 691
negin mohammadpoor 906
Negin Mohammadpoor 866
Negin Nokhandani 678
Nesa Rashidi 352
Neyshabouri 858
Nezafat Ferizi Maryam 148
N. Ghanizadeh 638
Nika Khoshnevis 583
Nikoo H 77, 724
Nikoo Hossein-khan nazer 205
Nikoo Hossein Khan Nazer 484
Nilofar Keyhanmehr 412
Niloofar Alvandpour 1020
Niloofar Basrehizad 583
Niloofar Hassannejad 365
Niloofar Sadat Ebrahimi 467
Niloofar Taghipour 186

Niloufar Keyhanmehr 152
Niloufar Rezvani 541
Niloufar Sadat Miri 478, 480, 482, 552, 795, 856, 857
NimaParvaneh 72
Nima Rezaei 73, 339, 340, 397, 1040
Nima Rostami Koshki 369
Nima Taefehshokr 166, 303
Niusha Amiri 484
N. Mojtabavi 131
Nodehi M 100
Nooshin Ahmadzadeh 130
Nooshin Askarani 1004, 1005, 1007, 1022
Norouz Delirezh 159, 430
Norouz DelireZh 845
NosratollahZarghami 309
Nour Amirmozafari 781
Nourizadeh M 608
Nouroz Delerizh 681
Noushin Lotfi 412, 836
NoushinLotfi 82
Nouzar Nakhaee 368
Nowruz Delirezh 24, 195, 473, 552, 855, 952
N. Rezaei 989
N. Seyed 267
N. Shekari 103

## O

ohan Garssen 119
Omid Emadian 647
Omid Kooshkaki 1025
Omid ramezani 1026
Omidreza Salehi 755
Ostadrahimi A 135
owsar Smaeilnejad 787

## P

Padide Ebadi 537
Padideh Ebadi 535
Pak F 608
Pantea Mohammadi 551
Parastoo Shakerin 617
Pardis Bayat 422, 423
Pardis Sadeghipour 318
Paria Jorbozehdar 283
Parinaz mahdavi 463
Parisa Amir Kalvanagh 161, 540
Parisa Esmaeilie 988
Parisa Khodabandeh Shahraki 625
Parisa Nourbakhsh 587, 1011
ParisaVahidi 93
Parisa Zafari 817, 818, 824, 825, 971, 991
Parissa Farnia 327
Parizad Bavandpour-Baghshahi 113
Parsova Tavasolian 339
ParvanehAfsharian 736
Parvaneh Farzaneh 717, 730, 1013, 1028, 1037
Parvaneh Jafari 546
Parvin Ayremlou 726
Parvin Mansouri 1017
Parvin Nikaein 648
Parviz Farzamifar 695
Parviz Kokhaei 158, 460, 540, 558, 631, 637, 1035
Parviz Zoulghadri 795
Pascale Kropf 318
Payahoo L 135
Payam Saadat 677
Payam Tabarsi 119, 120, 327, 388, 393, 394
Payman Tavakoli 653
Pedram Ahmadpoor 257, 259, 963, 964
Pedram Azimzadeh 349
Pedram Nazari 380
Pejman Allahvaisy 591
Pejman Allahveisey 117
Peter Krustrup 751
Peyman Bakhshaei 266
Phipatanakul W 26

P Jorbozedar 131
P. Kokhaei 638
Poorya Foroutan 920
Poorya foroutan pajoohian 463
Pooyanmehr Mehrdad 962
Pordel, S 77, 724
Pouria Karimi 563, 564, 567
Pouriya Dadebighlu 166
Poursani Shiva 404, 880
Pourya Tahmasebi 613, 899
Pouya Lahoori 562
Pouyan houshmandpour 997
Pro.AR.Khosravi 370
P. Safari 131

## R

Radjabian T 847, 848, 874, 875
Rafal Al-Saigh 712
Rafal J. Al-Saigh 123
Rafatpanah Houshang 782
Raheleh Shokohi Shoormasti 25
Rahele Miri 385
Rahim Hobbenaghi 195, 473, 855
Rahman Emamzadeh 710
Rahmani Kukia Nasim 911, 931
RahmaniKukia Nasim 307
Rahman Shokri 410
Ramazan Ali Sharifian 654
Ramazan Rezaei 80
Ramezan-Ali Sharifian 43
Ramin Farajpour 805
Ramin Ghahremanzadeh 173, 470
Ramin Lotfi 579, 586
RaminPourakbari 969
Ramin Shekarriz 45, 290, 305
RaminShekarriz 657
Ramin Yaghobi 535
Ramona Nitiu 238
Ramyar Azizi 954
Rana Ezzeddini 400, 655
RaphaelaSemper 238
Rashin Mohseni 65
Rasool Baharloo 283
Rasool Rahimi Junqani 150
Rasoul Baharlou 635
Rasoul Godini 50, 312, 699, 783
Rasoul Jafari 141
Rasoul Nasiri kalmarzi 282, 919
RasoulSalehi 138
Rasoul Zarrin 726
Rassoul Dinarvand 173
Rayhaneh Ahangar Parvin 432
Rayhaneh Ahangar-Parvin 102
Razie Alibeigi 308
Razieh Amini 863
Razieh Dawodi 374
Razieh Dilmaghanian 97
Razieh Farazmand 453
Razieh Nejadi 481
Razieh Rezaeianpour 586
Razie Mahmoodian 738
Raziyeh Abdolhamidi 1039
Raziyeh Karami 607
RaziyehKarami 605
Reihaneh Pirjani 81
Reina E Mebius 247
Reina E. Mebius 136
Reyhaneh Babaei Khorzoughi 168, 441
Reyhaneh Ghorbanpour 270
Reyhaneh Hoseinpoor 530
Reyhane Mirzaee 1001
Reza Aflatoonian 256
RezaAflatoonian 736
Reza Ahangari Cohan 929
Reza Alizadeh Navaei 820, 896

Reza Boostani 376, 377, 379, 384, 789, 808, 902
Reza Falak 8, 13, 40, 232, 256, 283, 832
Reza Farid Hosseini 18, 376, 378, 565, 788, 902
Reza Gharakhanlou 101
Reza Gheitasi 889
Reza Hadavi 56, 172, 319
Reza Hajiaghaee 157, 856, 857, 858
Reza Hajiasghar Sharbaf 657
Reza Hosseini Ghatar 603
Reza Hosseini-Ghatar 39, 43
Reza Jafari 256, 802
Reza Malk poor 489
Reza Mansouri 20, 59, 122, 363, 392
Reza Marvinam 320
RezaMirfakhraie 81
Reza Nadafi 136, 247
Reza Nedaeinia 138
Reza Norian 552
Reza Nosratabadi 249, 1009
Reza Pasandideh 271
Reza Ramazani Khoorshiddoost 583
Reza Rezaie 979
Reza Shahbazi 488
Reza Valadan 647
Reza yazdani 72
Reza Yazdani 73, 340, 341
Rezvan Mohammadi Nezhad 559, 950
Rezvan MohammadiNezhad 556
Rezvan Pilpa 48
R.Mansori 819
Robabeh Taheripanah 81
Robabe Latifi 856, 857
Roghayeh babaei 812
Roghayeh Pirzadeh 674
Rogier M. Reijmers 136, 247
Rokhsareh Sadeghi 207, 481
Rooholla Abdollahpour 752, 753
Roonak azizbeigi 933
Roonak Azizbeigi 534, 877
Rostam Golmohammadi 966
Rostami Rad M 732, 1036
Rouzbeh Sanaei 339
Roya Bishe Kolaei 752, 753
Roya Dolatkhah 351
Roya Ghods 40, 173, 513, 1016
Roya khanahmadi 935
Roya Khosravi Eghbal 266
Roya Najafi Vosough 966
RoyaShamsi 653
Roya sherkat 336
Roya Sherkat 74, 338, 387, 414
Roya Taleban 414
Roya Yaraee 444
Rozhin Jamali 861
Rozita Abolhasan 192
Rozita Doosti 525
R. Safaralizadeh 267
R. Yarmohamadi 798

## S

saam torkan 972
Saber Rokhafrooz 999, 1002
Saboor-Yaraghi AA 100
Sadegh Farhadi 660
Sadegh Fattahi 611
Sadegh Feizollahzadeh 181
Sadegh Feyzollahzadeh 584, 953
SadraAnsaripour 605
Saeed Alamian 197, 198, 548
Saeed Amel Jamehdar 815
Saeed Azandeh 510
Saeed Babaie 111
Saeede Ghasemi 830
Saeedeh Erfanin 286
Saeedeh Mehraban Moghadam 379

Saeedeh Torabi Goudarzi 107
Saeede Sadat Mirbageri 997
Saeede Shabanitabar 528
Saeed Farhadzadeh 166
Saeed Malek Hosseini 383
Saeed Mirzaee 938
Saeed Namaki 70, 134, 407
Saeed Nazeri 967
Saeed noorolyai 464, 729
Saeed Oraee Yazdani 244
Saeedreza Vazirian 289
Saeed Talebi 315
Saeed Zeinali Heris 879
Saeid Abediankenari 13, 19, 291, 792, 793
Saeideh Erfanian 888
Saeideh Milani 56, 172, 319
Saeideh Tabesh 279
Saeid Pishgoee 892
Saeid Taghiloo 99, 290, 820, 896
Safar farajnia 452
SafaTahmasebi 984
Safdar Mehdizadeh Shermeh 829
Safoora Nikzad 228
Safoora pordel 284, 285
Sahand Fattahi 303
Sahar Bayat 81
Sahar etemadi 906
Sahar Etemadi 866
Sahar Ghaffari 558
Sahar Karimzadeh 721
Sahar Khorasani 133, 251, 253, 399
Sahar Mashayekhi 149
Sahar Mirzaee 870
Sahar Mohammadi 770
Sahar Mosalla 856, 857
Sahar Raoofi Mohseni 448
Sahar Salimi 96
Sahar Shariati 298, 645
Sahar Tahaghoghi Hajghorbani 844, 854
Sahel Heydari 352, 503
Sahel Khazaee 722
Saied Abediankenari 654
Saied Bokaie 440
Saiedeh Erfanian 160, 346, 633, 634, 728
Sajad Dehnavi 18, 565
Sajad Rashidi 421
SajediKhanian 77
Sajjad Ahmadi Renani 434, 878
Sajjad Esmaeili 883
Sakineh Pirahmadi 965
SakoMirzaie 629
Saleh Ramazani Khorshiddoost 583
Saloomeh Fouladi 154
Samaeh Khorami 433
Samaneh Abdolmohammadi Vahid 945
Samaneh Arab 47
Samaneh Beigi 185
Samaneh Delavari 201, 616, 831
Samaneh Gholami 829
Samaneh H. Shabani 263, 978
Samaneh khorami 835
Samaneh Mahmood Aghdam 1037
Samaneh Mahmoud Aghdam 730
Samaneh Maleknia 52
Samaneh Salehi pour 762, 771
Samaneh Tokhanbigli 348, 443
Samane Mohamadzade 672
Saman Hosseinkhani 93
Saman Nikeghbalian 619
Saman Seyedabadi 883
SamanSoleimanpour 48
Samira Asgharzade 1049
Samira Derakhshan 295
Samira Ghasemi 1030

Samira Ghorbani 137, 252, 254, 408, 409
Samira Mirbaha 710
Samiramis Qavam 889
Samira Mohammadi Yeganeh 316, 690
Samira Torki 852
Samira Zand 775
Sanam Dolati 192, 209, 396, 523, 821, 940
Sanaz Ahmadi Gezeldasht 385
Sanaz Baghban Rahimi 304
SanazDehghani 256
Sanaz Hesami 525
Sanaz Jourghasemi 105, 774
SanazKeshavarz shahbaz 963
SanazKeshavarzshahbaz 257
Sanaz Keshavarz Shahbaz 258
Sanaz Rasolzadeh 840
Sanaz Rasolzadeh Nori 147
Sanaz Sheikhzadeh 195, 473
Sanaz Zarasvand Nia 271
Sara Abdolmaleki 266
Sara Aghakhani 725
Sara Ghafarpour 642
Sara Ghaffarpour 97
Sara Golbahari 775, 849, 893
Sara Hajimiri 48
S.A Rahim Rezaee 48
Sara Iranparast 762, 766, 771, 999
Sara Mohamadi Saani 879
Sara Mohamadnezhad 781
Sara Parsa 662
Sara Parsapour 95
Sara Rasouli 790
Sara Shirahmadi 907
Sara Soudi 8, 101, 237, 250, 334, 445, 508, 509, 665, 690, 813, 983
Sara Yaghotian 576
Sarira Shahnavaz 206, 479, 486
Sarira Shah Navaz 202
S.A.R Rezaee 815
Sayed Ali Hosseini Siyar 853
Sayed Hussain Mosawi 1055
Sayed Mohammad-Hassan Emami Najaffi Dehkordi 182
Sayyed Hamid Zarkesh Esfahani 57, 710
Sayyed Mohamad Hossein Ghaderian 81
Sayyed Vahab Azizi 102
S. Bayanolhagh 798
Sedigheh Amini Kafi Abad 794
Sedigheh Arbabian 466
Sedigheh Nabian 554
Sedigheh Pouya 67, 134, 407, 461, 998
Sedigheh Rahrotaban 295
Sedigheh Younessi Amirabad 90
Sedigheh Zakeri 262, 263, 541, 965, 967, 978
Sepideh Ashouri Movassagh 1013
Sepideh Haghdoost 416
Sepideh Khodaverdi 224, 503, 734
Sepideh Maralbashi 692, 693
SepidehMohseni 232
Sepideh Moradkhani 587, 1011
Sepideh Sadat Hosseini 411
Sepideh Shahbazi 985
Sepideh Soukhtezari 363
Sepide Khodaverdi 502, 504
Sepide Salehipour Bavarsad 271
Setareh Haghighat 544, 995
Setareh Moazen 670
setare Mamishi 800
Seyed Abdolrahim Rezaee 378, 379, 788, 1031
SeyedAbdolrahim Rezaee 660
Seyed Abdolreza Mortazavi-Tabatabaei 65
seyedAbolhassanShahzadeh Fazeli 730
Seyed Abolhassan Shahzadeh Fazeli 717, 1013, 1028, 1037
Seyed Ahmad Hosseini 280

Seyed Ali ghaffarikhalafmohammadi 994
Seyed Ali Javad Mousavi 283
Seyed Ali Mirhosseini 378, 788
Seyed Alireza Esmaeili 251, 253
Seyed-Alireza Esmaeili 133
Seyed Alireza Mesbah Namin 525, 1029
Seyed Alireza Razavi 279
Seyed Amir Hossein Jalali 556, 559, 950, 1038
Seyedamir Jalali 308
Seyed Amir Jalali 44, 268, 1021
Seyed Davood Hosseini 546
Seyedeh Forough Sadeghian-Kiadehi 99
Seyedeh Mahboobeh Mosavi Mehr 1029
Seyedeh Mehrnaz Mostatabzadeh 846
SeyedehNaemeh javid 622
Seyedeh Naemeh Javid 678
Seyed Ehsan Asadi 37
SeyedehTahereh Mohaddes 660
Seyedeh Zohreh Jalali 55
Seyede Rahele Tabatabaei Jafari 1020
Seyede Zeinab Hosseini 784
Seyed Fazlollah Mousavi 230
Seyed Hamid Aghajanzadeh 829
Seyed Hamidreza Monavari 809
Seyed Hamidreza Mortazavi 581, 585, 586, 600
SeyedHamidreza Mortazavi 579, 582
Seyed H. Ghaffari 666
Seyed Hojjat Hossaini 1002
Seyed Hossein Hoseinifar 1038
Seyed Hossein Hosseini 1047
Seyed Javad Hasheminia 723
Seyed Javad Mowla 623
Seyed. Latif Mousavi Gargari 374
Seyed Mahmoud Hashemi 33, 64, 67, 70, 97, 134, 185, 239, 243, 250, 300, 331, 332, 334, 407, 443, 445, 461, 509, 511, 515, 624, 663, 690, 983, 998, 1040
Seyed Mahmoud Hashemi, 64
Seyed.Mahmoud Hashemi 508
Seyed Mahmoud Masiha Hashemi 205
Seyed Mahmud Hejazi 758
Seyed Masih E'temad aubi 925
Seyed Masood Nabavi 1024
Seyed Mehdi Ebrahimi 587
Seyed Mehdi Sadat 765
Seyed Mehran Modaressadegi 74
Seyed Mehrdad Jalali 88
Seyed Mohammad HadiBagheri Rostami 590
Seyed mohammad hossein Musavi shenas 299
Seyed Mohammad Mehdi Peyghambari 896
seyed Mohammad Moazzeni 505
Seyed Mohammad Moazzeni 8, 222, 223, 496, 497, 500, 960
Seyed Mojtaba Amiri 824, 825, 991
Seyed Morteza Seifati 735
Seyed Nezamodin Hosseini 230
Seyed Nooreddin Faraji 61
Seyed Reza Mirhafez 138
Seyed Reza Mohebbi 801, 923
Seyed Rohollah Arab 378, 788
Seyed saadi hosseini 347
SeyedSaadihosseini 733
Seyed Shahabeddin Mortazavi Jahromi 427, 428
Seyed Shahabeddin Mortazavi Jahromib 362, 756
Seyed Shapoor Reza Shojaei 731
Seyed Vahid Ganjiani 555
Seyyed Abdolrahim Rezaee 385
Seyyed Amin Ayatollahi Mousavi 368
Seyyed Amir Yasin Ahmadi 389
Seyyede Masoume Athari 573, 574
Seyyed Meysam 333
Seyyed Meysam Abtahi 613, 899
Seyyed Meysam Abtahi Foroushani 361
Seyyed Meysam Abtahi Froushani 69, 425, 507, 627, 681, 842, 845, 846, 849, 850, 851, 862, 866, 884,

893, 894, 901, 903, 904, 906
Seyyed Mohammad Moazzeni 330
Seyyed Shamsadin Athari 573, 574
S.Faghihzadeh 97
Shaban Alizadeh 86
Shabnam Abtahi 649
Shabnam Ghazanfari-Sarabi 887
Shabnam Kazemian 801
Shabnam Shahrokh 188, 465
Shaden Kamhawi 265
Shadi Abbasnia 48, 815
Shadi Eghbalfard 495
Shadi Kesmati 411
Shadi Sadri 187
Shadi Tabibian 740
Shadi Zamanian 377
Shafiei Reza 148
Shaghayegh Askarian amiri 675, 677
Shaghayegh Derakhshani 115
Shaghayegh Haghjoo Javanmard 636
Shaghayegh Pezeshki Naraghi 483
Shaghayegh Tajik 25
Shaghik Barani 76
Shahabeddin Mortazavi Jahromi 865
Shahab Shahgaldi 223, 505
Shahdad Khosropanah 436, 721, 897
Shahin Bonakdar 444
Shahram Farhad 404, 880
Shahram Nazarian 374, 980, 982
Shahram Parvane 551
Shahram Samie 626
Shahram Shahabi 24
Shahrbanoo Rostami 651
Shahriar Alipour 822
Shahriar Zeighami 32
ShahriarZeighami 292
Shahriyar Dabiri 1034
Shahrokh Navidpour 551
Shahrooz Rasoul 240, 996
Shahryar Hashemzadeh 42
Shahrzad Nojehdehi 509
Shaker Ardakani M 608
Shakiba Y 608
Shamila D Alipoor 119
Shamila D. Alipoor 120, 393, 394
ShamimSarhaddi 178
Sharareh Bordbari 241
Sharifeh Khosravi 79
Shayan Mostafaei 94, 130, 363
Shayesteh Mehdinejadiani 268, 316
Shayesteh Z 804
Sheikh Sajjadieh Mohammadreza 139
Shekoofeh Moghadam 936, 938, 1012, 1018, 1019
Shideh Khangholi 632
Shideh Mehrmofakham 479, 484, 486
Shideh Mehr Mofakham 202
Shila Jalalpour 372
Shima Bahador 710
Shima Bastaki 193, 926, 927, 928, 930
Shima Bazaz 1012, 1018, 1019
Shima Farshbaf 403
Shima Khajouee Ravari 623
Shima Nahidi 420
Shima Shapoori 79, 520
Shima Sheikhi 1060
shirin Assar 825, 991
Shirin Farivar 49, 53, 652, 682, 683
Shirin Farjadian 531, 625
Shiva Borzouei 521, 522, 973
Shiva Farajpour 805
Shiva Irani 765, 929
Shiva Khezri 905
Shiva Mohammadi 1013, 1037
Shiva Poursani 130

Sho'aub Dehghani 591
Shohreh Fakhari 463, 629, 630, 920, 988
Shohreh Mohammadi 412, 836
Shohreh Nikoo 40, 92, 220, 221
Shokofeh Rahimpourkoldeh 974
Shokoofeh Almasi 806
Shokooh Salimi Moghadam 951, 958, 959
Shokrolah Farrokhi 387
Shokrollah Farrokhi 23, 284, 285
Sholeh Etehad asnaf 438
Siamak Asri Rezaei 196
Siavash Abedi 19
Siavash Mashhouri 507, 842
Sibylle Schneider-Schaulies 115
SimaBalouchi Anaraki 641
Sima Ghaffari 694
Sima Habibi 668
Sima Habibzadeh 144, 265, 318, 420, 838
Sima Kazemi 761
Sima Rafati 143, 144, 265, 318, 415, 420, 838
Sima Shahmohammadi Farid 293, 351
Simin Ahmadvand 28
Sina Abdolrahim Poor Heravi 337
Sina Alizadeh 765
Sina Nabi Zadeh 1063
Sina Taefehshokr 166, 303
Sina Vakili 898
S.Meysam Abtahi frushani 905
Sogand Nayeri 562
Sogol Mazhari 443
Soheila Ajdary 390
Soheila Arefi 352
Soheila Golchin 44
Soheila Rahmani 381
Soheila Rohani 707
SoheilaRohani 705
Soheyla Rohani 706
Soleimani S 673
Solmaz khansalar 383
Solmaz Khansalar 993
Solmaz Shirjang 30
Somaieh Kazemnejad 40, 220, 221
Somaieh Nasereslami 382, 803, 807
Somaye Aletaha 397
Somayeh Abolhassani 639
Somayeh Ahmadi afshar 159
Somayeh Ahmadiafshar 430, 901
Somayeh Behjat Khatouni 975, 977
Somayeh Ebrahimi Barough 529
Somayeh Fallahnejad 677
Somayeh Ghotloo 175, 200, 715
Somayeh Hamd Ghadareh 662
Somayeh Pourtalebi 639
SomayehRezaeifard 292
Somayeh Shamlou 60
Somayeh Zarrati 1055
Somaye Mami 359
Somaye pooranfar 755
Somayyeh Ghadimi 233
Soodabeh Aghapour 739
SoolmazHabibi 736
Soraya Bilvaieh 22
Sorayya Salimi 128, 405, 828
Soroor Erfanimanesh 778
Sousan Ghasemi 984
S. Rafati 267
S. Rostami 638
S. Sadati 989
S. Soudi 476
Su Ling Li 303
Sussan K. Ardestani 97
Suzan Amini 576

## T

Tabatabaei Yazdi SM 608

Taghi Golmohammadi 130
Taghi Naserpour Farivar 426
Tahereh Jalilvand 501
Tahereh Kalantari 68
Tahereh Mohammadi 224, 502, 504, 734
Tahereh Naji 92
Tahereh Soltantoyeh 39, 603
Tahereh Taheri 265, 420
Tahereh Zeinali 687, 689
Taher Elmi 1055
Taher Nejadsattari 25
Tajaddin akbarzadeh khiavi 713
Takayoki Yoshimoto 63
Talat MokhtariAzad 114
Tanja Konijn 136
Tannaz Bahadori 448
Tannaz Bahadori 603
Taravat Bamdad 382, 803, 807
Taravat Talebi 51
Tavakoli Hajar 404, 880
Taybeh Tavakoli 639
Tayebeh Mehdipour 451
Tayebeh Nowras 156
Tayebeh Radjabian 1060
Tayebeh Sohrabi 447, 976
ThureAdler 238
Tina Shahani 51
T. Kazemi 103
T. Mousavi 131
Tohid Kazemi 684, 692, 693, 694, 695
Tooba Ghazafari 1062
Tooba Ghazanfari 96, 97, 358, 359, 749, 882, 1058, 1059, 1060, 1061
Tooba Ghazanfari1 1057
Toomaj Sabooteh 389
Toran Sepehrzad 554
T. Taheri 267

V

Vahedeh Hosseini 268, 316
Vahid Baghdadi 356
Vahid Bagheri 242, 490
Vahid Baniasadi 174
Vahid Chaleshi 184, 188, 459, 460, 465, 468
Vahid Jajarmi 624
VahidKhazeShahgoli 42
Vahid Kia 316
Vahid Mashayekhi 144, 318
Vahid Mirzaee 372
Vahid Mohammadi 850, 894
Vahid R Haghaniat 757
Vahid Salimi 114, 266
Vahid Shayeghanejad 974
Vahid Shaygannejad 518, 1032
VahidShaygannejad 723
Vahid Yardel 1021
VahidYounesi 147
Vajiheh Ostadi 74, 336
Valiollah Mehrzad 636
Vazeh H 804
Vaziri Nezamdoust F 1042
Vedad Alkhamis 661, 916
Versa Omraninava 793
Versa OmraniNava 792
V. Eskandari1 989
Vida Hashemi 193, 400, 926, 927, 928, 930
Vida Homayouni 34, 302, 475
Vida Sheikh 521, 522, 973

W

Wytske J. Fokkens 23

X

Xanthi N. Stachtea 136

Y

Yadollah Farshi 740
YadollahOmidi 178

Yaftiyan A 716
Yaftiyan Atefeh 218, 219
Yahya Asemani 157
Yalda Zarafshan 1025
Yasaman Alimohammadi 921
Yasaman salimi 932
Yasaman Taslimi 144, 265, 318, 420
Yasamin Pahlavan 830
Yaser Azizi 860
Yaser jafarikhataylou 948, 949
Yaser Moghaddam 302
Yaser Panahi 212
Yaser Piralii 150
YasharAzari Key 303
Yasin Bagheri 1054
Yasmin Vahidi 32, 685
Yasmin Zare 625
Yazdani Neyshabouri Sh 804
Yekaninejad S 100
Youcef Shahali 278
Younes Pilehvar-Soltanahmad 165
Yousef Daneshmand pour 396
Yousef Mortazavi 263, 978
Yousef Tarverdizadeh 982
Yousef yousefzadeh 935
Yousof Tarverdizade 1050
Yunes Panahi 299

## Z

Zahra Adnani Sadati 336
Zahra Aghazadeh 428
Zahra akbari jonoush 59
Zahra Alizadeh Tabari 480, 482
Zahra Alizade Tabari 478
Zahra Amirghofran 155, 157, 289, 429, 431, 859, 993
Zahra Arabi 432
Zahra Asadi 882, 921
Zahra Asadzadeh 1043
Zahra Asghari 578
Zahra Badiei 976
Zahra Basiri 1020
Zahra Elyasi Gorji 717, 1013, 1037
Zahra Faghih 11, 28, 29, 32, 292, 297, 685
Zahra farzaneh 383
Zahra Farzaneh 116, 155
Zahra Fereydouni 439, 900
Zahra Gholami Mahmoodian 853
Zahra Hamidi Esfahani 340
Zahra Hashemi 390
Zahra Hooshyar 528
Zahra Hoseini Khah 854
Zahra Hosseinikhah 94
Zahra Kanannejad 217
Zahra karami 566
Zahra Kashani Khatib 86
Zahra Khademi 518
Zahra khosrowpour 508
Zahra Koolivand 355, 739
Zahra Madjd 513, 1016
Zahra Matluobi 161
Zahra Meshkat 138
Zahra Mirsanei 47, 668
Zahra Moeinibakhsh 583
Zahra Mohtasham Amiri 802
Zahra Mojtahedi 31, 294, 619
Zahra Momeni 532
Zahra Moradi 224, 502, 504, 734
Zahra Mozaheb 660
Zahra nekooee zadeh 537
Zahra Nori Sabzikar 196
Zahra Noroozbabaei 371
Zahra Norouzbabaie 440
Zahra Omidi 1062
Zahra Pourmoghadam 215
Zahra pourpak 20, 25

Zahra Pourpak 60, 278, 281
Zahra Radaee 863
Zahra Rashid 470, 471
Zahra RezaeiYazdi 125
Zahra Sadat Delbari 872, 885
Zahra Sadr 666
Zahra Samimi 401, 402
zahra Shaker 748
Zahra Shaker 656, 745
Zahra Sharifzadeh 66, 472
Zahra Soleimani 902
Zahra Taghipour 102
Zahra Taghpour 432
Zahra Telikani 521
Zahra Zeinaly 333, 627, 681, 851
Zakaria Vahabzadeh 907, 908
Zakiyeh Azadi 25
Zangeneh Mohamad Mahdi 962
Zarrin Minuchehr 227
Zatollah Asemi 403
Zeinab Barartabar 184
Zeinab Kavyani 630
Zeinab Mahmoodi 1046
Zeinab Rostamy 156
Zeinab Salehian 870
Zeynab Baghernejadan 21
Zeynab Fallah 1056
Zhima Akhavansales 734
ziba rahimi 353
zivar zangeneh 912
Zohair Mohammad Hasan 656, 745, 748
Zohre Eftekhari 273
Zohreh Babaloo 31, 128, 165, 405, 822, 828
Zohreh Bolandi 250, 983
Zohreh Hajheidari 526, 1027
Zohreh Hekmati 437
Zohreh Hesari 158
ZohrehHesari 558
Zohreh Koohini 19, 398
Zohreh Mortezaei 485
Zohreh Nasiri 459
zohreh rahimi 353
Zohreh Sharifi 231, 794
Zohreh Vahidi 133, 251
ZohrehVahidi 125
Zohreh Zareighane 488, 746
Zohreh Zarei Ghane 345, 1020
Zohre Mohammadi 169
Zohre Najafi 271
Zohre Poursina 377
Zohre Zaerieghane 966
Z. Rahmati 989
Z. Rahnemoon 989
ZS.Hasan 580
Zuhair M. Hassan Saraf 63
Zuhair Mohammad Hassan 300, 663,
Zuhair MohammadHassan 33
zuhair saraf hasan 932
Zuhair Saraf Hasan 161


# Actolukast

Montelukast 5mg Chewable tablet, 10mg tablet

- Once daily dosing
- Rapid onset of action compared to corticosteroids
- Safe for chronic use
- Safe in pregnancy (Category B)
- An alternative to steroids in children unable to use inhaled corticosteroids or suffering from poor growth

ACTOVERCO

No. 58, 8th St., Kooye Nasr (Gisha St.), Tehran, IR Iran, Postal Code: 1446863914, Tel: +98 (21) 4163 70 00
ACT.MKT.INT.07.1396.0231
www.actoverco.com


# fastover

**Tablets 30, 60, 120, 180 mg** FEXOFENADINE

- **Once daily administration, 24 hour relief**
- **Lowest sedative effect**
- **Multi-symptom relief**
- **Safe in children over 6 months**

ACTOVERCO

No. 58, 8th St., Kooye Nasr (Gisha St.), Tehran, IR Iran, Postal Code: 1446863914, Tel: +98 (21) 416 370 00
ACT.MKT.INT.07.1396.0230
www.actoverco.com

# INCOMPARABLE IMPRESSION,
more than your trust





**Suprimun**
Mycophenolate mofetil Tablet 500mg

First generic of mycophenolate mofetil with the most extended clinical experience launched in 2003

**EMA APPROVED**
European Medicines Agency

ACTOVERCO
Together for a healthy future

CLAUSEN

www.actoverco.com

# IMMUNOREGULATION

Volume 2. Supplement 1. April 2018
ISSN: 2588-5200