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## -Proliferation of a Pure Population of CD4+ T Cells Compared to Peripheral Blood Mononuclear Cells

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**Background & Objective:** T cell proliferation is believed to be dependent on several factors including the environment the cells are growing in and the cytokines and the cells present. In this study we aimed to compare the response of a subset of T cells, known as $CD4^+$ T cells with peripheral blood mononuclear cells (PBMC) in response to phytohemagglutinin (PHA). **Methods:** PBMCs were separated by density gradient centrifugation, using Ficoll (Histoprep). $CD4^+$ T cells were isolated by Dynal CD4 negative isolation kit and purity was checked by Flow cytometry (purity>95%). PBMCs and $CD4^+$ T cells, in three concentrations and each triplicate, were cultured in 96-well plates for 3 days in the presence or absence of PHA. Proliferation was assessed by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) proliferation assay. This technique offers a quantitative, convenient method for evaluating a cell population's proliferation in response to external factors. Yellow MTT is reduced to insoluble purple formazan in the mitochondria of living cells and is then solubilized by the addition of a detergent such as acidified isopropanol. The color is then quantified by spectrophotometric means and is directly proportional to the living cells' density. **Results:** PHA induces PBMCs and $CD4^+$ T cells to proliferate. $CD4^+$ T cells respond to PHA comparable to PBMC. This response is shown in 50000, 100000 and 200000 concentration of cells but best results are achieved in 100000 cells per well. **Conclusion:** This study shows that $CD4^+$ subset of T cells have proliferative capacity in response to PHA even in the absence of antigen presenting cells. The negative isolation kit used in this experiment retains the functional capacity of cells and these cells can be used for further studies.

2

# 2The Effect of *Urtica Dioica* on Lymphocytes Cell Viability of BALB/c Mice

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**Background and objective:** Lymphocytes are the most important cells in the specific immune response. Finding a material that modulate activity of these cells are very important. Some of the herbal medicines are effective for immune modulation. *Urtica dioica* extract is reported as an anti - inflammatory herb. In this research we evaluate the effect of Urtica extract in lymphocyte cell viability. **Methods:** This research performed on 30 male BALB/c mice with 8 weeks average age. Mice were divided into 6 groups; one as control and others take 10, 50, 100, 200 and 500 mg/kg/day of the extract. After two weeks orally prescription of the extract, in 15th day we sacrificed mice, separated their spleens and spleen cells were isolated and cultured for 48 hrs. Cell viability was evaluated using MTT assay. **Results:** MMT results indicated that spleen cells viability was increased after *Urtica dioica* extract exposure. This effect is appearing dose dependently. Mean OD of MTT test for control group was 0.1468 and by 10, 20, 50, 100, 200 and 5oo mg/kg were 0.1619, 0.2297, 0.2940, 0.3271, 0.1909 respectively. **Conclusion:** *Urtica dioica* show significant immune stimulatory effects on spleen cells of mice. Further studies are needed to clarify exact immunomodulator components and their mechanism.

3

# 3 Comparison of Heparin and Monocyte Conditioned Medium (MCM) for the Induction of Maturation in Monocyte-Derived Dendritic Cell (mDC)

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**Background & Objective:** Dendritic cells (DCs) can induce tumor- or pathogen-specific T cell responses in humans. Several laboratories have developed culture systems including maturation factors for human DC from peripheral blood monocytes. We comprehensively compared standard maturation stimulus, autologous monocyte-conditioned medium (MCM), with heparin for their ability to promote uniformly mature DCs that elicit T cell responses. **Methods:** A short (4-day) priming of plastic adherent monocytes with granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin-4 (IL-4) followed by 48 hour-incubation in MCM, heparin or both to generate fully mature and stable DC. Phenotypic and functional analysis were carried out using anti CD14 and anti CD83 monoclonal antibodies, and mixed lymphocyte reaction (MLR) respectively. **Results:** We found that fully matured DCs with large amount cytoplasm and copious dendritic projections were visible at the end of culturing period in the presence of MCM, heparin and MCM with heparin. Thus, DCs generated with this three maturation factors are nonadherent and have typical satellite morphology. Flow cytometric analysis using anti-CD14 (monocyte marker) and anti-CD83 (Mature DC Marker) revealed that expression of CD14 is decreased in particular in MCM with heparin treated DCs, and expression of CD83 was the highest when MCM and heparin are used as maturation factor. MCM and MCM with Heparin treated DCs showed stronger mixed leukocyte reaction than heparin alone. **Conclusion:** these results support the use of the MCM and heparin as maturation factor could results in functionally mature monocyte derived DCs in comparison to either MCM or heparin alone.

4

# 4 Effect of TGF- β on Dendritic Cell Generation and Foxp3 Expression in $CD4^+$T Cells

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**Background & Objective:** Dendritic cells (DCs) are professional antigen presenting cells that have an important role in regulation of immune response. In this study, ability of dendritic cells evaluated in development of regulatory T cell. **Methods:** In an experimental study, peripheral blood mononuclear cells isolated and DCs generated in presence GM-CSF, IL-4 and TGF-beta in comparison with control. Mixed leukocyte reaction performed with use of DCs and $TCD4^+$ Lymphocytes. Cell proliferation level and expression of Foxp3 evaluated on lymphocytes by Flow cytometry. **Results:** $CD4^+CD25^+Foxp3^+$ T cell level in culture media of mixed leukocyte reaction significantly different from control ($P< 0.05$). In addition, Cell proliferation of T lymphocytes decreased in comparison with control $P< 0.05$. **Conclusion:** It is concluded that use of TGF-beta molecule has an important role in differentiation of inhibitory dendritic cell and T regulatory development. In addition production of this cell can be affect in preventing of unwanted immune response such as, autoimmune diseases.

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# 5 Immunosuppressive Effect of Adipose Derived Mesenchymal Stem Cells on Peripheral Blood Mononuclear Cells

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**Back ground & Objective:** Mesenchymal stem cells (MSC) compromise a subpopulation of cells in different tissues and they have capacity of differentiation into different mesenchymal tissue invitro. They are known to secrete a number of soluble factors implicated in immune regulation. Here we assessed the immunostimulatory or suppressive effects of MSCs on peripheral blood mononuclear cells (PBMCs) proliferation. **Methods:** Normal adipose derived MSCs were plated 24h prior to co culture with PBMCs in the ratios of 1:20, 1:10 and 1:5 for MSC: PBMC. In another culture condition the serial concentrations (10%, 30% and 50%) of MSC culture SN were added into culture and cell proliferation was studied 3 and 7 days post culture by Flow cytometry. **Results:** At day 3 post culture MSC induced suppression was 1.3, 3.3 and 4.2 fold decrease respectively for1:20, 1:10 and 1:5 ratios in which the presence of MSC/PBMC as 1:10 and 1:5 resulted in a statistically significant ($P< 0.05$) decrease in PHA-induced proliferation. Although at day 7 post culture, the MSC induced suppression was significant in all ratios. Suppression of the proliferative response of PBMCs occurred in a dose dependent manner. In overall, there was acceptable suppression of PBMC proliferation especially by highest concentration of MSC's SN (2 and 2.4 fold suppression for day 3 and 7 post culture. However the suppression was not statistically significant due to variations in our results but that suppression was dose dependent. **Conclusion:** My results showed that SN of one week cultured MSC could suppress PBMCs proliferation; however that suppression was less significant compared to suppression by MSCs in the coculture. These results could confirm several studies that express when MSCs were replaced by MSC culture supernatant the inhibitory activity was reduced; this was suggested that both contact dependant and independent mechanisms by soluble factors operate in MSC induced suppression.

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# 6 Evaluation of Immunosuppressive Effects of Human Adipose Derived Mesenchymal Stem Cells in the Presence of Costimulatory, Agonistic Antibodies

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**Background & Objective:** Mesenchymal stem cells (MSCs) are a non-hematopoietic population with the capacity of self renewal and differentiation. Contact dependent and secretory factor derived from MSCs have been proposed as possible mechanisms of their immunosuppressive effect. Here we assessed whether provision of costimulation could restore lymphocyte proliferation under the influence of MSC suppression. **Methods:** Normal adipose derived MSCs were propagated and maintained in culture. CFSE-labeled peripheral blood mononuclear cells (PBMCs) were co-cultured with MSCs or MSC's culture supernatant (SN) and activated by PHA with or without addition of anti-CD28 and/or anti-CD137 (4-1BB) antibodies. Proliferation/suppression was evaluated after 7 days culture in CD4 and CD8 sub-populations. IFN-γ, TGF-β and IL-10 cytokines were measured by ELISA. **Results:** Provision of neither anti-CD28 nor anti-4-1BB antibodies could restore lymphocyte proliferation in CD4 and CD8 sub-populations after MSC induced suppression. However, costimulation by these antibodies could relatively overcome SN suppression in CD4 and better in CD8 cells. Greater effects were observed by anti-CD137 or dual treatment. Presence of costimulatory Abs could increase IFN-γ but reduced TGF-ß and IL-10 production. Presence of costimulatory Abs could not significantly affect cytokine production. **Conclusion:** Using anticostimulatory Abs, especially anti-4-1BB revived higher percentages of CD8 cells compared to CD4. This difference could be due to down regulation of CD28 by activated lymphocytes and proposed preferential response of CD8 cells to anti-4-1BB .Based on recent findings, increased IFN-γ production by PBMCs could enhance the suppressive function of MSCs. This may explain the inability of anti-costimulatory Abs in proliferation recovery. Reducing TGF-ß and IL-10 concentration by costimulation is not sufficient to abolish suppressive effect of MSCs. These results suggest that lack of costimulation expression by MSCs is not the only mechanism of MSC suppression and other mechanisms are involved as well.

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# Evaluation of the Effect of Thyme Constituents on Mouse Lymphocyte Proliferation

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**Background & Objective:** In recent years chemical constituents of different medicinal plants have been evaluated for their immunomodulatory activity. Efforts have been applied to study this effect of plants in order to identify the new potential compounds as immunosuppressive agents. To study the immunomodulatory properties of two constituents of thyme, an important member of Labiatae family of plants. **Methods:** The effect of thymol and carvacrol on the mitogen-induced mice splenic lymphocytes was investigated. Cells were separated from spleen of BALB/c mice and treated with and without suboptimal dose of Concanavalin A as the mitogen and different concentrations of the compounds. After 48 hours, lymphocyte proliferation was measured by BrdU incorporation assay. **Results:** Treatment of mice with increasing concentrations of carvacrol in the absence of mitogen decreased the proliferation of cells. Addition of both compounds on the culture of mice splenocytes activated with mitogen, dose-dependently decreased the proliferation of cells. **Conclusion:** The decline in lymphocyte proliferation due to the compounds indicates their possible immunoinhibitory effects on cell-mediated immune responses. More studies in this regard are in progress.

8

# Tolerance Induction by CD40 Silenced Dendritic Cells through Antisense and Antibody Blocking

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**Background & Objective:** One of the valuable tools for inhibiting the specific gene expression is antisense technique. In the present study, the effects of antisense against CD40 mRNA and antibody blocking effect against CD40 on the function of dendritic cells (DCs) were investigated. **Methods:** The DCs were separated from the mice spleens and then cultured in vitro. By the means of Lipofectamine2000, antisense was delivered into the cells and the efficacy of transfection was estimated by Flow cytometry. Also the mRNA expression and protein synthesis were assessed by real time- PCR and Flow cytometry, respectively. The DCs were transfected with 6μM antisense and 2μl Lipofectamine2000. **Results:** The obtained results showed that CD40 expression reduced in the DCs as much as 22% (antisense1) and 24% (antisense2) and in the BCL1 cell line as much as 20% (antisense1) and18% (antisense2). By Annexin V and PI staining, we could evaluate the transfected cells' viability. The inhibition and blocking of CD40 gene expression were associated with the increasing of IL-4 secretion, shifting the DCs to stimulate Th2 cytokine production from the allogenic T cells. In addition, the DCs with reduced CD40 expression showed poor allostimulatory effects in MLR. **Conclusions:** These results suggest that CD40 pathway has a critical role in the generation of tolerogenic DCs, and support that down regulation of CD40 is effective to inhibit allostimulatory function.

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# HLA-II Gene Polymorphisms in Turkish Speaking People of Iran (Qashqaees and Turkmens in Comparison to Azeries

**9**S Safi, Sh Farjadian

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**Background & Aim:** Anthropology is the science of studying the origins and relationships of human beings according to the physical, socio-cultural, linguistic, archeology and molecular characteristics. Different genetic markers are used in molecular anthropology. Because HLA genes are highly polymorphism and some of HLA alleles have different frequencies among ethnic groups and some of alleles are exclusively found in distinct populations, these genes can be used for understanding the genetic history of human populations and reconstruction of their migration in the past time. The aim of this study was to investigate the genetic relations between Qashqaees, Turkmens, and Azeris, Turkic speaking people of Iran, based on the polymorphism of HLA-II genes in Qashqaees, and Turkmens and to compare these results to the HLA information of Azeris and other ethnic groups of Iran. **Methods:** The distribution of HLA-DRB1 alleles was determined by PCR-SSP while DQA1 and DQB1 alleles were detected by PCR-RFLP. Data were analyzed by Arlequin version 3.1, DISPAN, MEGA 4. **Results:** DQA1*0101/2 in Qashqaees (38.1%) and Turkmens (32%), DQB1*0301 in Qashqaees (21.6%) and DQB1*0201 in Turkmens (24%), DRB1*11 in Qashqaees (22.1%) and DRB1*15 in Turkmens (17.7%) were the predominant alleles. DRB1*11-DQA1*0501-DQB1*0301 (17.3%), and DRB1*03-DQA1*0501-DQB1*0201(11.5%) were the most common haplotypes in Qashqaees and Turkmens, respectively. The first haplotype was also reported as the most common haplotype in Azeris while, both of the mentioned haplotypes were frequent in other Iranian ethnic groups. The results of ANOVA showed a little genetic differentiation among Qashqaees, Turkmens, and Azeris. The results of Neighbor Joining tree showed a close genetic relation between these three subpopulations and other ethnic groups of Iran which all placed in the same cluster. **Conclusion:** The results of this study showed that Turkic speaking people of Iran, in contrast to their common linguistic characteristics were not that close to each other when compared to other Iranian ethnic groups.

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## The Effect of *Pistacia khynjuk* on Humoral Immune System in Wistar Rats

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**Background & Objective:** Plants from genus Pistacia with different species such as *Pistacia atlantica*, *Pistacia Vera* and *Pistacia khynjuk* are herbal medicine. Antibacterial and anti-inflammatory effects of these plants have been confirmed. The aim of the current study was to find the effect of Pistacia khynjuk on humoral immune system of Wistar rats. **Methods:** Forty male Wistar rats (200-250 gr) were randomly allocated in four groups of ten animals and orally received 10 mg/kg of the extract of nucleus, cutin and fruit of Pistacia khynjuk respectively, everyday for two weeks. The control group received only placebo. Immunoreactivity was induced with using BCG vaccine with Frumd´s Complete Adjuvant (IP). The titer of IgG and IgM were measured after the treatment using ELISA method. Moreover the cervical lymph nodes and spleen of animals were excised and the volume and density of the primary and secondary follicle was evaluated by steriology. **Results:** The differences in the mean level of IgG and IgM between the treated and the control animals were not significant (P> 0.05). Also, the mean volume and density of the primary and secondary follicle of the first three groups in comparison with the control animals was not significant (P> 0.05). **Conclusion:** Findings of this study showed that the *Pistacia khynjuk* has not any direct effect on the activity of humoral immune system and increasing of antibody level in Wistar rats.

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## MT-DNA and HLA-II Gene Variation in Ethno-Linguistic Isolates of Iran

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**Background & Objective:** However the results of HLA class II gene diversity revealed a close genetic relation among eleven Iranian ethnic groups with some genetic similarities to Italians; since HLA genes are under strong selection by local infectious agents, mtDNA control region was considered in this study to trace maternal lineage. **Methods:** The variation of the first hypervariable segment (HVS-I) of the mitochondrial DNA was analyzed among 718 Iranians belonged to fourteen different ethnic groups: Pars, Azeri, Kurd, Lur, Balooch, Gilak, Mazandarani, Qashqaee, Turkmen, Armeni, Zoroastrian, Arab, Jew, and the people of Qeshm Island. **Results:** The results of this study showed that, haplogroups H and U were the most common among Iranians while haplogroupe W (24.6%) was exclusively detected within Baloch people. Iranians are maternally more related to Caucasians, Near Eastern, and Mediterraneans than other Asians, Europeans, or Africans. The results of AMOVA revealed no differentiation among the 14 ethnic groups and about 97.31% of the total variance was attributed to variation within populations. The results of SAMOVA also showed no geographical structure among these ethnic groups. **Conclusion:** According to the results of MDS; Zoroastrians, Jews, Baloochis, and the people of Qeshm can be considered as outliers. These populations also revealed a remarkable degree of long term evolution in phylogenic analysis. Although the results of this study cleared some obscure points about the genetic relation among Iranian ethnic groups, further data based on complete mtDNA sequencing as well as analysis of paternally transmitted Y chromosome in combination with autosomal gene variations are required to solve the genetic history of the Iranians.

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## Intracellular Cytokines of Natural Killer Cells in Patients with Allergic Rhinitis

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**Background & Objective:** Allergic rhinitis is a common disorder with great morbidity. Type 2 cytokines have a crucial role in development of allergies. Recently, natural killer (NK) cells have attracted attentions as producers of type 2 cytokines. The aim of the present study was to evaluate NK cells and their IL-4 and IFN-γ production in allergic rhinitis patients compared to healthy controls. **Methods:** Eighteen patients with allergic rhinitis and 12 age-sex matched healthy controls were included in this study. Patients with any other allergic diseases were excluded. Percentage of peripheral blood NK cells and their intracellular IFN-γ and IL-4 were measured by three-color flowcytometry. **Results:** The percentage of NK cells was 15.2 ± 10.9 in patients and 12.2 ± 3.3 in controls, but it was not significantly different. In patients 49 ± 14.3% of NK cells were IL-4 producers, while in controls 27.2 ± 8.7% of NK cell were IL-4 positive. The percentage of IL-4 positive NK cells was significantly higher in patients compared to healthy controls ($P< 0.001$). However, IFN-γ positive NK cells were slightly more in patients than controls but the difference was not statistically significant (in patients 53.2 ± 15.4%, in controls 49.5 ± 13.5%). **Conclusion:** This study confirms the presence of different types of NK cells in allergic rhinitis regarding their cytokine profile. Type 1 NK cells which secrete IFN-γ were similar in patients and controls while type 2 NK cells producing IL-4 were significantly higher in patients. The role of NK cells in pathogenesis of allergic conditions is highlighted.

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# Incidence of Allergy in Sabzevar

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**Background & Objective:** *Artemisia vulgaris* and *Salsola kali* are common plants in North hemisphere (e.g. in Iran). The pollen of *Artemisia vulgaris* is major aeroallergen in late summer usually 10-14% patients that suffering from pollinosis in summer have allergy to *Artemisia vulgaris*. The pollen of Salsola Kali is major aeroallergen in spring usually 50-66% patients that suffering from pollinosis in spring have allergy to Salsola Kali. **Methods:** This study was carried out on 50 patients with allergy. Skin rick test was used for determining allergic patients with allergy to *Artemisia vulgaris* and *Salsola kali* . **RESULTS:** It was shown that 27 patients (54%) were allergic to *Artemisia vulgaris* and 48 patients (96%) were allergic for Salsolaents Kali. **Conclusion:** This study was shown that incidence of these allergies in Sabzevar is more common than usual.

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# CpG-ODN Co-Administration of *Chenopodium Album* Allergen on PBMCs Responses of Patients with Allergic Rhinitis

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**Background & Objective:** Such reports suggest that Allergic Rhinitis (AR) is one of the most common chronic diseases in the industrialized world. Moreover, the economic burden and morbidity associated with AR are substantial. Our purpose was to investigate whether the Th1 enhancing properties of CpG-ODN co-administration homemade crude (*Che a*) allergen could be used to alter the Th2 dominated immune response of PBMCs of patients with AR in vitro. **Methods:** To determine these effects, we quantified cytokine responses characteristic of Th2 immunity (IL-4, IL-13), Th1 immunity (IFN-γ), as well as IL-10, a cytokine sometimes linked to regulatory T-cell populations and Che a specific IgE. **Results:** We demonstrated that Th2 responses were selectively redirected toward Th1 responses, with significant increases in IFN- γ, IL-10, and significant decreases in IL-4. *Che a* specific IgE had not significant alteration. **Conclusion:** CpG-ODN co-administration homemade *Che a* allergen has enhanced Th1 biased immunogenicity. It may offer a more effective approach for allergen immunotherapy than currently available methods.

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## Allergy to Fungi, Pollens and Mite in Allergic Patients: Mashhad, Iran

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**Background & Objective:** Allergy to fungi and mites, as well as pollens is important in aggravation of allergic diseases such as allergic rhinitis and asthma. In this study, diversity of fungal allergy is evaluated among allergic patients. **METHODS:** In a cross sectional study, 88 allergic patients were selected randomly from those referring to allergic clinic of Ghaem hospital, Mashhad, Iran. Skin prick test was performed with extracts of different fungi (Aspergillus, Alternaria, etc.), grass, weed, tree, and mite. **Results:** From the 88 patients, allergic asthma was present in 50 patients, allergic rhinitis in 52, eczema in 24, and urticaria in 23 cases, as there exists significant overlap between them. Sensitization to more than one allergen (poly-sensitization) was found in 45% of cases. Sensitization to tree, weed, grass, and fungi (Alternaria) were found in 58.6%, 49.4%, 47.8%, and 48.9% respectively. Only 27.4% had sensitization to mite. **Conclusion:** As the pattern of sensitization to allergens is quite different from several other studies performed in other countries, it seems necessary that a national multi-centre survey in this case should be performed.

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## Alternaria and Allergic Asthma

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**Background & Objective:** Alternaria is a genus of fungi that as an allergen may affect allergic asthma. Inhalation of Alternaria spores is shown to be important. Presence of Alternaria in the nasal mucous might be a trigger of allergic process in the allergic asthma. Thus, we evaluated relation between detection of Alternaria in the nasal mucous and allergic asthma and its severity. **Methods:** In this case-control study, 58 patients with allergic asthma were selected non-randomly in the asthma clinic according to the inclusion criteria- positive symptoms and signs, indicative pulmonary function test, and presence of atopy documented by skin prick test. They were compared with a matched control group (50 volunteer with none of the above criteria) for detection of Alternaria in their nasal mucous, by direct microscopy, staining, and culture. Within the asthma group, severity was defined and relation with detection of Alternaria was assessed. **Results:** Detection of Alternaria in the nasal mucous was significantly related to allergic asthma ($P< 0.001$). However, its relation with the severity of asthma was not significant. Furthermore, relation between detection of Alternaria in the nasal mucous and some features of severity of asthma such as FEV1/FVC, number of admissions in the hospital, etc. was not significant. **Conclusion:** This study verifies previous investigations showing relation between asthma and airborne concentration of Alternaria. Although in this study, presence of Alternaria in the nasal mucous had no relation with severity of the disease, further investigations probably with higher sample size is needed to confirm this result. By the way, it seems rational to avoid exposure to Alternaria in allergic asthma setting.

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## Relation between Alternaria and Allergic Rhinosinusitis

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**Background & Objective:** Artemisia spp. are important medical plants in the world. Cancer cell toxicity of fractions and compounds from different Artemisia species had been shown. **Methods:** In present study, three samples of Iranian Artemisia khorassanica were collected. Cytotoxicity of their isolated fractions were studied on cancer cell lines. Ethanol, ethylacetate, dichloromethan and hexan fractions were isolated from three Artemisia samples of different places in Khorassan province. Gastric (AGS), colon (HT-29), breast (MCF-7) and cervix (Hela) cell lines were incubated with different concentrations of fractions for 72 h. Then, cytotoxicity was measured using MTT assay and reported as IC 50. **Results:** All fractions showed strong inhibitory effects on cancer cells in a dose-dependent manner. But, it was different for same fractions from three samples. The most strong fractions were ethylacetate of sample 1, dichloromethane of sample 2, dichloromethane of sample 3 and hexan fraction from sample 1 of Artemisia khorassanica. MCF-7 and Hela were the most sensitive cell lines. **Conclusion:** With regard to significant toxicity of isolated fractions, they could be evaluated in prevention and treatment of different cancers.

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# Effect of Hydroalcoholic Extract of *Astragalus gypsicolus Maassoumi* & *Mozaffarian* on Immunological Factors (IFNγ ,IL4) in Early Sensitized Mice Induced by Ovalbumin

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**Background & Objective:** The genus Astragalus is a very large group of more than 2,000 species distributed worldwide, and is commonly known as Gavan in Iran. This genus is widely distributed, about 800 species in Iran. Currently, much of the pharmacological research on Astragalus is focused on its immune-stimulating polysaccharides and other active ingredients useful in treating immune deficiency conditions such as balance of Th1/Th2 cytokines. The present study was aimed to investigate the effect of *Astragalus gypsicolus Maassoumi & mozaffarian* on Th1/Th2 cytokines in allergy mouse model in comparison to control group. **Methods:** Hydro alcoholic extract of *Astragalus gypsicolus Maassoumi & Mozaffarian* assessed by phytochemical tests to recognize the main active constituents including flavonoids and terpenoids. Mice were sensitized with subcutaneous injection of ovalbumin and aluminum hydroxide. Efficiency of sensitization was assessed by blood IgE levels. After sensitization, two doses of extraction (250 mg/kg & 500 mg/kg) was injected intraperitoneally until day 14.on day 14, mice were challenged with intraperitoneal injection ovalbumin. IL-4 and IFN-γ levels in bronco alveolar lavage (BAL), was assessed by ELISA kits. The results were analyzed with one-way variance (ANOVA) and TUKEY test to assess meaningful difference between various groups. ($P< 0.05$) **Results:** Phytochemical tests shows, *Astragalus gypsicolu Maassoumi &Mozaffarian* possesses the main active constituents of genus Astragalus which are responsible for its therapeutic effects including terpenoids and flavonoids. Intraperitoneal injection of *Astragalus gypsicolus Maassoumi & Mozaffarian* was able to decrease IL-4 and increase IFN-γ. **Conclusion:** This herbal species has the potential effect to modulate the balance of Th1/Th2 cytokines in allergy.

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# Assessment of Prevalence of Food Allergy in Patients Referring to Afzalypoor Hospital in Kerman Province

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**Background & Objective:** Food allergy is an unusual reaction of immune system toward foodstuffs. Prevalence of food allergy has estimated about 10% in infants and less than 2% in adults. Food allergens are proteins or glycoproteins with about (15-70) KD molecular weight. Formation of allergic response is often with production of IgE, sensitization of mast cells and basophils and finally production of different inflammatory mediators such as histamine. Clinical signs of allergy include: urticaria, itching, anaphylaxis shock. For diagnosis the type of allergy, different types of test such as skin prick test can be used. **Methods:** this study is done on 31 allergic patients in afzalypur hospital in Kerman province, referring to afzalypur hospital. After clinical examining and taking history of patients, skin prick test was performed in order to find out IgE and measure of wheal and flare. **Results:** analyzing of data showed that there is significant relation between age of patients and food allergens. Also there is a significant relation be observed between range of histamine and allergens. But there was no significant relation between gender and food allergen. Analysing of data showed that among food stuffs performed on pateint, egg yolk had the most prevalentse, and then Egg (white), kiwi, peanut, strawberry, banana were important. **Conclusion:** Studying on prevalence of food allergen are valuable by this surveys, the allergens which are more prevalent can be determined and also appropriate measure in order to can be performed.

20

## Component-Resolved Diagnosis of Grape Allergy with Purified Natural Allergens

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**Background & Objective:** During the last decade, limitations of using crude extracts for the diagnosis of fruit allergy, has led to efforts to purify the natural allergens or to produce their recombinant forms. In this study, we purified four formerly identified allergens of the grape extract and evaluated their IgE reactivity with grape allergic patients' sera. **Methods:** Grape crude extract was fractionated on a column of DEAE sepharose, and the bonded proteins were eluted by increasing amounts of NaCl in a Tris based buffer. The collected fractions were concentrated by liophylaization and their purity was checked by SDS-PAGE and silver staining. Each purified fraction was then subjected to ELISA and immunblotting assays to determine their reactivity with specific IgE in patients' sera. Correlations were assessed by spearman rank test. **Results:** Based on the results of ELISAs, specific IgE levels to the crude extract were significantly correlated with those of chitinase ($r=0.71$, $P< 0.04$) or gluconase ($r=0.78$, $P< 0.02$). Furthermore, there were strong correlation between specific IgE levels to lipid transfer protein (LTP) and skin prick test (SPT) to grape crude extract ($r=0.81$, $P< 0.01$). **Conclusion:** Our results indicate that grape allergic patients' sera contain a high level of specific IgE to chitinase and gluconase; however, considering the highly significant correlation between specific IgE levels to LTP and SPT results, it appears that LTP is a major allergen of grape.

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# A Preliminary Study of Serum Allergen-Specific IgE Antibodies in Pediatric Patients with Allergy

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**Background & Objective:** The most common chronic diseases of childhood are allergic diseases such as asthma, atopic dermatitis, allergic rhinitis and food allergy. The prevalence of allergic disease has increased in the last decade in the world. During the past several years, immunoassays for specific IgE antibodies have been refined to permit reporting results in mass units. Thus quantitative immunoassays for IgE antibodies may be an adjunct to skin tests. **Methods:** A total of 26 pediatric outpatients with suspected allergic disease were recruited from the Razi Pathobiology Laboratory, Karaj, Iran during 2008-2009.Serum IgE levels were estimated and specific IgE antibodies were assayed using EAST system for 30 food allergens and aeroallergens. The immune profile entailed estimation of Age antibodies against various inhalation and food allergens with the help of the Enzyme-Allergo-Sorbent Test (EAST) which was performed using Euroimmune® kits (Medizinische Labordiagnostica, Germany).The Euroline test kit provides a semiquantitative in vitro assay for human IgE antibodies to inhalation and food allergens in serum. The kit contains test strips coated with parallel lines of 30 different allergen extracts. **Results:** Serum IgE was elevated in 80% of the patients. Seventy two percent of the children were positive to one or more food allergens. Our results showed the principal foods involved in allergic reactions are: egg white, egg yolk, wheat flour and potato. For inhalation allergens mainly are grass and mugwort. **Conclusion:** We emphasize that measurement of serum specific IgE antibodies may be relevant tool in the accurate diagnosis in pediatric patients.

22

## Cytokine Gene Variants and Respiratory Factors in Asthmatics

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**Background & Objective:** Asthma is a multifactor inflammatory disorder and chronic bronchial inflammation resulting in airway obstruction in various degrees. Cytokines and other inflammatory mediators are important factors in asthma pathophysiology and airway remodeling. In this study we evaluated role of cytokine polymorphisms and in lung functions. **Methods:** In 81 asthmatic patients ARMS-PCR method was used to characterize IL-6 G-174C, TNF-α-A308G, TGF-β T+869C and IFN-γ T+874A polymorphisms and PCR-RFLP using AvaII restriction enzyme for IL-4 C-590T. A complete clinical history, physical examination, and pulmonary function test (PFT) in a standard fashion were performed for all subjects. **Results:** IL-6-174C allele ($P< 0.05$), TNF-α-308GG genotype ($P< 0.005$) and TNF-α-308G allele ($P< 0.005$) were associated with reduced and TNF-α-308GA genotype ($P< 0.002$) with increased FEF25-75 value in asthmatics. IFN-γ +874AA genotype caused decrease in FVC factor ($P< 0.05$). **Conclusion:** This study showed that IL-6, TNF-α and IFN-γ lower producer variants are associated with reduced pulmonary capacities. These genetic variants maybe by affecting on cytokine synthesis participate in airway wall thickening and obstruction by both structural remodeling and inflammation. Further studies with consideration of local, serum and systemic examination of cytokine proteins and other different inflammatory agents and airway pathology will be needed.

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# Constructing an Allergenic Hybrid Molecule from Three Individual Allergens from *Chenopodium Album* Pollen

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**Background & Objective:** *Chenopodium album* pollen as aeroallergen is a common cause of allergic respiratory diseases in Iran. Considering the difficulties of using natural extracts for diagnosis, developing of the genetically engineered allergenic molecules would be useful for diagnostic and therapeutic purposes. The aim of this study is therefore to design a hybrid molecule from three different allergens of *C. album* pollen. **Methods:** Using in silico studies, overlapping extension primers were designed for fusion of coding fragments of the three allergens from *C. album* pollen. After amplification of each individual allergen fragment with specific primers, hybridization of the three fragments was carried out by means of overlapping extension PCR technique. Secondly, hybrid DNA was inserted into pET21b+ expression vector, expressed in *E. coli* BL21-CodonPlus (DE3)RIL expression host, and recombinant hybrid protein was purified by Ni-NTA chromatography. Finally, the allergenicity of this molecule was evaluated by ELISA and western blotting. **Results:** Electrophoresis of the PCR products *Che a 1* and *Che a 3* allergens showed that there were 507 bp and 267 bp bans on agarose gel respectively, when two PCR products combined to each other constructed che a1-3 fragment with 774 bp. Connection of this fragment with *che a 2* (399 bp) allergen developed a hybrid allergen with 1173 bp. DNA of this molecule successfully entered into the pET21b+ vector. Finally, sequencing and analysis with the NCBI blast showed that the hybrid allergen was correctly cloned. After expression and purification, the produced recombinant protein was confirmed to have an approximately 45 KDa via SDS-PAGE. Moreover, immunoblotting and ELISA showed that this recombinant hybrid allergen was IgE reactive with 7/10 of sera from *C. album* pollen allergic patients. **Conclusion:** In our study, we successfully fused the three allergen fragments of the *C. album* pollen with a confirmed IgE reactivity. Our results suggest that this molecule can be used in the diagnosis of allergy and that it can be converted into a hypo-allergenic protein for immunotherapy.

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# Correlations and Reproducibility between Histamine, IL-4 and IL-13 Generation Activated with either Anti-Human IgE or IL-3 from Human Basophils

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**Background & Objective:** Human basophils play a key role in allergic diseases such as asthma and in a variety of immunological disorders. Basophil is a major source of interleukin-4 (IL-4) and IL-13 and that the generation of these cytokines can be induced by IgE-dependent and non-IgE-mediated mechanisms. Time and stimulus-dependent differences in the regulation of IL-4 and IL-13 could be having relevance to the biological effects of these cytokines. The aim of the present study was, activation of basophils and evaluation the extent of histamine, IL-4, and IL-13 generations. **Methods:** Basophils were prepared by Percoll gradients (purity, 10 ±3%, n=25). The release of histamine, cytokines was assessed activated with either anti-human IgE (1/1000 or 1/10000, 4 h or 24 h) or IL-3 (100 ng ml-1, 24 h). Histamine was measured by fluorometric technique. To measure IL-4 and IL-13 in supernatants, ELISA kits were used. Results were analyzed statistically, using ANOVA. **Results:** With anti-IgE, there was no significant correlation between the extent of either IL-4 ($r=0.24$, $P \approx 0.35$) or IL-13 ($r=0.47$, $P \approx 0.098$) and histamine release. With IL-3 from different donors showed that the extent of IL-13 correlated with histamine ($r=0.44$, $P< 0.05$). There was no correlation between the extent of IL-4 and the degree of either histamine ($r=0.077$, $P \approx 0.72$) or IL-13 ($r=0.162$, $P \approx 0.5$). The reproducibility of cytokines isolated from the same donor, but on different occasions, indicates that the ability of anti-IgE to cause cytokines was consistently similar for a given donor. In contrast, the response to IL-3 showed greater variability in the extent to which cytokines were generated. **Conclusion:** The pathways leading to IL-3-triggered histamine release and IL-13 generation show similarity. Donor-dependent differences may be responsible for this wide range in the extent of releasibility. Our data show that the ability of IL-3 to release cytokines from basophils showed a greater range.

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# Association between Asthma and Allergic Rhinitis in Jondishapoor and Chamran Universities, Ahwaz

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**Background & Objective:** Asthma is a chronic inflammatory disease that occurs in people regardless of age. Epidemiologic surveys and clinical reports have documented that allergic rhinitis coexists with asthma in many patients. The aim of this study was to determine association between allergic rhinitis and asthma. **Methods:** A cross-sectional, descriptive, questionnaire-based study was conducted in Ahvaz universities from Feb 2007 to May 2007. A total of 3500 students, 19 to 35 years of age were evaluated by a self administrated screening questionnaire evaluated for asthma and allergic rhinitis. The data were analyzed with SPSS. **Results:** The average age of students was 21.9 ± 3.20 and 56% was female and 44% male. About 2/3 (62.9%) are living in Khuzestan and 1/3 in other cities of Iran. The definition of asthma and allergic rhinitis was based on the following question: Has your physician ever told you that you have asthma or allergy? According to our finding, the prevalence of asthma and allergic rhinitis was 2.7% (94 case) and 35.6% (1226 case) respectively. From total of 94 asthmatic individuals 65(63%) also had allergic rhinitis. Subjects with allergic rhinitis were more likely to be males, OR = 2.23 (1.30-3.72), P< 0.005, and to have allergic symptoms. The highest prevalence of nasal symptoms was reported during the spring with 49.9%. **Conclusion:** Allergic rhinitis is the most common comorbidity with asthma and physicians should be mindful for treatment of asthmatic patients.

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# Prevalence of Atopic Eczema in 6-7 and 13-14 Years Old Children in Ahwaz – an ISAAC Study

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**Background & Objective:** The existence of geographic variation in the frequency of atopic eczema and related symptoms has been supported by many studies. However little is known about the atopic eczema prevalence and associated symptoms in Ahvaz city. This study aims to estimate the prevalence of atopic eczema and related symptoms in Ahwazian school children aged 6-7 and 13-14 years. **Methods:** A cross-sectional study of 3000 students aged 6-7 and 13-14 yrs was conducted from Oct 2008 to Apr 2009 by using the International study of Asthma and Allergy in Childhood (ISAAC) phase one questionnaire. Subjects were selected from 32 schools by random cluster sampling method; data were analyzed using SPSS-15 soft ware. **Results:** of 3000 questionnaires 2930 were completed and returned (The response rate was 97.5 %). The prevalence of itchy rash ever was 8.4% among Ahvazian schoolchildren. Sex difference was not significant, while by age 13-14 yr old students showed higher frequency of rash ever ($P< 0.05$). The 12 month itchy rash was reported by 5.3%; and frequency of itchy flexural rash was 3.7% among students. Rash clearance in the past yr was reported by 4% of subject. The prevalence rate of diagnose of eczema (eczema ever) was higher in 13-14 yr old students (7.2 vs. 4.1, $P< 0.0001$), and sleep disturbance was reported by 2.2% with no sex or age difference. **Conclusion:** The prevalence of atopic eczema in Ahvazian schoolchildren was moderate compared with other regions of Iran, but it was lower than that reported from Arab neighboring countries.

27

# Interleukin-8-251 A/T and CXCR2+ 1208 C/T Genes Polymorphisms in Chronic Rhinosinusitis

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**Background & Objective:** IL-8 is one of the pro-inflammatory cytokines and can play an essential role in the pathogenesis of chronic rhinosinusitis (CRS) as well as nasal polyposis (NP). The ability of individuals in producing IL-8 is partially determined by IL-8 −251 A/T polymorphism. Hence, the aim of the present study is to investigate the association between IL-8 −251 A/T and CXCR2 +1208 C/T genes polymorphisms and susceptibility to CRS and NP. **Methods:** 245 CRS patients and 204 healthy controls were included in this study. CRC patients were categorized by the existence or lack of NP. IL-8 promoter −251 A/T and CXCR2 +1208 C/T gene polymorphisms were genotyped via allele specific PCR (AS-PCR) method. **Results:** While no remarkable difference was demonstrated between patients and controls for both CXCR2 +1208 C/T and IL-8 -251 A/T polymorphisms, a significant increase in IL-8 -251 AA genotype was detected in CRS patients with NP compared to those without NP (29.3% and 16.2%, respectively; $P< 0.05$). Interestingly, this association is getting far stronger when non-asthmatic CRS patients were only taken into consideration ($P< 0.005$). **Conclusion:** The results of the present study indicate that inheritance of IL-8 -251 A allele is significantly associated with NP development in CRS patients. Therefore, NP formation might be a result of the exposure to an intense inflammatory environment which is much likely in genetically susceptible CRS patients.

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## Role of Dendritic Cells on Pathogenesis of Lung Emphysema

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**Background & Objective:** Chronic obstructive pulmonary disease and emphysema (COPD) is characterized by chronic airway inflammation. Cigarette smoke (CS) has been considered a major player in the pathogenesis of COPD. The inflamed airways of COPD patients contain several inflammatory cells including neutrophils, macrophages, T lymphocytes, and dendritic cells (DCs). The relative contributions of these various inflammatory cells to airway injury and remodeling are not well documented. Myeloid and plasmacytoid dendritic cells (mDCs, pDC) are crucial immune cells detecting microorganisms and linking innate and adaptive immunity. mDC are antigen presenting cells and pDC are intermediate cells. They produce large amounts of IFN-gamma after stimulation with CpG motifs and are also antigen presenting cells. The antiviral effect exerted by IFN-gamma is due to the induction of IFN response genes. **Methods:** In current study we investigated the effects of cigarette smoke extract (CSE) on mouse bone marrow derive myeloid dendritic cells (mDC) and human pDC. In addition, we assessed CSE-induced changes in cDC function in the mixed lymphocyte reaction (MLR) examining $CD4^+$ and $CD8^+$ T cell proliferation. **Results:** CSE induces the release of the chemokines CCL3 and CXCL2 (but not cytokines), via the generation of reactive oxygen species (ROS) in mDC. In a mixed-leukocyte reaction assay, CS-primed DCs potentiate $CD8^+$T cell proliferation via CCL3. In contrast, proliferation of $CD4^+$T cells is suppressed via an unknown mechanism. The CS-induced release of CCL3 and CXCL2 by DCs may contribute to the influx of $CD8^+$T cells and neutrophils into the airways, respectively. In pDCs, we observed that CSE augmented the production of IL-8 and suppressed the release of TNF-α, IL-6 and IFN-α. Moreover, CSE suppressed PI3K/Akt signalling in pDC. **Conclusion:** our data indicate that CSE has both the potential to diminish anti-viral immunity by downregulating the release of IFN-α and other pro-inflammatory cytokines while, at the same time, augmenting the pathogenesis of COPD via an IL-8 induced recruitment of neutrophils.

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# Study of Expression of Human Truncated Recombinant CD23 (FcεRII) Fragments by RBL Cells

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**Background & Objective:** The RBL-2H3.1 cell line was developed as a model for the study of mast cells. RBLs originate from a basophil like solid tumor that occurs in a β-chloroethyl-amine-treated rat.CD23 (FcεRII), low-affinity receptor for IgE, has been widely implicated in the synthesis of IgE as well as in IgE-mediated immune and inflammatory functions. There are two forms of CD23 in humans, CD23a and CD23b, their cell expression and functional activities are different. Aim of this project is to express defined truncated fragments of (FcεRII) / CD23- and to assess the physical and biological properties of these truncated fragments with regard to: IgE independent mast cell /basophile secretayogue activity. **Methods:** CD23a and b minus RGD sequence and also the whole things of CD23a and b have been cloned into pIRES vector for transfection and subsequent evaluation of IgE in functional assays. Overlap PCR was used to produce the different truncated fragments of CD23 molecule, consisting of CD23a and b minus the RGD sequence (adhesion part). Their DNA and protein was purified and obtained the characterization by electrophoresis. CD23 a and b minus RGD sequence and their whole things was transfected into the RBL cells (Rat Basophilic Leukemia) in order to study of expression of EGFP and biological activity by FACS (Fluorescence activated cell sorting) and FACS analysis for investigating the interaction of the IgE with different expression of CD23. Binding of IgE-Fc fragments to cell surface and expressed human FcεRII/CD23 (low affinity receptor for IgE) were assessed using Flow cytometry to detect the binding of IgE to cell surface receptors, using a biotinylated anti-IgE, followed by a streptavidin phycoerythrin conjugate. **Results:** It was shown that RBL cells can express CD23a and b (whole things) and CD23a and b minus RGD sequence in pIRES vector with unstable form. **Conclusion:** The RBL cells could express both forms of CD23.

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# Scrutinize the Effect of Cancerous Cell Line in a Mouse Model of Allergic Asthma

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**Background & Objective:** In general, cancer and allergy are the two major conditions in adults and children which lead to mortality and morbidity. Several studies have shown that site of chronic inflammation are often associated with the establishment and growth of malignancy. Still there are major questions which should be answered concerning the Allergo-Oncology. Therefore we decide to explore the effect of cancerous 4T1 cell lines in BALB/c females after the induction of allergic asthma. **Methods:** We induced allergic asthma with soluble OVA without adjuvant. After the induction of disease we introduce the 4T1 malignant breast cancer cell line to those mice via intravenous injection. Mice were aerosol challenged several times after the injection of cancerous cell line to see the recruitment of inflammatory and malignant cells to the lungs. Mice were evaluated for inflammation in the lung and bronchi and also lungs were examined for mucus hyper secretion. **Results:** We found that inflammatory infiltrate to the lung of mice with allergic asthma after the injection of cancerous 4T1 cells is four times increased compare to the mice with allergic asthma alone. These infiltrate is composed of eosinophils, lymphocytes and macrophages. The total number of inflammatory infiltrate increased 3 times and the percentage of eosinophils increased is increased 4 times but the percentage of macrophages decreased vigorously. **Conclusion:** We can conclude that presence of cancerous cells in combination with allergic asthma detriment the allergic asthma in mice.

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## Long-Standing Lymphocytes in a Mouse Model of Allergic Asthma

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**Background & Objective:** Although life-long immunity against pathogens is beneficial, immunological memory responses directed against allergens are potentially harmful. Because there is a paucity of information about Th2 memory cells in allergic disease, we established a model of allergic asthma in BALB/c mice to explore the generation and maintenance of Th2 memory. **Methods:** We induced disease without the use of adjuvants, thus avoiding Ag depots, and found that unlike allergic asthma in mice immunized with adjuvant, immunizing with soluble and aerosol OVA resulted in pathological lung lesions resembling human disease. To test memory responses we allowed mice with acute disease to recover and then re-exposed them to aerosol OVA a second time. **Results:** Over 400 days later these mice developed OVA-dependent eosinophilic lung inflammation, airway hyperresponsiveness, mucus hypersecretion, and IgE. Over 1 year after recuperating from acute disease, mice had persistent lymphocytic lung infiltrates, Ag-specific production of IL-4 and IL-5 from spleen and lung cells in vitro, and elevated IgG1. Moreover, when recuperated mice were briefly aerosol challenged, we detected early expression of Th2 cytokine RNA in lungs. **Conclusion:** Taken together, these data demonstrate the presence of long-lived Th2 memory cells in spleen and lungs involved in the generation of allergic asthma upon Ag re-exposure.

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# Association between Chronic Urticaria and the Results of Skin Prick and H-Pylori Abs Tests in Patients Referred to the Immunology and Allergic Clinic of Zahedan in 1385-87

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**Background & Objective:** The chronic urticaria defined as skin wheal, erythema and itching intense, caused by allergic reactions to variety of allergens for at least 6 weeks in adults. On the other hand, this is presumably believed that chronic infections such as gastrointestinal infection with gram negative bacillus pathogen as *Helicobacter pylori* agents, have been implicated in the causation of chronic urticaria and this may make the possibility of an association between chronic urticaria and *H. pylori* infection that is contribute to the several acute and chronic diseases. Antibody mainly serum IgG and IgA were reacting against this bacteria of infected patients and the aims of present study were to study the results of skin prick test and amount of antibodies in 563 allergic chronic urticaria patients, refereed to the Amir-Al Momenin and Ali Asghar hospitals of Zahedan-Iran. **Methods:** The method of present study was analytic and descriptive study and was carried out on two separated groups of healthy control and chronic urticaria patients evaluated along with an equal number of age and sex matched controls for presence of *H. pylori* infection, admitted at the immunology and allergic clinic of Zahedan city in 1385-87. The study group was consisted of 563 and had been screened for analyzing complete history, complete blood counts with differential, skin prick test and measuring serum IgG and IgA by commercial SRID and nephlometry assay. **Results:** From 563 study population, 76% were female and the rest were male aged between 8 and 59 years old. Based on the results of skin prick test, 82.5% of the study group had highly flare and wheal reactions with food allergens such as curry, tomato, white and yellow eggs, walnuts, banana and wheat and the rest (17,5) had highly positive reactions with aeroallergens such as dust house mite, threes, cladosporium and weeds. But only 12% in control group showed positive skin prick test mainly with aeroallergens. Increased amount of serum IgG level was observed in 79% in study group, whereas only 17,5% of healthy control group had increased amount of serum IgG level. High concentration of serum IgA was obtained in 82% of patients and 9% in normal control. **Conclusion:** The results of this study support the association of chronic urticaria and *H. pylori* infections indicating the high response of patients to the infection. Further studies are required to find better understanding of the causal relationship with the other environmental elements.

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# The Impact of School and Domestic Indoor Air Pollution on Respiratory Symptoms among Primary School Students from Different Socio-Economic Backgrounds (Low and High) within the Perth Metropolitan Area

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**Background & Objective:** Although genetic background and environmental exposure seem to be the key factors for the development of respiratory symptoms, socio-economic status (SES) may also contribute to the development of those illnesses in children (Rona 2000). To investigate the extent to which socio-economic factors may contribute to the increased prevalence of respiratory symptoms and asthma in Australia we studied respiratory symptoms and asthma among primary school students from low and high socioeconomic backgrounds. A cross sectional study to determine the impact of school and domestic indoor air pollution on respiratory symptoms among primary school students from different socio-economic backgrounds (low and high) was conducted within the Perth metropolitan area. The study was carried out in three stages: 1) Questionnaire survey, 2) Indoor air quality monitoring in schools, 3) Indoor air quality monitoring in houses.
**Methods:** We studied 104 primary school students from low and high socioeconomic areas of Perth metropolitan between 2007 and 2008. The respiratory symptoms and asthma were assessed with a standardized questionnaire. Schools and domestic environmental monitoring took place in winter and summer in order to determine seasonal differences in concentrations of studied air pollutants. For this purpose, 11 primary schools with low and high socio-economic backgrounds were selected. Domestic air qualities were monitored in 90 houses from each area of low and high socio-economic status. SES was derived from means of more than two indicators including education and income. The Australian Bureau of Statistics also determined the areas of low and high socio-economic status. Exposure levels to some primary indoor air contaminants including Volatile Organic Compounds [(VOCs) ($\mu g/m^3$)], formaldehyde (HCHO) ($\mu g/m^3$) and particulate matter with size 2.5 microns in diameter ($PM_{2.5}$) ($\mu g/m^3$) and PM(10) were measured in domestic and schools environments. Indoor temperature (TºC) and relative humidity (RH) (%) were also monitored. Multivariate analyses were then used to quantify the effect of relevant factors on the prevalence of respiratory symptoms. **Results:** Socioeconomic status is a comprehensive index that refers to a broad range of factors, such as level of social communities, income, education, parental occupations and living conditions. School children from low socioeconomic groups showed more respiratory symptoms in this study. Those who had higher SES had fewer asthma and respiratory symptoms. We conclude that low socioeconomic status is itself a risk factor for respiratory symptoms and asthma among schoolchildren. **Conclusion:** Asthma and respiratory symptoms were more common in low socioeconomic status groups. There was no significant support for the hygiene hypothesis.

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## Molecular Effects of Estrogen in Patients with Systemic Lupus Erythematosus

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**Background & Objective:** Systemic lupus erythematosus (SLE) is a disease with unknown etiology. The pathogenic role of sex hormones and apoptosis in SLE, have often been discussed, but the mechanisms by which this might occur is not completely understood. We studied the effects of estrogen in the pathway of induced apoptosis in Iranian SLE patients. **Methods:** T lymphocytes from 35 SLE patients (23 female with mean age of 28 years, and 12 male with mean age of 30 years), and 20 age matched normal control (10 females with mean age of 29 years and 10 males with mean age of 30 years) were isolated and cultured in the presence of 10-8M 17-β estradiol. The expression levels of Fas, FasL, Bcl-2, Caspase 8, and Caspase 9 RNAs were determined semi quantitatively in comparison to the expression level of Beta-actin RNA. **Results:** Estrogen exposure did not have any significant effects on the expression levels of Fas, Bcl-2 and Caspase 9 both in SLE patients and controls. However the expression levels of FasL, and Caspase 8 were significantly increased in SLE patients, but not in controls. **Conclusion:** This suggests the probable involvement of extrinsic apoptosis pathway in estrogen induced apoptosis in SLE.

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## The Role of PDCD1 Gene Promoteric Allele A with Risk of Rheumatoid Arthritis

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**Background & Objective:** Programmed cell death 1 (PDCD1), a negative T cell regulator to maintain peripheral tolerance, induces negative signals to T-cells during interaction with its ligands oretheref and is a candidate gene in the development of autoimmune diseases such as Rheumatoid Arthritis .Thus the association of PDCD1 polymorphism with the risk of Rheumatoid Arthritis was investigated among Iranian patients and healthy controls. **Methods:** Genomic DNA was extracted from the whole blood samples using DNA Purification kit (DNG –plus). A total of 81 Iranian Rheumatoid Arthritis patients and 188 healthy controls were genotyped using PCR- RFLP method for PD1.1 (-538) A/G Promoter .polymorphism. **Results:** The A allele of the PD1.1 polymorphism located on Promoter of PDCD-1 Gene was significantly more frequent in Iranian Rheumatoid Arthritis patients than controls (2.9% versus 0.8%, $P< 0.05$, odds ratio 3.735, 95% CI=0.956–14.588).There were no significant difference in PD1.1G/G genotype ($P \approx 0.084$, OR=0.308, 95% CI=0.076–1.256) , PD1.1A/A genotype ($P \approx 0.390$, OR=2.580, 95% CI=2.241–2.969) and PD1.1G/A genotype($P \approx 0.167$, OR=2.681, 95% CI=0.629–11.432) between Rheumatoid Arthritis cases and controls. **Conclusion:** Our results showed that The A allele of the PD1.1 (-538) polymorphism located on Promoter of PDCD1Gene increase susceptibility to Rheumatoid Arthritis in Iranian Patients.

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# HLADRB1, Circulating Th1/Th2 Cytokines and Immunological Homunculus in Atherosclerotic Plaque Instablization

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**Background & Objective:** Plaque Instablization is a critical event in Coronary Atherosclerotic disease destination. This study was designed to investigate the correlation between HLA-DRB1allels and Th1/Th2 type cytokines in atherosclerotic plaque instablization. **Methods:** Th1/Th2 type cytokines have been determined in serum samples of 31 subjects with unstable angina and 27 subjects with chronic stable angina as well as 24 individuals as normal control. Then 24 subjects who had skewed serum levels of Th1/Th2 type cytokines were selected and more than 100 alleles of HLA DRB1 locus has been typed in those subjects by SSP-PCR. **Results:** The mean concentration of serum LDL-cholesterol in normal control group was 142.046(pg/ml) and their mean age was almost like the patient groups (59 year old). This means these people are at risk of coronary atherosclerotic disease but the mean serum concentration of IL-10 in these subjects was higher in comparison to two patient groups.0.33 +-0.59 pg/ml versus.064 +0.3 pg/ml in unstable angina pectoris group($P< 0.028$) and $0.22 \pm 0.6$ pg/ml in chronic stable subjects. There was no statistically significant difference among the groups in serum levels of other desired cytokines in this study.3 of 9 individuals of normal control subjects were HLA-DR16 positive .No one of the 9 subjects with chronic stable angina nor the 6 individuals with unstable angina pectoris were HLA-DR16 positive. **Conclusion:** This preliminary study shows there is no strong correlation between Th1 /Th2 type cytokines and HLADRB1 in atherosclerotic plaque instablization, however, HLA-DR16 and IL-10 through immunological homunculus theory may work in prevention of this phenomena.

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# Cytokine Pattern in the Blister Fluid and Sera of Patients with Pemphigus Vulgaris

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**Background & Objective**: Pemphigus vulgaris (PV) is a chronic autoimmune blistering disease of the skin, in which loss of adhesion between keratinocytes is caused by autoantibodies. Autoantibodies have clearly been shown to be pathogenic. Because autoantibodies are also found in uninvolved skin, further mechanisms may be important in the development of pemphigus lesions. The goal of this study was to investigate the other immunopathological aspects of PV by determining the levels of serum and blister fluid cytokines in patients with PV. **Methods:** Twenty-three patients with PV and a control group consisting of 24 healthy individuals were examined. Serum IL-2, IL-6 and IL-12 were measured in the two groups by the ELISA technique. **Results:** The levels of IL-2 in the sera of most patients and controls were undetectable. Nevertheless, the mean concentration of IL-2 only in the sera of two patients was 85.82 pg/ml. However, the mean concentration of IL-2 in the blister fluids of patients was 41.45. The same was roughly observed for IL-2 levels in the blister fluids of controls. The mean concentration of IL-6 was significantly increased in the sera of patients, compared with controls (169.50 vs. 75.62 pg/ml). The same was observed for IL-12 (135.33 vs. 86.28 pg/ml). **Conclusion:** The results suggest the predominance of TH2 cytokines (IL-6) in PV and that IL-12 may play an important role in the pathogenesis of PV.

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## The Role of PDCD1 Gene Promoteric Allele A with Risk of Rheumatoid Arthritis

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**Background & Objective:** Multiple Sclerosis is an autoimmune disease, affecting central nervous system mostly in young adults. Th17 cells play an important role in the pathogenesis of MS. IL-21 is a cytokine that is produced by Th17 cells and functions as a autocrine growth factor for Th17 cells. Considering the importance of IL-21 in Th17 cell development, IL-21 gene could be considered as a candidate gene for susceptibility to MS. Therefore, the aim of the present study is to determine the association of two IL-21 gene polymorphisms (IL-21 C5250T in exon 3 and IL-21 G1472T in the second intron) with susceptibility to MS. **Methods:** IL-21 C5250T and IL-21 G1472T polymorphisms were analyzed by PCR-RFLP and Allele specific PCR method in 206 Iranian MS patients (151 females, 55 males) and 203 healthy controls (151 females, 52 males), respectively. MS patients were characterized based on Poser criteria. **Results:** We found a significant association between IL-21 G1472T polymorphism and susceptibility to MS development ($P \approx 0.009$). In fact, genotype frequencies in patient groups were TT=18.4%, TG=54.8% and GG=26.6% while in control group were TT=31.52%, TG=45.8% and GG=22.6%. Interestingly, this polymorphism showed a significant association with clinical types of MS ($P \approx 0.00001$). In fact, inheritance of GG genotype was higher in RR-MS patients (24.6%) compared to SP-MS (16.4%) or PP-MS (6.7%). However, the other polymorphism did not show any association with susceptibility to MS, disease type and other clinical parameters such as age at disease onset or EDSS. **Conclusion:** Among the two polymorphisms of IL-21 gene, G1472T polymorphism was significantly associated with MS development. Other studies to clarify the association of this polymorphism with MS susceptibility in other populations and IL-21 levels would be desired.

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# The PDCD-1 Gene Promoter Polymorphism Is Not Associated with Iranian MS Patients

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**Background & Objective:** Programmed cell death 1 (PDCD1), a negative T cell regulator to maintain peripheral tolerance, induces negative signals to T-cells during interaction with its ligands and therefore is a candidate gene in the development of autoimmune diseases such as Multiple sclerosis. Thus the association of PDCD-1 polymorphism with the risk of MS was investigated among Iranian patients and healthy controls. **Methods:** Genomic DNA was extracted from the whole blood samples using DNA Purification kit (DNG – plus). A total of 107 Iranian MS patients and 188 healthy controls were genotyped using PCR- RFLP method for PD1.1 (-538) G/A polymorphism in Promoter of PDCD-1 gene. **Results:** We didn't observe any AA genotype in either case or control. There were no significant difference in PD1.1G/G genotype ($P \approx 0.480$, OR=0.562, 95% CI=0.111–2.836), PD1.1G/A genotype ($P \approx 0.671$, OR=1.779, 95% CI=0.353–8.973) between MS cases and controls. The G or A allele frequency of the PD1.1 polymorphism did not show differences between the patients and the controls ($P \approx 0.482$, OR=1.768, 95% CI=0.354–8.837).we also did not find any significant association between PD1.1 G/A polymorphism and there Type of MS Disease. **Conclusion:** Our results showed that the PD1.1G/A polymorphism is not associated with MS susceptibility in Iranian patient.

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# Evaluation of Nonnominal Alleles for Immunoglobulin G1M(X) and G1M (A) Allotypes in B Lymphocytes of Rheumatoid Arthritis Patients and Normal Individuals

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**Background & Objective:** Associations or linkages between polymorphisms of Gm allotypes-mendelian genetic markers of immunoglobulin gamma heavy chains-and susceptibility to autoimmune diseases, including diseases with immuno-pathological pathogenesis were reported in several studies. Anti Gm's in rheumatoid arthritis (RA) patients detect products of genes such as G1m (a) and G1m(x) allotypes. Commonly, these anti Gm's are specific for other individual immunoglobulin allotypes. Reasons are given for the contention that such anti Gm's in patients with RA are indicative of expression of nonnominal allotypes. Productions of antibodies against nonnominal immunoglobulin allotypes in RA patients suggests that immune system of these patients has been exposed to such foreign allotypes. In this study we searched for nucleotide sequences specific for nonnominal G1m(x) and G1m (a) copies in individuals homozygous for these alleles. **Methods:** DNA of B lymphocytes from 28 RA patients and 25 normal controls were analyzed with a Real Time PCR technique with SYTO® 9 and SAbiosciences primers. **Results:** We found nonnominal G1m(x) sequences in 10 of 12 (83%) tested G1m(a) homozygous patients with anti G1m(x) antibody in serum [and 1 of 11 (9%) healthy individuals] ($P< 0.001$) and nonnominal G1m(a) sequences in 15 of 16 (93%) tested G1m(x) homozygous patients with anti G1m(a) antibody in serum [and 2 of 14 (14%) healthy individuals] ($P< 0.001$). Results also showed that nonnominal Ig sequences were present in very low copy numbers (1-2 copies per cell). **Conclusion:** The origin of such a low copy number of Ig gene fragments may be explained by genes transferred from one individual to another by means of a virus-mediated capture such as some B cell virus and insertion mechanism of Ig gene fragments generated by normal Ig switch-associated gene excision process. This process-transduction and occurring after the tolerance induction period-may lead to a chimaeric state of the B cell compartment in RA patients.

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# Anti-*Saccharomyces Cerevisiae* Antibodies in Tunisian Patients with Crohn's Disease

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**Background & Objective:** In Western Europe and USA, the presence of anti-*Saccharomyces cerevisiae* antibodies (ASCA) in Crohn's disease (CD) patients and their healthy relatives suggests that ASCA may be influenced by genetic and/or environmental factors. So the aim of this study was to assess the prevalence of ASCA in Tunisian patients with CD or ulcerative colitis (UC), and unaffected family members, in relation to clinical phenotype. **Methods:** Seventy-seven patients (39 CD, 38 UC), 66 healthy relatives of CD patients, 16 relatives of UC patients and 70 healthy controls were studied. ASCA were quantified with a new isotype-specific ELISA test involving an antigenic extract from S. cerevisiae strain W303 and by the original test which detects total immunoglobulins against *S. cerevisiae* SU1 mannan. **Results:** The isotype-specific ASCA W303 test was more sensitive than the ASCA SU1 test for immunoglobulin detection, although the specificity of the two tests was identical (91%). A high percentage of patients with CD (72%) and their unaffected family members (35%) were ASCA-positive in contrast to UC patients (16%) and their relatives (0%) and controls (8.6%). ASCA were shown to be independent of disease activity, but were associated with ileal location. Some antigens and/or isotypes discriminated patients depending on sex or age at diagnosis. **Conclusion:** This study confirms the antigenic heterogeneity of *S. cerevisiae* strains in their ability to detect ASCA. It suggests that ASCA are markers of immunoregulatory disturbance in CD, independent of ethnic/cultural differences between Europe, USA and North Africa.

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# Association of NALP1 A/T Gene Polymorphism with Susceptibility to Vitiligo in Iranian Patients

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**Background & Objective:** Vitiligo is a chronic disorder that causes depigmentation in patches of skin. NALP1 contains a NACHT domain, an LRR domain (leucine-rich repeat), and a Pyrin domain. Proteins with a NACHT domain or an LRR domain are known to be involved in apoptosis or inflammation. NALP1 is widely expressed in skin associated cells, namely Langerhans' cells. Therefore, NALP1 polymorphism could be considered as a proper candidate gene for susceptibility to vitiligo, a goal that is aimed in this study. **Methods:** NALP1 A/T polymorphism (resulted in histidin to leucine substitution at amino acid 155) was investigated by Allele specific PCR method in 187 Iranian vitiligo patients (108 females, 79 males) compared to 249 healthy controls (129 females, 120 males). **Results:** Genotype frequency in patient groups were AA=34.01%, AT=32.48% and TT=33.51% while in control group were AA=32.69%, AT=32.69% and TT=34.62%. The results showed no significant difference between cases and controls ($P \approx 0.56$). No significant association between NALP1 A/T polymorphism and response to treatment ($P \approx 0.45$), disease progression during last year ($P \approx 0.64$) and age at disease onset ($P \approx 0.48$) were detected. However, there was an approximate significant association between NALP1 A/T polymorphism and disease severity ($P \approx 0.08$). **Conclusion:** There is a probable association between NALP1 A/T polymorphism and vitiligo in Iranian patients.

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# Association of 3’ UTR A/C Polymorphism in the IL-23 Receptor Gene with Susceptibility to Multiple Sclerosis

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**Background & Objective:** Multiple Sclerosis (MS) is an autoimmune disease, affecting central nervous system (CNS) mostly in young adults. IL-23 is expressed predominantly by phagocytic cells and induces IFN-γ secretion by T cells. Therefore, IL-23 plays an important role in the pathogenesis of MS. Until now, multiple SNP has been detected in IL-23 receptor gene and their associations with some autoimmune diseases have been reported. In the present study, being first reported, the association of A/C polymorphism in the 3’ UTR of IL-23R gene (rs10889677) with susceptibility to MS was investigated. **Methods:** IL-23R gene (rs10889677A/C) polymorphism was investigated by PCR-SSP (polymerase chain reaction with specific primers) method in 197 Iranian MS patients (147 females, 50 males) and 211 healthy controls (155 females, 56 males). MS patients were characterized based on McDonald criteria. **Results:** Genotype frequency in patient groups were AA=34.01%, AC=32.48% and CC=33.51% while in control group were AA=32.70%, AC=33.70% and CC=33.60%. The results showed no significant difference between cases and controls ($P \approx 0.95$). No significant association between IL-23R polymorphism and disease disability, progressive index and age at disease onset was also detected. However, there was significant difference in frequencies of AA and AC+CC between patients with RR-MS and SP-MS+PP-MS ($P< 0.05$). In fact, inheritance of AA genotype was higher in RR-MS patients (38.6%) compared to SP-MS+PP-MS patients (24.13%). **Conclusion:** The results of the present study showed that while 3' UTR polymorphism in the gene of IL-23R does not affect the susceptibility to MS, this polymorphism could determine the clinical type of MS.

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# Evaluation of the 844C/T Polymorphism in the Fas Ligand Promoter in Iranian Rheumatoid Arthritis Patients

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**Background & Objective:** Dysregulation of apoptosis–related genes in the immune system which is pursuant in this genes polymorphisms leads to autoimmune disease including rheumatoid arthritis. FasL-844C>T (a putative motif for CAAT/enhancer- binding protein) polymorphism, play a crucial role in the apoptotic signaling pathway and is essential for forming of the homeostasis and proper immune response, it is postulated that FasL-844 C>T polymorphism may influence the clearance of autoreactive T cells in RA. **Methods:** In this study, we analyzed polymorphism in 120 patients with rheumatoid arthritis and 112 age and gender-matched unrelated healthy controls, Genomic DNAs were obtained from Peripheral blood mononuclear cells from RA patients and healthy controls, and the polymorphism was examined with the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. and Frequency in distribution of the polymorphism was compared the patients and the healthy controls. **Results**: The frequency of this polymorphism was higher in the cases than controls (52.5% versus 43.7%). **Conclusion:** Our results demonstrated that between the120 RA patients being heterozygous in FASL-844C/T (OR=1.42, CI= 0.92 to 1.52, P ≈ 0.18) promoter region polymorphisms was not statistically associated with the RA in Iranian patients.

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# TNF-Related Apoptosis Inducing Ligand (TRAIL) Gene Polymorphism in Iranian Patients with Multiple Sclerosis

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**Background & Objective:** TNF-related apoptosis inducing ligand (TRAIL) has been indicated that cause apoptosis of autoreactive T cells, and thus, it is a strong candidate gene for involvement in the development of autoimmune disease. We studied single nucleotide polymorphisms (SNPs) in the coding region of the gene at position _1595 C>T in Iranian patients with MS (multiple sclerosis). **Methods:** After isolation of Genomic DNA from whole blood samples by DNA extraction kit we genotyped the 107 case patients and 112 control subjects using polymerase chain reaction–based restriction fragment length polymorphism (PCR-RFLP) method. We analyzed the genotype and allelic frequencies of TRAIL_1595C>T in patient and healthily controls. **Results**: We found that the presence of the CC genotype at position of TRAIL _1595C>T (OR; 1.32, 95% CI; 0.76 to 2.3, genotype moderately is associated with a high risk of MS in Iranian patient (66.4%versus60.0%) although it is not statistically significant. We did not found association between TRAILC/T, T/T genotypes and risk of multiple sclerosis in Iranian patient. **Conclusion:** Genetic polymorphisms in the death pathway gene TRAIL appear to be associated with an increased risk of developing multiple sclerosis. Further study in the future is needed to confirm this study.

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# Polymorphism in Enhancer of PDCD1 Gene is not Associated with Rheumatoid Arthritis in Iranian Patients

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**Background & Objective:** Programmed cell death 1 (PDCD1), a negative T cell regulator to maintain peripheral tolerance, induces negative signals to T-cells during interaction with its ligands oretheref and gene is a candidate in the development of autoimmune diseases such as rheumatoid arthritis. Thus the association of PDCD1 polymorphism with the risk of RA was investigated among Iranian patients and healthy controls. **Methods:** Genomic DNA was extracted from the whole blood samples using DNA Purification kit (DNG –plus). A total of 120 Iranian RA patients and 188 healthy controls were genotyped using PCR- RFLP method for PD1.3 (+7146) A/G polymorphism in enhancer of PDCD-1 gene. **Results:** There were no significant difference in PD1.3/GG genotype (P ≈ 0.552, OR=0.844, 95% CI=0.482–1.477), PD1.3 G/A genotype (P ≈ 0.591, OR=1.168, 95% CI=0.663–2.057) and PD1.3A/A genotype (P ≈ 0.748, OR=1.571, 95% CI=0.097–25.363) between RA cases and controls. The G or A allele frequency of the PD1.3 polymorphism did not show differences between the patients and the controls (P ≈ 0.541, OR=1.175, 95% CI=0.700–1.971). **Conclusion:** Our results showed that the PD1.3G/A polymorphism is not associated with RA susceptibility in Iranian patients.

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## IL-27 mRNA Expression in Multiple Sclerosis

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**Background & Objective:** Multiple sclerosis (MS) is a complex inflammatory, demyelinating disease of the CNS. Although the pathogenesis is incompletely understood, MS is believed to be a T cell-mediated autoimmune disease. Th1 immune responses were thought to mediate inflammatory demyelination in MS, however, there is growing evidence implicating IL-17-producing T helper cell population (Th17) in the pathogenesis of MS and other autoimmune diseases. IL-27 is a novel heterodimeric cytokine that up-regulate early phase of Th1 responses through IL-12R. However, more recent studies have demonstrated anti-inflammatory properties of IL-27 signaling. So the aim of this study was to investigate functional correlation between IL-27 gene expression and MS activity, we examined mRNA expression of IL-27 at the effector phase of MS. **Methods:** 35 MS patients (new cases) and 35 healthy subjects were evaluated for IL-27 mRNA expression. After total RNA isolation and cDNA synthesis, we used real-time (RT) PCR for evaluation of IL-27 mRNA. 18sRNA was used as internal control. **Results:** Our study showed 1.5% significant increase ($P< 0.005$) in IL-27 mRNA expression in MS patients compare to the healthy controls. **Conclusion:** IL-27 has pleiotropic roles in immune responses. Recent two studies demonstrated that IL-27 suppresses Th17 immune responses. Another study showed that expression of IL-27 and its receptor subunit, correlated with disease course in a chronic model of Experimental Autoimmune Encephalomyelitis ( EAE) . Denise et al (2007) showed that expression of IL-27 subunits was maximal during severe disease, declined during remission, and increased again when relapses began. This study demonstrates increased IL-27 mRNA level in active MS patients. Future studies will show the exact role of IL-27 in MS and whether we could use therapeutic potential of this Th17 suppressive cytokine in MS and other autoimmune diseases.

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# Prolactin Decreases Apoptosis of PBMCs in SLE Patients via Fas/Fas Ligand Pathway

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**Background & Objective:** SLE is a chronic autoimmune disease characterized by antibodies to nuclear antigens. SLE has a marked female predominance, with a 9:1 female to male ratio. Sex hormones, mainly estrogen and prolactin are implicated in modulating the immune response. The elevation of prolactin level can be seen in 20% of women with a high titer of anti-DNA antibody. A common feature of SLE is the breakdown of tolerance to self antigens. In patients with SLE, increased numbers of apoptotic lymphocytes and macrophage have been reported. This could result from defective signaling, execution, or burial phases of apoptosis, thus delaying completion of the death program. **Methods:** In this study we evaluated effect of prolactin on apoptosis of PMBC in lupus patients. The study group was comprised of 21 SLE patients and 20 healthy controls. Prolactin concentration of all study subjects was measured by ELISA. After treatment of PBMCs with different concentration of prolactin (10ng/ml & 40ng/ml) Flow cytometry analysis with annexin V and PI was performed. Then expression ratio of several gens, including Bcl2, Bax, Fas and Fas ligand was assessed by real time PCR. **Results:** Prolactin concentration of patients was higher than healthy controls ($P \approx 0.034$) and 24% of lupus patients had hyperprolactinemia. Apoptosis in lupus patients was higher than control group ($P< 0.005$). Prolactin inhibits apoptosis in lupus patients at both concentration, but increases apoptosis in healthy controls. There is no significant difference between two groups in Bcl2 and Bax expression. However, in control group, Fas and particularly Fas Ligand, has higher ratio of expression. **Conclusion:** These findings suggest that prolactin influence apoptosis via Fas/Fas ligand pathway and might lead in defect in self tolerance by inhibiting apoptosis of autoreactive thymocytes and immature B cells.

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# Detection Serum Levels of Anti-Ganglioside Antibodies in Children with Guillain-Barne Syndrome by EUROLIN EIA and Evaluation with ELISA

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**Background & Objective:** This study aimed to determine Serum levels of anti-Ganglioside antibodies in children Guillain-Barne syndrome by EUROLIN EIA and evaluation with ELISA method .**Methods:** In this study the 50 children with Guillain-Barre syndrome (GBS) between July 2006 to July 2008 were successively admitted to Tabriz Pediatric Hospital in the northwest of Iran were studied and 30 children who for various reasons other than GBS disease with random sampling as a control group Were selected . Quantity of antibodies measured by ELISA method using commercial kit factory Buhlman (Switzerland) and methods of western blot commercial kit was used German uroimmun . **Results:** Mean age of patients was 5.3±3.8 and the control group 5.4 ± 3.4 years. Ganglioside antibodies (IgG) by ELISA method in GBS patients and controls, respectively 16(32%) and 1 (3.3%) were diagnosed and antibodies against seven Ganglioside by western blot method (at least one) in 28 (56%) were found positive GBS patients while in the control group were all negative. Western blot and ELISA method sensitivity 56% and 32% and specificity respectively 100% and 97% was found (P< 0.001). **Conclusion:** specificity and sensitivity of two methods western blot and ELISA in comparison with clinical criteria of GBS was measured. Western blot method revealed specificity 100% and sensitivity 56%. It is noteworthy that measurement of the seven kinds of antibodies (GD1b, GT1b, GQ1b, GM1, GM2, GM3, GD1a) by western blot method could be done easily and with lower cost. Is recommended both IgM, IgG antibody against Ganglioside evaluate in the future.

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# Quantitative Analysis of IL-17A and IL-17F Gene Expression in Patients with Multiple Sclerosis

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**Background & Objective:** Multiple Sclerosis (MS) is considered as an immune-mediated inflammatory disease of central nervous system (CNS). Patchy demyelination and infiltration of mononuclear cells in CNS are the main pathologic characteristics of MS. Pro- inflammatory cytokines could have important role in MS pathogenesis. IL-17, as an inflammatory cytokine, supposed to have a key role in autoimmune diseases like MS. This study has been designed to show the role of IL-17A and IL-17F gene expression in MS patients. **Methods:** Blood samples from 35 MS patients (new cases) and 35 healthy subjects were collected. After total RNA isolation and cDNA synthesis, mRNA expression of IL-17A and IL-17 F, and 18sRNA (internal control) were evaluated by Real-time PCR. **Results:** Data analysis showed significant increase of IL-17A (2%) and IL-17 F (1.6%) mRNA expression in MS patients ($P< 0.005$). **Conclusion:** Recent studies demonstrated an IL-17-producer $CD4^+$ T cell subpopulation, termed Th17, distinct from Th1 and Th2.The predominant function of IL-17 is thought to be as a proinflammatory mediator. Locally, IL-17 stimulates production of IL-6, nitric oxide and prostaglandin E2 (PGE2) which have synergy with other inflammatory cytokines such as IL-1β, tumor necrosis factor (TNF)-α, IFN-γ. Increasing levels of IL-17A and IL-17F mRNA in our study, represent the important inflammatory effects of these IL-17 subpopulations in pathogenesis, diagnosis and treatment follow up of MS.

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# Study of the Cytokine Secretion Pattern by Treatment of Fumaric Acid Esters in Peripheral Blood Lymphocytes of Multiple Sclerosis Patients

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**Background & Objective:** Fumaric acid esters (FAEs) have been reported to induce a Th1 to Th2 shift as one of their therapeutic effects in psoriasis patients. Objective: The present study was performed in order to investigate the effects of two metabolites of FAE including dimethylfumarate (DMF) and methylhydrogen fumarate (MHF) on the cytokine pattern of antigen- and mitogen-stimulated peripheral blood mononuclear cells (PBMCs) of multiple sclerosis (MS) patients. **Methods:** Blood samples of 20 RR-MS patients were obtained and the PBMCs were stimulated with myelin basic protein (MBP) as the major antigen involved in the pathogenesis of MS, and phytohemagglutinin (PHA) as a mitogen. Cells were cultured in the presence of 1 and 10µg/ml of DMF and MHF. The percentage of $CD4^+IL\text{-}4^+$ and $CD4^+IFN\text{-}\gamma^+$ cells was determined by means of intracellular cytokine staining using Flow cytometry. **Results:** This study showed that in MS patients, the percentage of $CD4^+IL\text{-}4^+$ cells was significantly increased in the presence of DMF and MHF when PBMCs were stimulated by MBP ($P< 0.05$). The same significant result was obtained by PHA stimulation ($P<0.05$). No significant difference was observed for the extent of increase in the percentage of $CD4^+IL\text{-}4^+$ between DMF and MHF. In terms of $CD4^+IFN\text{-}\gamma^+$ cells, the percentage of these cells did not significantly differ between the cultures stimulated with MBP or PHA in the presence and absence of the drugs. **Conclusion:** As the findings of the current study showed, both DMF and MHF effectively increased IL-4, whereas they did not significantly changed IFN-γ level, indicating the role of these drugs in immune deviation of Th1 toward Th2 cytokines. These results may show the beneficial effects of FAE metabolites in treatment of RR-MS patients.

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# Quantitative Analysis of TGF-β Gene Expression in Patients with Multiple Sclerosis

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**Background & Objective:** Multiple sclerosis (MS) is an immune-mediated inflammatory disease that attacks myelinated axons in the CNS, destroying the myelin and the axon in variable degrees. The cause of the disease likely involves a combination of genetic susceptibility and an environmental trigger. Activated T lymphocytes play an important role in the pathogenesis of multiple sclerosis (MS). These T cells secrete both pro- and anti- inflammatory cytokines. Transforming growth factor-beta (TGF-β) is a potent regulatory cytokine with diverse effects on hemopoietic cells. The pivotal function of TGF-β in the immune system is to maintain tolerance via the regulation of lymphocyte proliferation, differentiation, and survival. Defects in TGF-β expression or its signaling in T cells correlate with the onset of several autoimmune diseases. The decreased production of TGF-beta (anti-inflammatory cytokine) by lymphocytes in MS patients has been observed, which may play an important role in the mechanisms and manifestations of MS. The aim of this study was to evaluate the quantitative expression of Transforming growth factor-β in MS patients. **Methods:** 35 MS patients with Relapsing-Remitting and 30 healthy and ethnic matched controls were included in this study. Quantitative Real Time-PCR was performed to investigation of TGF-β and β-actin gene expression. **Results:** Data analysis showed that TGF-β (1.2%) mRNA expression in MS patients was not significantly different from healthy individuals ($P \approx 0.265$). **Conclusion:** In this study, the decreased expression of TGF-β gene in MS patients compared to controls did not observed .In summary, our data indicated that TGF-β maybe doesn't have influence in development of MS in this population. But to confirm this finding, other complete studies are required.

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# Quantitative Analysis of IL-8 Gene Expression in Patients with Multiple Sclerosis

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**Background & Objective:** Multiple sclerosis (MS) is an autoimmune disease of the central nervous system destroying myelin, oligodendrocytes, and axons. Combinations of genetic and environmental factors play important roles in the induction of MS. IL-8 plays important roles in CNS development, modulation of neuronal survival and excitability.IL-8 may play an important role in the trafficking of leukocytes to the CNS and may be involved in the pathogenesis of MS. Therefore, the aim of the present study was to investigate the mRNA expression of IL-8 in patients with Multiple Sclerosis. **Methods:** 35 MS patients with Relapsing-Remitting and 30 healthy and ethnic matched controls were included in this study. Total RNA was extracted from peripheral blood and cDNA synthesized. Then Real time-PCR was performed to investigation of IL-8 and β-actin gene expression. **Results:** Data analysis showed that IL-8 mRNA expression (1.6%) has been significantly increased in MS patients ($P \approx 0.005$).However, there was no significant association between different clinical findings (EDSS score, progression index, disease onset age) and IL-8 gene expression. **Conclusion:** Our data indicate that the higher expression of IL-8 maybe related to MS development. The upregulation of IL-8 in MS patients suggests that IL-8 cytokine should be evaluated as a potential diagnostic biomarker, and maybe in treatment follow up of MS. However, similar investigations in different populations are recommended to clarify the role of IL-8 expression in susceptibility to MS.

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# In Vitro Effects of Sodium Benzoate (NaB) on Production of IL-4 and IFN-γ by CD4+ Cells in Patients with Multiple Sclerosis (MS)

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**Background & Objective:** NaB is used as a therapeutic agent to treat the acute hyperammonaemia. Also the effect of NaB in deviation of immune responses toward the Th2 cells in EAE has been documented. In the present study we sought to determine the effect of NaB on Th1/Th2 balance in MS patients and healthy controls. **Methods:** The effect of different concentrations of the NaB (100, 500 and 1000 µg/ml) on the peripheral blood mononuclear cells of 20 relapsing remitting-MS patients and 8 healthy controls was evaluated in the presence of mitogen (PHA) or specific antigen (MBP). For determining the percent of cytokine producing cells, intracellular cytokine staining was performed. The samples were evaluated by FACScalibur flow cytometer. **Results:** Our result showed NaB can significantly increase the production of IL-4 in the presence of PHA and MBP in both patients ($P< 0.005$ and $P< 0.005$, respectively) and controls ($P< 0.05$ and $P< 0.05$ respectively). Also the percentage of $CD4^{+}IFN\text{-}\gamma^{+}T$ cells was $1.08 \pm 0.5$ after stimulation with PHA while addition of NaB (1000 µg/ml) to the culture results in reduction of the percent of these cells to $0.89 \pm 0.43$ ($P< 0.005$). **Conclusion:** Our data suggest that NaB, an approved preservative by FDA that can be administered simply by drinking water with no considerable cost, might be a useful candidate in treatment of relapsing remitting-MS.

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## Therapeutic Approach by Aloe Vera in Experimental Model of Multiple Sclerosis

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**Background & Objective:** Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) that leads to an inflammatory demyelination, axonal damage, and progressive neurologic disability that affects approximately 2.5 million people worldwide. The aim of the present research was to test the therapeutic effect of Aloe Vera in experimental model of multiple sclerosis. **Methods:** All experiments were conducted on C57BL/6 male mice aged 6-8 weeks. To induce the experimental autoimmune encephalomyelitis (EAE), 250 µg of the MOG 35-55 peptide emulsified in CFA was injected subcutaneously on day 0 over two flank areas. In addition, 200 ng of pertussis toxin in 100 µl PBS was injected i.p on days 0 and 2. The therapeutic protocol was carried out intragastrically using 120 mg/kg/day Aloe Vera from 7 days before to 21 days after EAE induction. The mice were sacrificed 21 days after EAE induction. The brains of mice were removed for histological analysis and their isolated splenocytes were cultured. **Results:** The results indicated that treatment with Aloe Vera caused a significant reduction in severity of the disease in experimental model of MS. Histological analysis showed 3 ± 2 plaques in Aloe Vera treated mice compared with 5 ± 1 plaques in control group. The density of mononuclear infiltration in the CNS of Aloe Vera treated mice (500 ± 200) was significantly less in comparison to 700 ±185 cells in control group. Moreover, the serum level of Nitric Oxide (NO) in treatment group was significantly less than control animals. The level of IFN-γ in cell culture supernatant of treated mice splenocytes was lower than control group, whereas decrease in serum level of IL-10 in treatment group was not significant in comparison with control mice. **Conclusion:** These data indicate that Aloe Vera therapy can attenuate the disease progression in experimental model of MS.

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# Islet Cell Autoantibodies in Patients Younger than 20 Years of Age with Recently Diagnosed Diabetes in Northwest of Iran

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**Background & Objective:** Type 1 diabetes mellitus (T1D) is a heterogeneous disorder, resulting in most cases from an autoimmune mediated destruction of pancreatic β-cell, which leads to an absolute insulin deficiency. It is well known that genetic as well as environmental factors contribute to the pathogenesis. Glutamic acid decarboxylase (GAD) is one of the autoantigens that trigger β-cell specific autoimmunity. Islet cell autoantibodies (ICA) has been utilized to diagnose type 1 diabetes because this antibody circulates in most patients before and at the onset of the disease, and frequently declines following diagnosis of disease; but GADAs appear to remain positive for long periods of time. The objective of the present study was to assess the prevalence of β-cell autoantibodies such as glutamic acid decarboxylase-65 antibodies (GADAs) and islet cell antibodies (ICA) among patients younger than 20 years of age with recently diagnosed diabetes in northwest of Iran. **Methods:** From 2006-2008, 163 patients were enrolled in this study. They were clinically classified into two groups: 136 with Type 1 diabetes (T1D) and 27 with Type 2 diabetes (T2D). Serum levels of GADAs, ICA and C-peptide were determined with enzyme linked immunosorbent assay (ELISA) kits. Fasting blood glucose and HbA1c levels were also determined. **Results:** The prevalence of GADAs in T1D patients was 33.1%, slightly lower than that of ICA 35.3%. Forty-eight patients (35.3%) with T1D were positive for ICA compared to only one (3.7%) in T2D patients. The overall occurrence of any autoantibody in T1D patients (60.3%) was significantly higher than that of T2D patients (18.5%) ($P< 0.001$). There was a statistically different association with family history of diabetes among the autoantibody positive versus autoantibody negative patients with T1D ($P< 0.01$). **Conclusion:** The prevalence of autoantibodies in Iranian patients with T1D is very similar to that reported for diabetic patients in other non-Caucasian ethnic groups.

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# Association of the FCRL3 Gene Polymorphism with Rheumatoid Arthritis in Iranian Patients

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**Background & Objective:** Rheumatoid Arthritis (RA) is a complex disease, the hallmark of which is synovial joint inflammation. The heritability of RA has been estimated to be in the order of 60%, suggesting a substantial contribution for genetic factors. The FC receptor-like (FCRL-3) gene is one of the genes that recently have shown a significant association with RA. The objective of this study was to determine the possible role of FCRL3-3(-169C/T) and FCRL3-4(-110A/G) gene polymorphisms in development of RA in Iranian patients. **Methods:** FCRL3-3 polymorphism was investigated in 356 patients with RA and a control group consisting of 272 healthy subjects. FCRL3-4 polymorphism was assessed in 286 patients and 310 healthy normal subjects. DNA was extracted from peripheral blood mononuclear cells and genotyping was performed using PCR-RFLP method. **Results:** A significant difference between the patients and controls in FCRL3-4 genotype was observed. The frequency of genotypes in patients were AA 65.73% , AG 32.17 % and GG 2.1%, whereas in healthy control it was AA 57.1% , AG 34.83 % and GG 8.07% (P< 0005). There was also a significant difference between allele frequencies in controls and patients (P< 0.05). No significant difference in the genotype distribution and allele frequencies of FCRL3-4 between patients and controls was found. **Conclusion:** Results of this study showed an association between FCRL-3 polymorphism at position -110A/G and susceptibility to RA in Iranian patients, indicating the role of FCRL-3 in development of RA.

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# Antiapoptotic Effects of Dehydroepiandrosterone (DHEA-s) in Lymphocytes from Systemic Lupus Erythematosus (SLE) Patients

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**Background & Objective:** Apoptosis or programmed cell death excessively conserved among all cells and tissues. Patients with SLE have excessive amount of apoptotic dying cells in peripheral blood and recently suggested that sex hormones are related with the quantity of apoptosis. The abnormal sexually dimorphic prevalence of autoimmune diseases such as SLE suggests that sex hormones are important role and involved in the pathogenesis of SLE. Sex hormones may have various protective and apoptotic effect for peripheral blood cells one of them is (DHEA-s). Dehydroepiandrosterone (DHEA) and its sulfated ester (DHEAs) are among the most abundant steroid hormones synthesized by the adrenal glands and brain. These hormones have many biological effects and diverse influences in various types of cells, tissues and organs. Dehydroepiandrosterone may have immunomodulatory role as well as positive effects on PBL cells. However, the detailed molecular mechanism and signaling pathways of DHEA(s) have not yet been clarified. In this study, we investigated and compared the apoptotic effects of these hormones on PBL cells from SLE patients and healthy controls in cell culture. **Methods:** 20 SLE patients and 20 age and sex matched healthy controls participated in this study. PBL cells isolated and treated with 7.5 µmol of DHEAs for 24h in cell culture medium. Apoptosis measured by flowcytometry, using FITC conjugated Annexin V and Propidum Iodide (PI). **Results:** In the presence of DHEAs, the percent of FITC+/PI–cells which correlated to early apoptotic cells in SLE patients was decreased with $P<0.05$ whereas in healthy controls there was not considerable alteration. **Conclusion:** DHEAs decreased the percent of early apoptotic cells in SLE patients, but not in healthy controls. DHEAs may be a promising chemopreventive drug for. Systemic lupus erythematosus (SLE) patients and other autoimmune diseases.

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# Quantitative Analysis of Gene Expression of IL-27 in Patients with Multiple Sclerosis

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**Background & Objective:** Multiple sclerosis (MS) is considered an immune-mediated inflammatory disease of central nervous system (CNS). Patchy demyelination and infiltration of mononuclear cells in CNS are characteristic for MS. Cytokines are soluble proteins that are involved in the regulation of immune responses. Increased systematic as well as intrathecal production of both pro- and anti-inflammatory cytokines is a common findings in MS. In this experiment we studied the mRNA expression of IL-27 cytokine in MS patients with quantitative RT PCR. **Methods:** Blood samples were collected from 35 MS patients (new case) and 35 healthy subjects. After total RNA isolation and cDNA synthesis real-time PCR was done in 20 µl volume/well for IL-27 gene and 18sRNA (internal control). **Results:** Data analysis showed that IL-27 (1.5%) mRNA expression has been significantly increased in MS patients ($P< 0.005$). **Conclusion:** Our results showed the importance of this cytokine in diagnosis and treatment follow up of MS.

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# Quantitative Analysis of Gene Expression of IL-17 in Patients with Multiple Sclerosis

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**Background & Objective:** Multiple sclerosis (MS) is considered an immune-mediated inflammatory disease of central nervous system (CNS). Patchy demyelination and infiltration of mononuclear cells in CNS are characteristic for MS. Cytokines are soluble proteins that are involved in the regulation of immune responses. Increased systematic as well as intrathecal production of both pro- and anti-inflammatory cytokines is a common findings in MS. In this experiment we studied the mRNA expression of IL-17A and IL-17F cytokines in MS patients with quantitative RT PCR. **Methods:** Blood samples were collected from 35 MS patients (new case) and 35 healthy subjects. After total RNA isolation and cDNA synthesis real-time PCR was done in 20 µl volume/well for IL-17A and IL-17F genes and 18sRNA (internal control). **Results:** Data analysis showed that IL-17A (2%) and IL-17F (1.6%) mRNA expression has been significantly increased in MS patients ($P< 0.005$). **Conclusion:** Our results showed the importance of these cytokines in diagnosis and treatment follow up of MS.

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# Quantitative Analysis of Gene Expression of IL-23 in Patients with Multiple Sclerosis

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**Background & Objective:** Multiple sclerosis (MS) is considered an immune-mediated inflammatory disease of central nervous system (CNS). Patchy demyelination and infiltration of mononuclear cells in CNS are characteristic for MS. Cytokines are soluble proteins that are involved in the regulation of immune responses. Increased systematic as well as intrathecal production of both pro- and anti-inflammatory cytokines is a common findings in MS. In this experiment we studied the mRNA expression of IL-23 cytokine in MS patients with quantitative RT PCR. **Methods:** Blood samples were collected from 35 MS patients (new case) and 35 healthy subjects. After total RNA isolation and cDNA synthesis real-time PCR was done in 20 µl volume/well for IL-23 gene and 18sRNA (internal control). **Results:** Data analysis showed that IL-23 (1.8%) mRNA expression has been significantly increased in MS patients ($P< 0.005$). **Conclusion:** Our results showed the importance of this cytokine in diagnosis and treatment follow up of MS.

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# Survey of Relation between the Onset Age of Type 1 Diabetes and Celiac Disease in Children and Adolescents

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**Background & Objective:** Celiac disease (CD) is a chronic enteropathy caused by hypersensitivity to gluten. Most studies have showed more prevalence of CD in the patients with Diabetes Mellitus type 1. Both diseases are autoimmune and their incidence is related to inheritance and environmental factors. The aim of this study is the survey of relation between CD prevalence and diabetic age onset. **Methods:** In a cross-sectional descriptive study, 135 children with Diabetes Mellitus type 1 referring to Tabriz children hospital endocrine department and clinic between 2006 and 2008 were selected. After filling individual identity of patient and the measurement of weight and height, the serum level of anti-tissue Trans glutaminase IgA antibody (A-tTG-A-IgA), anti endomisial IgA antibody (AEA-IgA) and anti-gliadin IgG antibody (AGA-IgG) were measured. In the case that A-tTG-A either AEA alone or with AGA was high, small intestinal biopsy was preformed. The data was analyzed using SPSS, version 16 software. **Results:** 28 of 135 patients with diabetes mellitus type 1, were serologically positive for celiac. Confirmed celiac prevalence based on biopsy was 6.8%. From diabetic age onset and celiac incidence point of view there was not any significant relation (P≈ 0.996). **Conclusion:** Celiac Disease in type1 diabetic patients dose not have correlation with the onset age of type 1 diabetes and diabetic patients should be followed up from celiac point of view during treatment and prevention.

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# Prevalence Survey of Anti-Tissue Transglutaminase and Anti-Endomesial Antibody in Type 1 Diabetic Children and Adolescents

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**Background & Objective:** Colorectal cancer is a major cause of world wide morbidity & mortality and is the second most common cause of cancer death. Colorectal cancer is often diagnosed at a late stage with poor prognosis. Vascular endothelial growth factor (VEGF) is a neo-angiogenesis with great importance for tumor growth, which has a direct effect on vascular endothelial cell proliferation and migration. C3a is also diagnostic factor in determining colon cancer. The aim of the study was to measure the VEGF and C3a level in patients with colorectal cancer. **Methods:** One hundred and nine patients with colorectal cancer, including 64 Men and 45 women. (At an average age of 54 years) were enrolled into the study. VEGF and C3a level of 109 patients with colorectal cancer were determined using ELISA method. Only 55 Patients with elevated serum VEGF and C3a were followed up after 3 months, because of death of the rest. **Results:** our results demonstrate that VEGF is a suitable diagnostic tumor marker in patients with colorectal cancer. A combination of the serum tumor markers C3a and VEGF can significantly increase the pre-operative diagnostic. VEGF and C3a serum level slowed significantly difference pre- and post – treatment [(mean 385.7 pg/ml, 262.2 pg/ml; 2.2 ng/ml, 1.8 ng/ml) ($P< 0.001$) ($P< 0.005$)]. **Conclusion:** both VEGF and C3a are useful markers to predict future metastasis, survival, and response to the treatment.

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# Quantification of Regulatory T Cells in Peripheral Blood of Patients with Systemic Lupus Erythematosus

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**Background & Objective:** Regulatory T cells (Tregs) are supposed to stop immune responses in the course of immune activation. However, chronic activation of immune system in systemic lupus erythematosus (SLE) and many other autoreactive disorders are evidence of mal-function of this system. Therefore, it is plausible to quantify presence of these cells in different diseases. **Methods:** Forty and one patients with diagnosis of SLE were enrolled in this study. Patients were divided into two groups of patients with active and inactive disease based on the disease activity score. Flow cytometry analysis was used to determine the frequency of regulatory T cells in peripheral blood according to high expression of CD25 and intracellular Forkhead/winged-helix, (Foxp3). Further 30 healthy individuals considered as control group. **Results:** Significantly less $CD4^{+}CD25^{hi}$ regulatory T cells were detected in active patients compared to healthy individuals. The percentage of $CD4^{+}CD25^{hi}$ cells was inversely correlated with the SLEDAI disease score in patients with active disease. Patients with active disease had lower frequencies of $CD4^{+}Foxp3^{+}$ cells. However, increased frequencies of $CD4^{+}Foxp3^{+}$ T cells were observed in peripheral blood of patients with inactive disease compared with active patients or healthy individuals. Moreover, a significant difference between the proportion of $CD4^{+}CD25^{-}Foxp3^{+}$ population in healthy controls and patients with active disease was shown. **Conclusion:** Presence of lower frequencies of Tregs in patients with SLE could be evaluated as an immune turbulence and could be employed as a target for immunotherapeutic manipulation. However, controversies need to be resolved.

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# Investigation of IL-17 and COX2 Genes Expression in Peripheral Blood Leukocytes of Vitiligo Patients

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**Background & Objective:** Vitiligo is a pigmentation disorder which inflammatory mediators such as cytokines have a pivotal role in disease's pathogenesis. Previous studies have demonstrated that there are increased expressions of proinflammatoty cytokines in the skin and peripherals blood of patients with vitiligo. Interleukin 17 (IL-17A) is a proinflammatory cytokine which is involved in the induction of several proinflamatory mediators such as COX2. The aim of this study was to investigate the gene expression of IL-17 and COX2 in peripheral blood leukocytes of vitiligo's patients. **Methods:** peripheral blood leukocytes of 15 patients with vitiligo and 15 healthy controls was separated using gradient density centrifugation method. After total RNA isolation and cDNA synthesis, IL-17 and COX2 gene expression was quantified by Real-Time RT-PCR. **Results:** There were no significant differences in IL-17 and COX2 genes expression in lymphocytes of patients with vitiligo compared with control group ($P< 0.05$). However there was an increased IL-17 and COX2 genes expression in neutrophils of patients than controls, but it did not reach to the significant level ($P< 0.05$). We could not find any differences in IL-17 and Cox2 gene expression between clinical diseases subtypes, sex and age. There was a significant relationship between IL-17 and COX2 genes expression in the neutrophils of patients ($P< 0.01$, $r=0.80$). **Conclusion:** Although in the present study we found not find any significant increases in IL-17 and COX2 genes expression in peripheral blood leukocytes of vitiligo patients compared with control group, however our results showed that an increased expression in IL-17 and Cox-2 genes in neutrophils of patients with vitiligo, , suggesting these two factors are involved in inflammatory process. Further studies with large sample sizes, might help to clarify the role of these factors in the pathogenesis of diseases.

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# Association of Immunoreceptor Programmed Cell Death 1 (PD-1) -538G/A and 7146 G/A Polymorphism with Susceptibility to Multiple Sclerosis (MS) in Iranian Patients

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**Background & Objective:** PD-1 exerting inhibitory functions on T-cells and plays a crucial role in attenuating T-cell responses in EAE (animal model of MS). Several functional single nucleotide polymorphisms were reported in PDCD1 gene. The aim of the present study was to investigate the association of functional pd-1 -538 G/A and pd-1 7146G/A polymorphisms with susceptibility and prognosis of multiple sclerosis. **Methods:** Two hundreds and seventy nine MS patients and 300 ethnic matched controls were included in the present study. Genotyping was performed using polymerase chain reaction—restriction fragment length polymorphism (PCR-RFLP). **Results:** The results of the present study showed a significant difference in distribution of pd-1 7146G/A genotypes between cases and controls ($P < 0.00005$), while no differences was observed between genotypes of pd-1 -538 G/A polymorphisms in patients compared to controls ($P \approx 0.76$). Moreover, there were no association between above mentioned polymorphisms and disease types, age at disease onset and progression index. **Conclusion:** It could be concluded that the functional pd-1 7146G/A polymorphisms might affected the susceptibility to multiple sclerosis in Iranian patients.

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# Association of Proliferator-Activated Receptor-Gamma (PPAR-γ) PRO12ALA Polymorphism with Susceptibility to Multiple Sclerosis (MS)

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**Background & Objective:** PPARs are members of a family of nuclear receptor transcription factors which act in the regulation of immune and inflammatory responses. Among them the expression in central nervous system and lymphoid organs are also well documented and its product is generally involved in suppression of genes encoding pro-inflammatory molecules. Therefore, Pro12Ala polymorphism in the PPAR-γ gene could be considered as a proper candidate gene for susceptibility to MS. **Methods:** Two hundreds and fifty four Iranian patients with an unequivocal clinical diagnosis of MS according to McDonald criteria along with 217 ethnic matched healthy controls were enrolled randomly in this study. Pro12Ala polymorphism in the PPAR-γ gene was determined using PCR-RFLP method. Genotype distributions were compared using the $\chi 2$- test. **Results:** A significant difference in the distribution of Pro12Ala polymorphism was observed between cases and controls ($P< 0.05$) while no significant association was determined between this polymorphism and different clinical forms of MS, disease age of onset, Expanded Disability Status Score (EDSS) and progression index. **Conclusion:** Significant increases in GG genotype in patients compared to controls resulted in decreases expression of PPAR-γ and increase the chance of susceptibility to MS.

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# Annexin V and Anti-Annexin V Antibodies: Two Interesting Aspects in Acute Myocardial Infarction

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**Background & Objective:** Myocardial infarction is the combined result of environmental and personal factors. Prothrombotic factors might play an important role in this phenomenon. Annexin V (ANV) is a calcium-dependent glycoprotein widely present in various tissues exerting a potent anticoagulant effect in vitro by reducing plaque adhesion and aggregation. Anti-Annexin V antibodies (aANVAs) are detected in various diseases like rheumatoid arthritis, systemic lupus erythematosus and anti-phospholipid antibody syndrome. The study of ANV in Acute Myocardial Infarction (AMI) might shed light on hypercoagulability mechanisms in the pathogenesis of acute coronary syndromes. This study was conducted to investigate the association of plasma ANV, aANVAs and anti-cardiolipin antibodies (aCLAs) with AMI. **Methods:** This study recruited 45 patients with the diagnosis of AMI according to WHO criteria in their first 24 hours of admission. 36 matched individuals were studied as the control group with normal coronary artery angiography. Plasma levels of ANV, aANVAs and aCLAs were determined by enzyme-linked immunosorbent assay and the results were compared. **Results:** Plasma ANV levels in the patients with AMI on admission were significantly lower than those in the control group ($P< 0.005$). Positive test for aANVAs were found to be present in a significant number of our patients ($P< 0.005$). The studied groups were similar in their rate of patients with positive aCLAs tests. ANV, aANVAs and aCLAs were not correlated with hypertension, diabetes mellitus, hyperlipidemia, sex, age and smoking. **Conclusion:** Our findings suggest that low plasma ANV levels along with positive aANVAs tests in patients with AMI are indicative of hypercoagulable state that is not related to the traditional cardiovascular risk factors.

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## Anticardiolipin Antibody in Acute Myocardial Infarction

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**Background & Objective:** Bipolar disorder (BD) is a chronic severe mood disorder that has been consistently demonstrated to have a strong inherited component. IL-1RA protein has been widely investigated and found to be associated with different human neurodegenerative disease. The aim of this study was to investigate IL-1RN genotype and its associations with different phases of BD disease in a group of Iranian subjects. **Methods:** Totally, 135 patients meeting DSM-IV-TR criteria for bipolar disorder and 182 controls matched to the cases on age, sex and geographic population were admitted to the study. The severity of disorder was assessed by using HDRS and YMRS for depression and mania phases, respectively. IL-1RN polymorphism was analyzed by amplifying the polymorphic region using SSCP- polymerase chain reaction. **Results:** IL-1RN1/2 heterozygote genotype was more prevalent in BD patients than controls (45.2% vs. 30.8%, P< 0.05). Further stratification of the BD patients into acute and chronic disease subgroups revealed a strong association between IL-1RN1/2 heterozygous and chronicity of disease (OR 3.8; 95% CI (1.8-7.9); P< 0.005) and also in subjects with chronic depression phase [(OR 8.4; 95% CI (2.5-27.8); P< 0.0005)]. **Conclusion:** These findings suggest that the IL-1RN (86bp) n polymorphism might be a genetic susceptibility risk factor for bipolar disorder in an Iranian population.

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## BAFF, a Promising Target for Treating Autoimmune Diseases

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**Background & Objective:** Since BAFF has been identified as a critical factor for B cell maturation and survival, convincing evidence indicates that deregulation of BAFF is involved in pathogenesis of B-cell related autoimmune diseases. Therefore, BAFF can be a promising therapeutic target for treating autoimmune diseases. The antagonists of BAFF can take the forms of monoclonal antibodies against BAFF or BAFF receptor-IgG Fc fusion proteins. Blockade of BAFF activity significantly improves the symptoms of autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis both in animal models and clinical trials. Therefore, BAFF-targeting therapy is a promising approach to treat B-cell related autoimmune diseases. **Methods:** We had previously expressed and purified human BAFF (hBAFF), and obtained one hybrid that produced the monoclonal antibody by immunizing mice with the recombinant BAFF. **Results:** Although this mAb could recognize both recombinant and membrane-bound BAFF, it failed to neutralize BAFF activity. **Conclusion:** It is significant to screen more hybrids and obtain neutralized anti-BAFF mAbs.

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# Serum and Cerebrospinal Fluid Uric Acid Levels in Multiple Sclerosis Patients

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**Background & Objective:** According to the critical role of the free radicals like nitric oxide (NO), peroxynitrite (PN) in pathogenesis of multiple sclerosis (MS) and the uric acid (UA) function as strong scavenger of these free radicals. It is proposed that the levels of UA in serum (serum/UA) and in cerebrospinal fluid (CSF/UA) of patients can be important in pathogenesis and treatment of MS. **Methods:** therefore, we measured serum and cerebrospinal fluid uric acid levels in 12 new case of patients with MS (mean age 28.83 ± 0.99, range 24-37, 4 male, 8 female) and 10 age and gender matched controls (mean age 27.65 ± 0.81, range 25-35, 4 male, 6 female). **Results:** results showed that mean of serum/UA (3.32 ± 0.31mg/dl) level was significantly (P< 0.001) decreased when compared with the control (5.52 ± 0.27mg/dl). CSF/UA levels (0.15 ± 0.03mg/dl) in MS patients in compared with controls (0.24 ± 0.03mg/dl) were also decreased (P< 0.01). **Conclusion:** It is concluded that UA may protect against the development of MS and support hypothesis that higher serum and CSF uric acid might be beneficial in the future treatment of MS.

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# Association of CTLA4 Gene Polymorphism in Iranian Patients with Ankylosing Spondylitis

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**Background & Objective:** Cytotoxic T-lymphocyte antigen 4 (CTLA-4) is a costimulatory molecule expressed by activated T cells. This study was performed to investigate the allele and genotype frequencies of CTLA4 gene polymorphisms in Iranian patients with ankylosing spondylitis (AS). **Methods:** One hundred and fifty-seven patients with AS and 103 controls were included in this study. Polymorphisms of CTLA4 gene at positions +49 (in exon 1), -318, and -1,147 (in the promoter region) were studied on the genomic DNA using PCR restriction fragment-length polymorphism method. **Results:** The frequencies of the T allele at position -1147 in the patients with AS was significantly increased in comparison with the control group (11% vs. 5%, P< 0.005); whereas the frequencies of C allele at the same position were significantly decreased in the patient group (89% vs. 95%, P< 0.005). Comparison of genotype frequencies at this position showed that the frequency of CT genotype in comparison with other genotypes was overrepresented in the patient group (20% vs. 8%, P< 0.05), while the CC genotype in comparison with other genotypes was decreased (79% vs. 91%, P< 0.05). There was no significant difference on frequencies of genotypes at the positions -318 and +49. **Conclusion:** This study could suggest an association between specific allele in the promoter region of CTLA4 gene and AS disease.

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# Administration of Isoproternol, a β-Adrenoceptor Agonist, Prevents the Development of Multiple Low dose Streptozotocin Induced Diabetes Mellitus in BALB/c Mice

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**Background & Objective:** Insulin Dependent Diabetes Mellitus (IDDM) is an autoimmune disease that is believed to be resulted from shifting of immune responses toward Th1 pattern and production of pro-inflammatory cytokines. Recent trends in the treatment of this disease have been to reverse the imbalance toward the Th2 immune responses and to decrease the production of pro-inflammatory cytokines. In the other hand, lymphocytes express β-adrenoceptors and stimulation of these receptors causes a shift towards the Th2 profile. Thus, the aim of this study was to evaluate the effect of a β-adrenoceptor agonist (isoproterenol) administration on the development of Multiple Low Dose Streptozotocin (MLDS) induced diabetes mellitus (a model for IDDM) in mice. **Methods:** Twelve BALB/c mice were divided into two groups: the treatment group and the control group. MLDS was induced by the injection of streptozotocin for five consecutive days in all mice. The treatment group received isoproterenol (3mg/kg BID) for 12 days (1 day before, 5 days along with and 6 days after the induction) and the control group received normal saline for the same period. Fasting blood sugar (FBS) was measured before the induction and on days 14 and 21. Blood was drawn for assessment of serum concentration of TNF-α on day 21. **Results:** The level of FBS was significantly lower in the treatment group than that of the control group. Furthermore the mice of the treatment group had less serum TNF-α than that of the control group. **Conclusion:** Up to our knowledge, this is the first study showed administration of a β-adrenoceptor agonist could prevent the development of MLDS induced diabetes mellitus in mice most likely through decreasing the production of pro-inflammatory cytokines.

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# Association of Serum Levels of IL-17 with Eczema and Xerosis in Sulfur Mustard Exposed Patients

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**Background & Objective:** Sulfur mustard (SM) is one of a class of vesicant chemical warfare agents with the ability to form blisters on the exposed skin. skin tissue is at a high risk when exposed to SM. IL-17 is a pro-inflammatory cytokine that shown to be mainly produced by activated $CD4^+$ cells to induce the secretion of variety of cytokines and chemokines. The aim of this study was to evaluation of correlation between serum levels of IL-17 and skin complications such as eczema and xerosis in SM exposed patients 20 years after exposure. **Methods:** In a historical cohort study, Sardasht-Iran Cohort Study (SICS), 343 SM exposed participants were studied 20 years after exposure and were compared with 124 control participants. All participants were examined by a dermatologist, and the serum concentrations of IL-17 were measured by ELISA technique. **Results:** Xerosis and eczema were the most incident skin problems in this study (eczema: 27.6%, xerosis: 24.6%). In comparison of serum levels of IL-17 between eczematous SM exposed and nonexposed subjects decreased levels of IL-17 ($P< 0.05$) was seen in exposed group. However in exposed subjects without eczema there were lower levels of IL-17 ($P< 0.005$) compared to control group. In exposed subjects without xerosis there were lower levels of IL-17 ($P< 0.005$) compared to control group but there was no significant difference between exposed and nonexposed subjects with xerosis. **Conclusion:** we cannot speculate on the specificity of these results, and further investigations will be required to define the role of serum IL-17 in the pathogenesis and their correlation with clinical severity of eczema and xerosis in participants that have exposed with SM.

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# The Effect of MS14 on IL-1β, IL-2 and IL-10 Production and DTH Test of BALB/c mice

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**Background & Objective:** The immune system intervenes in etiology and pathophysiology of many diseases, and carries out its application by producing various mediators including different cytokines. INF-γ, IL-2, and IL-12 are the major cytokines produced by Th1 cells, contributing to cell-mediated inflammatory immune responses. IL-10 had been grouped as Th2- type cytokine, but it is now considered as regulatory cytokine. During inflammation, injury, immunological challenge or infection, IL-1β is produced and contributes to the inflammatory response. DTH reactions are antigen-specific, cell-mediated immune responses which, depending on the antigen involved could be beneficial or harmless. In this study the effect of MS14, an herbal – marine preparation, were studied on IL-1β, IL-2, IL-10 and DTH in BALB/c mice in vivo. **Methods:** MS14 have been orally administered (100 mg/kg) to 6-8 weeks old female BALB/c mice for 5 days. Peritoneal macrophages and spleen lymphocytes were obtained from mice and cultured. PMA+LPS and ConA were used to stimulation macrophages and splenocytes respectively. After 21 h and 43h, levels of IL-1β, IL-2, IL-10 in supernatant of peritoneal macrophages and spleen lymphocytes culture assayed by ELISA kit. DTH was measured 24 h after the last immunization of mice. **Results:** MS14 at the dose of 100 mg/kg for 5 days induces a decrease in IL-1β production of peritoneal macrophages at the presence and absence of stimulator (70%, 50% decrease respectively). It has also caused about 20%reduction of IL-2 production of spleen lymphocyte at the presence of ConA. However significant increase was observed in IL-10 production of lymphocytes at the presence of ConA. MS14 had no effect on DTH test. **Conclusion:** According to diverse functions of studied cytokines, we can conclude that MS14 acts as an immunomodulator toward Th2 responses and possess anti-inflammatory properties.

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# Cloning of Human Interferon-γ1 (IL-29) cDNA from Monocyte- Derived Dendritic Cells and Investigation of Its Expression and Bioactivity

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**Background & Objective:** Type III Interferons (IFNs) are novel members of the interferon family, which contains three ligands: IFN-γ1(IL-29), IFN-γ2(IL-28A) and IFN-γ3(IL-28B).These three ligands use the same unique heterodimeric receptor which is completely different from type I & type II IFN receptors while they share many functional characteristics with IFN-α/β. Like type I IFNs, they are expressed in response to virus and TLR agonists and exhibit several features such as antiviral, antiproliferative, antitumor and immunomodulatory activities. Dendritic cells are an important source of IFN secretion. In this study, Monocyte-derived Dendritic cells as one of human DC subsets, utilized to clone the IFN-γ1 cDNA and subsequent experiments performed to confirm the expression and bioactivity of this cytokine. Materials and **Methods:** PBMCs were isolated from human blood by Ficoll Paque density gradient centrifugation. Monocytes were isolated and incubated with GM-CSF and IL-4 for 5 days, then LPS added as DC maturation factor. Flow cytometry analysis using CD1a and CD83 antibodies performed to confirm maturing DCs. Total RNA was extracted from maturing Monocyte-derived DCs by Qiazol reagent and cDNA was synthesized thereof.PCR was carried out applying specific primers designed for IFN-γ1.PCR product was cloned into the pTZ57R/T cloning vector and subsequently sub-cloned into eukaryotic expression plasmid pcDNA3.1+ under CMV promoter so called as pcDNA3.1+/ IFN-γ1. Identifying expression of the construct, pcDNA3.1+/ IFN-γ1 was transfected into HEK-293T cell line. Medium containing secreted IFN-γ1 was collected 36 and 72 hours post infection and quantified using ELISA development kit. To assess whether secreted cytokine is active biologically, the induction of typical IFN-induced genes, MxA and 2',5'-OAS, measured by real-time PCR in A549 cell line known as a responsive cell line containing specific receptor for IFN-γ1. **Results & Conclusion:** Successful cloning, expression and confirmed bioactivity of IFN-γ1 gene can be the first step for further investigations related to several and unique biological characteristics of this new discovered cytokine and obtaining new therapeutic approaches for cancer, viral infections, autoimmune and allergic disease.

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# Transforming Growth Factor-Beta2 Levels in Human Ovarian Cancer Cell Cultures Treated with Aques and Alcoholic, and Essential Oil of *Trachyspermum copticum* (L.) Link (Zenian)

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**Background & Objective:** *Trachyspermum copticum* (L.) Link (called Zenian in Persian) is a native plant to Iran, Egypt, Afghanistan and India and its aqueous and alcoholic extracts exert cytotoxic effects on human ovarian cancer cell cultures. Our aim was to study the Transforming Growth Factor-beta2 (TGF-beta2) levels in supernatant of human ovarian cell [A2780, sensitive and resistant to Cisplatin] cultures treated with aqueous and alcoholic, and essential oil of dried seeds of *Trachyspermum copticum* (L.) Link. **Methods:** Human ovarian cancer cells (S and CP) treated with different concentrations (from 50 to 7000 µg/ml) of aqueous and alcoholic extracts. Using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide test (MTT) and calculating cytotoxic percentage of each extracts after overnight incubation, non cytotoxic concentrations of extracts were selected. Non cytotoxic concentrations of aqueous extract for treatment of sensitive cells were 50, 100, 200, and 1000 µg/ml; and for resistant cells were 50, 100, 200, and 2000 µg/ml. Non cytotoxic concentrations of alcoholic extract for treatment of sensitive and resistant cells were 50, 100, and 200 µg/mL. Essential oil excluded from the study because of its extremely cytotoxic effects on cells even with very low concentrations. TGF-beta2 concentrations in supernatants were measured using enzyme-linked immunosorbent assay (ELISA). Results were analyzed with comparing mean test. P value less than 0.05 was considered statistically meaningful. **Results:** Treatment of sensitive cells with 200µg/ml, and resistant cells with 50 and 2000 µg/ml of aqueous extract associated with very less concentrations of TGF-beta in supernatants comparing with other extract concentrations ($P< 0.001$). But there was no meaningful association between the treatment of cells with alcoholic extract and TGF-beta2 concentrations in supernatants. **Conclusion:** Treatment of human ovarian cancer cells (A2780, sensitive and resistant to Cisplatin) with some concentrations of aqueous extract associated with diminished levels of TGF-beta2 in supernatants comparing other concentrations. Considering critical role of TGF-beta2 in growth and metastasis of tumor cells, further studies for determining the chemical components of aqueous extract and mechanism(s) of its effects on TGF-beta2 levels should be done.

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# IL-4C-590T Gene Variants in Patients with Gall Stone

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**Background & Objective:** Gallstone is a common biliary disorder that etiology may be multifactorial in some cases with several risk factors. Immune responses and inflammatory cytokines are important in this disease and cytokines has been detected in bile fluid. IL-4 is a Th2 cytokine could regulate inflammatory and cellular immune responses. Polymorphism in IL-4C-590T promoter can affect cytokine production and probably play a role in gall stone formation. **Methods:** Population studied in this study were 87 unrelated gallstone patients with clinical and laboratory characteristics. 120 normal subjects were matched as age and ethnicity with patient group. DNA extraction was done and for evaluation of polymorphism of IL-4 in -590 position, PCR-RFLP method by AvaII restriction enzyme was used. **Results:** There were not a significant difference in distribution of IL-4C-590T polymorphism between patient group and normal control group. Statistical analysis indicated each TT, CT and CC genotypes versus other genotypes ($P \approx 0.093$ and OR=1.717; $P \approx 0.178$ and OR=1.545; $P \approx 0.429$ and OR=0.779 respectively) and also C versus T allele ($P \approx 0.811$ and OR=0.934) were not different. **Conclusion:** This study shows that IL-4C-590T variants are not risk factor for gallstone formation. IL-4 had several role and functions in immune responses but this studied polymorphism in its gene has not a crucial role in immune events that participate in gallstone formation.

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# Serum Levels of Interleukin (IL)-17 in Patients with Acute Myocardial Infarction and Unstable Angina

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**Background & Objective:** Recent evidence suggests immunologic factors may play an important role in the pathogenesis of cardiovascular diseases. The aim of this study was to evaluate the serum levels IL-17 in patients with acute myocardial infarction and unstable angina. **Methods:** In this descriptive study, totally 60 patients with IHD as having acute myocardial infarction (AMI; n=30) or unstable angina (UA; n=30) and 30 sex- and age- matched healthy subjects as a control group were enrolled to study. Serum samples of participants were tested for the levels of IL-17 by use of ELISA. Statistical analyses have been done by using ANOVA and t-test. **Results:** The mean serum levels of IL-17 was 6.68 pg/ml ± 1.2 in AMI group, 5.48 pg/ml ± 1.01 in UA group and 2.07 pg/ml ± 0.60 in healthy control group. Statistical analyses showed that the mean serum concentrations of IL-17 in AMI and UA groups were significantly higher than that observed in healthy control group ($P< 0.005$ and $P< 0.04$, respectively). Moreover, the mean serum levels of IL-17 in total IHD patients (6.08 pg/ml ± 0.79) was significantly higher than that observed in healthy subjects ($P< 0.002$). No significant difference was observed between AMI and UA groups regarding the mean serum concentrations of IL-17. **Conclusions:** These results demonstrated higher serum levels of IL-17 patients with AMI and UA groups. Accordingly, Th17-associated mechanisms may participate in the pathogenesis of IHD.

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# Serum C-Reactive Protein Levels a Biomarker for Differentiation of Ischemic from Hemorrhagic Stroke

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**Background & Objective:** Cerebrovascuar accidents rank first in the frequency and importance among all neurological disease. Although a number of studies had shown increased level of the high sensitive C-reactive protein (hs-CRP) in patients with ischemic stroke, the association of increased hs-CRP with various type of stroke especially the assessment hs-CRP level in ischemic and hemorrhagic stroke has not been investigated. In the present study, we assessed the concentration of hs-CRP in patients with documented ischemic and hemorrhagic stroke in the first 24 hours of the onset of symptoms. **Methods:** Thirty-two patients with Ischemic and hemorrhagic stroke were evaluated at neurology department of Poursina hospital. The presence of baseline vascular risk factors, including hypertension, diabetes mellitus, hypercholesterolemia, obesity, and smoking, was determined. The blood samples were then collected and routine hematology and biochemistry tests were done. hs-CRP levels were determined using a highly sensitive immunonephelometric method. **Results:** In this cross sectional study, the age of patient varied from 45–85 years (Mean 70.9 ± 9.4). Serum level of hs-CRP in Ischemic patients was 18.92 ± 11.28 and in hemorrhagic group was 2.65± 1.7. This relationship was statistically significant (P< 0.0001). **Conclusion:** The finding of this study reveal that types of stroke were related to elevated hs-CRP levels. Moreover, it may be concluded that hs-CRP might be considered as a usefully adjunct method for the initial diagnosis of the type of stroke.

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# Blood Serum Levels of IL-1β, IL-6 and TNF-α in Patients on Maintenance Hemodialysis

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**Background & Objective:** Dialysis provides effective and safe treatment of ESRD, but patients who are maintained on chronic dialysis are at risk for cardiovascular disease. One major risk factor for cardiovascular disease in adult patients with ESRD is chronic inflammation. Cytokines are essential mediators of immune response and inflammatory reactions. During a hemodialysis (HD), cytokines are released mainly by monocytes activated by endotoxin-type compounds in dialyzer fluid, Complement factors and direct contact with dialyzer membrane. Aim of this study was to examine effects of the duration of HD therapy upon systemic profile of the pro-inflammatory cytokines (IL-1 β, TNF-α and IL-6) in patients on regular maintenance HD. **Methods:** The study included 43 CRF patients, aged 59.32 ± 14.43 years, on regular HD maintenance therapy for mean 26.44 ± 41.29 months and 43 age and sex matched healthy controls. It was designed to assess serum levels of inflammatory cytokines: IL-1β, IL-6 and TNF-α in CRF patients on regular maintenance HD. **Results:** The serum IL-1β, IL-6 and TNF-α level were statistically significantly higher in patients than in the controls. There were statistically significant positive correlations between the duration of HD therapy and serum levels of the inflammatory cytokines. **Conclusion:** Elevated serum IL-1β, IL-6 and TNF-α levels in our CRF patients on regular maintenance HD indirectly confirm importance of HD in amplification of the chronic inflammation substantially depend on the duration of dialysis treatment.

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# Comparison of Serum Cytokine Changes after Short Term Circuit Resistance Training in Active and Inactive Females

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**Background & Objective:** Elevated levels of serum biomarkers such as cytokines have been independently associated with cardiovascular risk. However, the changes of these biomarkers after short term circuit resistance training in females are unknown, as is their association with cardiovascular disease. Purpose: to compare the effects of short term circuit resistance training on TNF-α, IL-6 and IL-8 serum concentrations in active and inactive females. **Methods:** 43 university female students (mean age 22/74 ± 3/9 year, weight 59/91 ± 9/8 kg , height 165/9 ± 0/04 cm , BMI 21/73 ± 3/1 (kg/m2) , PBF 26/47 ± 5/01 , Vo2max 38/65 ± 5/43 (ml/kg/min) who had complete health , were randomly assigned to four groups; active experimental (AE, n = 8) active control (AC, n= 8) inactive experimental (NE, n= 13) inactive control (NC, n = 14) . Subjects performed short term circuit resistance training, 5 times per week (10 sessions) for 2 weeks with free weights and weight training machines including : chest press , leg extension , sit-up, lat. pull down , front row , foot raising , back extension , leg curl., During the first week the training were done at % 40 of their one-repetition maximum (1RM ) for 15 repetitions and 3 sets . The intensity of training was increased to %50 1RM, during the second week, but other properties of training remained constant. Before and 48 hours after the last training session , fasting and resting blood samples were collected to measure, IL-8, IL-6, and TNF-α concentrations by ELISA. **Results:** Inter group comparison showed: IL-6 and IL-8 concentrations in all groups and TNF-α concentration with the except of inactive experimental group was reduced. In between group comparison; there were significant differences on TNF-α, IL-6 and IL-8 ($P< 0.05$). **Conclusion:** It seems that short term circuit resistance training can reduce inflammatory condition and possibly the risk of diseases related to cytokines.

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# Regulatory T Cells and IL-10, IL-17 and IFN-γ Producing T Cells Frequencies in Kidney Allograft Recipients with Donor Bone Marrow Cells Infusion

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**Background & Objective:** Type and dynamics of cytokines in the allograft are considered as the crucial factors in altering the tissue function and in reducing T cell responses leading to anergy, apoptosis or induction of regulatory T cells. The aim of this study was to assess the influence of donor bone marrow cells infusion (DBMI) on the frequency and function of regulatory T cells (T reg) and on the cytokine profiles in kidney allograft recipients. **Methods:** 27 living unrelated renal allograft patients were included in this study as DBMI (n=14) and control (n=13) groups and followed up 2 years retrospectively. Peripheral blood mononuclear cells (PBMCs) from all patients were obtained at the end of second year post operatively and the percentages of $CD25^{+}foxP3^{+}$ and $CD3^{+}CD8^{+}CD28^{-}$ Tregs were measured using flowcytometery. Also, the frequency of IL-10, IL-17 and IFN-γ producing cells separately were determined using ELISPOT analysis using peptides corresponding to HLA-DR mismatched alleles between donor and recipients and phytohemaglutinine (PHA) as stimulators. **Results:** a significant decrease in the number of IFN-γ producing cells were found in DBMI patients compared to controls ($P< 0.05$). Also, an increase in the frequency of IL-10 producing cells ($P \approx 0.07$) and a decrease in the rate of IL-17 producing cells ($P \approx 0.18$) were observed. The mean number of IFN-γ /IL-10 producing cells was significantly higher in DBMI patients versus controls ($P< 0.05$). The mean difference for the frequencies of $CD4^{+}CD25^{+}FoxP3^{+}$Tregs and $CD3^{+}CD8^{+}CD28^{-}$ T cells between both groups were 0.5% and 4.5% respectively and higher percentages for those Tregs were shown in DBMI patients ($P \approx 0.12$ and $P \approx 0.36$). **Conclusion:** These findings suggest that the donor bone marrow cells infusion could stimulate partially the regulatory mechanisms based on the presence of lower number of inflammatory cytokines producing cells concurrently with the higher percentage of regulatory cells in peripheral blood late after transplantation.

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# Serum Levels of IL-10, IL-17, TGF-β1 and IFN-γ and Expression Levels of IL-10 and TGF-β1 Genes in Renal Allograft Recipients with Donor Bone Marrow Cells Infusion

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**Background & Objective:** Cytokine storm generated by alloimmune response after transplantation can lead to either the graft survival or the rejection of graft. The aim of this study was to evaluate the serum levels of IL-10, IL-17, TGF-β1 and IFN-γ and expression levels of IL-10 and TGF-β1 in renal allograft recipients with donor bone marrow cells infusion (DBMI). **Methods:** 28 living unrelated kidney recipients were divided in DBMI patients (n=15) and controls (n=13) and followed up two years retrospectively. Both groups received the same baseline conventional immunosuppressant. Fourteen healthy subjects included in this study as normal control group. Gene expression analysis for IL-10 and TGF-β1 cytokines relative to the mRNA level of β-actin as a reference gene was performed in the PBMCs from all patients at the end of second year post-transplant using quantitative fluorescence real-time PCR. Also, serum levels of IL-10, TGF-β1, IFN-γ and IL-17 in different groups were measured by ELISA method at the same time. **Results:** Both groups of patients showed increased levels for IL-10 mRNA expression and IL-10 serum compared with normal control group, and it was statistically significant for the expression levels between control patients and normal subjects ($P < 0.05$). Serum levels of IFN-γ and IL-17 were higher in control patients compared to normal control. DBMI patients depicted significant lower serum levels of TGF-β1, IL-17 and IFN-γ in compare with normal subjects ($P \approx 0.05$ and $P < 0.03$ for TGF-β1 and IL-17 respectively). Also, the serum levels of IL-17 and IFN-γ in DBMI group were lower but not significant versus control patients. **Conclusion:** Increased levels of IL-10 mRNA expression and low serum levels of pro inflammatory cytokines (IL-17 and IFN-γ) as well as better clinical outcomes in DBMI patients could be somehow indicative for immunomodulatory effects of this approach in kidney allograft patients.

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# The Impact of KIR-HLA Mismatch on HLA-Identical Sibling Hematopoietic Stem Cell Transplantation Outcome

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**Background & Objective:** Natural killer (NK) cell allogenic reaction, defined as lack of interaction between donor inhibitory KIRs and recipient HLA class I molecules, may affect T-cell depleted hematopoietic stem cell transplantation (HSCT) outcome. However, influence of donor and recipient activating KIRs in of HSCT has poorly been investigated. **Methods:** This retrospective analysis has studied the importance of NK alloreactivity, based on 'missing ligand' model and 'gene-gene' model, for 78 recipients (n=40 with acute myeloid leukemia, AML; and n=38 with acute lymphoid leukemia, ALL) undergoing non-T-cell depleted HSCT from HLA-identical sibling donors. In this study, we set up a new combined KIR-HLA genotyping assay to detect KIR and HLA ligand genotypes in transplant pairs. Then, overall survival (OS), disease-free survival (DFS), and relapse were evaluated in recipients. **Results:** No impact of 'missing KIR ligand' was found on OS, DFS, and relapse for AML and ALL recipients. However, presence of additional activating KIR genes in donor genotype compared to those of AML recipient was associated with a higher two-year DFS in a multivariate analysis ($P< 0.01$). Interestingly, a significant increase in two-year OS was revealed in AML recipients who received stem cells with KIR2DS3 ($P< 0.005$) or KIR3DS1 ($P< 0.001$). Moreover, existence of KIR3DS1 in donors was associated with an improved DFS in AML recipients ($P< 0.005$). **Conclusion:** These findings may imply that 'missing KIR ligand in recipients is of little importance in our matched non-T-cell depleted HSCT outcome. However, HLA-identical sibling donors with KIR2DS3 and/or KIR3DS1 significantly increase survival in AML recipients.

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# The Comparison of Immune Response Factors in Exercisers and Non-Exercisers

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**Background & Objective:** Exercise in different situation and with different quality can change the Immune response accordingly. Many studies documented that systemic exercise can support the immune responses while discontinues exercise weakens it. The current study was designed to analyze the Immune properties amongst exercisers compared to non exercises. **Methods:** 20 exercisers as case group together with 20 non exercisers as control group were randomly selected from each 6ml blood was taken after 90 minutes exercise was ordered. CD3, CD4, CD8, CD19, CD56 and IgG titter were all measured using flowcytometry and Nephlometry analyses. **Results:** The mean CD3 level was 62.64 and 69.5 in men and women of case group and totally 64.33 in control group. The mean CD4 level was 41 for men, 41.83 for women and 42.86 for control group though the mean CD8 level for men was 19.5, women 27 and control group 20.33. The level of CD19 was 11 for men, 9.6 for women and finally 13.6 for control group. The mean value of CD56 was 7.92 for men, 7.83 for women and 6.39 for control group. The mean titer of IgG was 14.09, 14.49 and 14.14 for men, women and control group respectively. **Conclusion:** As the mean OD value of CD3 and CD8 and the CD4/CD8 ratio are all significantly different among exercisers than non-exercisers indicating that immune system can be affected dependent on the nature and quality of exercise.

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# Determination of Effect Exercise Training on Spirometric Indexes in Asthma Patients

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**Background & Objective:** In asthmatic children, Spirometry has been used as a common diagnostic test in asthma intensity. Aim: the aim of this study is to investigate the effect acute exercise Spirometry indexes in asthma patients. **Methods:** 15 overweight males (mean age: 13 years; range: 10-16 years) with mild-to-moderate asthma and 15 overweight males with non-asthmatic participated in this study. Forced expiratory volume in one second (FEV1) and Forced Vital Capacity (FVC) and FEV1/FVC) were measured in baseline for two groups. Then these indexes were measured after exercise test on cycle ergometer (YMCA standard protocol) in asthma group. The findings are compared by T test in two groups ($P< 0.05$). **Results:** The statistical analysis showed that spirometric variables were lower in asthma than non-asthmatic groups. Also, these variables (FEV1, FEV1/FVC) were significantly increased after exercise protocol ($P< 0.05$). **Conclusion:** These results suggest that lung function in overweight asthma patients is lower than overweight healthy people and physical training can improve respiratory volumes in these patients.

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# Correlation between Deficiency of Immunoglobulin Subclass G3 and Immunoglobulin GM Allotypes

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**Background & Objective:** The genes for the constant heavy chains of IgG are located on chromosome 14 and were further studied by identifying allelic, alternative Gm allotypes-mendelian genetic markers of immunoglobulin gamma heavy chains. These were defined by different epitopes for three of the IgG subclasses, G1m(a) and G1m(x) for IgG1, G2m(n) and G2m(f) for IgG2 and G3m(g) and G3m(b) for IgG3. A few researches show that immunoglobulin Gm allotypes are associated with the outcome of some immunodeficiency. In this study we searched for alternative Gm allotypes in patient with IgG3 deficiency and normal individuals. **Methods:** The Gm allotypes were investigated in 63 patients: 46 females and 17 males, in whom serum IgG3 was below 0.35 g/l and 68 healthy individuals with a sensitive competitive ELISA method. For quantitation of the Gm allotypes competitive ELISA was performed with specific monoclonal and polyclonal antisera and purified myeloma proteins of different Gm allotypes. Concentrations of the other IgG subclasses were within the age-related normal ranges. **Results:** The distribution of the IgG1 genetic markers G1m(a) and G1m(x) differed markedly from that observed in normal individuals ($P< 0.001$). Thus G1m(a) was present in 60 subjects as compared to an expected 36, and phenotype G2m(-f) in 34 subjects as against an expected 8. The mean IgG3 concentration was numerically lower in the G2m(-f) group than in the G2m(+f) cohort, and individuals with IgG3 levels 0.10 g/l were more frequent in the G2m(-f) group. **Conclusion:** Among Iranians as same as Caucasians, G3m(g) is in linkage disequilibrium with G1m(a) and our interpretations is that the haplotype G1m(a); G1m(x) G2m(n) G3m(g) is markedly increased in individuals with IgG3 deficiency.

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# Appointment of $CD4^+$ $CD8^+$ Cells and Percentage in Patients Infected With HIV

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**Background & Objective:** CD4 T-Lymphocyte Counts and CD4/CD8 Ratio Have Proven to be a standard Laboratory marker for disease Progression and Future Decision making in patient infected with HIV. Flow cytometry technique used viruses Antibody for detect cellular index. In this study appointment relationship between $CD4^+$ $CD8^+$ count and those percentage In Patients infected With HIV. **Methods:** this was a retrospective cross- sectional study. Participated subject were 244 patients who had positive serologic HIV test result, confirmed via western blot, between 1386 and 1387. $CD4^+$, $CD8^+$ were stained using dual label antibodies and analyzed with BD Flow cytometry. **Results:** our data showed that: in $HIV^+$ subtypes the percentage of $CD3^+$, $CD4^+$, $CD8^+$ cells is 74.57% - 30.95% and 54.59%. The absolute is 1426, 433 and 918 Cells/µl reported. CD4 absolute cunt reported Division in group: CD4 <200 cells/µl =15.5% and CD4 >200 cells/µl = 85.5% of 244 patients. **Conclusion:** $HIV^+$ subjects and their immune condition could be followed up by Flow cytometry technique. Appointment of relation between T cells and percentage can effective help decision making for anti- retrovirus treatment.

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# IgG and IgM Anti-*Toxoplasma gondii* Antibodies in Patients with HIV Positive in Khorasan Razavi Province, Northeast Iran

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**Background & Objective:** Toxoplasmosis is a cosmopolitan disease. Our aim was to evaluate the prevalence of toxoplasmosis in patients with acquired immunodeficiency syndrome (AIDS) in Mashhasd (Northeast Iran). **Methods:** 121 HIV positive patients were recruited in this cross-sectional study and investigated for IgG and IgM anti- toxoplasma antibodies with ELISA. **Results:** The seropositivity of IgG anti-toxoplasma antibodies was in 62 (51.2%) patients and there were 3 cases (2.5%) with IgM antibodies. **Conclusion:** Diagnosis of toxoplasmosis and immediate treatment in these patients is essential. Along with the support of our significant findings, primary chemoprophylaxis should be routinely given to any HIV-positive patients with Toxoplasma seropositive status, in order to prevent the risk of developing life-threatening secondary reactivation of toxoplasmosic encephalitis in association with AIDS.

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# Comparison Human and Mouse Antibody in Detection of HIV p24 Antigen

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**Background & Objective:** To compare human and mouse Antibody in detection of antigen p24, we design a simple and sensitive Enzyme immune assay for detection of this antigen. **Methods:** Three hundred negative samples from blood donor were tested with HIV third-generation kit and also the 30 positive samples that confirm by immunoblot and PCR were collected from AIDS Research center. We used a human monoclonal antibody as capture and a human monoclonal antibody labeled with biotin as tracer. All samples were tested by p24 antigen designed kit with ELISA method. In order to eliminate effect of antigen-antibody complex, different buffers was used, that best answer was obtained with 1.5 M Glycin buffer(pH=2). To compare sensitivity and specificity, at the same time mouse monoclonal antibody as the coating antibody was used and compared with human monoclonal antibody. **Results:** Twenty one of thirty positive samples were positive in designed kit with human monoclonal antibody and 18 samples in designed kit with mouse monoclonal antibody. Before pretreatment of samples with Glycin buffer Diagnostic sensitivity were 70% and 60% for human monoclonal antibody and mouse monoclonal antibody respectively. After antigen-antibody dissociation, 28 and 27 samples were positive with human monoclonal antibody and mouse monoclonal antibody respectively. (Diagnostic sensitivity, 93% and 90% respectively). Analytical sensitivity of assay by WHO antigen and recombinant antigen was 1 U/ml and 2 pg/ml with using of human monoclonal antibody respectively, that with use of mouse monoclonal antibody this value was 4 U/ml and 8 ng/ml respectively. The pretreatment of antibody positive sera and members of BBI panels that have positive for antigen and antibody and PCR test with 1.5M Glycin buffer cause increase of diagnostic sensitivity(70% against 93%). **Conclusion:** Human Antibody has a high analytical sensitivity and specificity rather than mouse antibody.

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# Examination of Correlation between Plasmacytoid Dendritic Cell Count and CD4+ and CD8+ Lymphocytes Count among Human Immunodeficiency Virus Infected Patients

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**Background & Objective:** Plasmacytoid dendritic cells are responsible for primary antiviral immunity. These cells are important in controlling HIV infection. The number of circulating plasmacytoid dendritic cells among human immunodeficiency virus infected patients was investigated and correlation between those with $CD4^+$ and $CD8^+$ lymphocytes was evaluated. **Methods:** This descriptive study was performed among 53 patients with HIV infection who referred to central laboratory of Iranian blood transfusion organization and 30 healthy controls. Two color flow cytometric analysis were performed for separating of plasmacytoid dendritic cell and then statistical analysis was performed for determining mean difference and coloration coefficient. Univariate one-way analyses of variance were performed for pairwise mean comparisons and correlation between parameters were performed by Pearson rank test. **Results:** Progressive decreases of plasmacytoid dendritic cells in parallel with CD4 positive lymphocyte were seen. The plasmacytoid dendritic cells count correlated with $CD4^+$ T cells count ($r=0.574$, $P< 0.001$) but did not show a significant correlation with $CD8^+$ T cell count ($r=0.177$, $P \approx 0.19$). **Conclusion:** Relationship between $CD4^+$ lymphocyte count and plasmacytoid dendritic cell count may reflect a dependent homeostasis between these cells in HIV patients.

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## Increased Serum Levels of Soluble CD30 in Patients

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**Background & Objective:** Common variable immunodeficiency (CVID) is a heterogeneous group of disorders, characterized by hypogammaglobulinemia and increased susceptibility to recurrent pyogenic infections, autoimmunity, and malignancies. **Methods:** Twenty-five cases with CVID (18 males and 7 females) and 25 healthy volunteers were investigated in this study. Soluble CD30 (sCD30) serum levels of the subjects were measured and compared. **Results:** Serum levels of sCD30 in the patients with CVID were significantly increased in comparison with controls (36.93 ± 32.38 vs. 5.27 ± 1.32 U/ml, $P< 0.005$). The group of patients with splenomegaly and reversed ratio of $CD3^+CD4^+$ T cells/$CD3^+CD8^+$ T cells had the highest serum levels of sCD30 (66.01 ± 43.34 U/ml) in comparison with other patients ($P< 0.05$). **Conclusion:** High levels of sCD30 in the CVID patients with splenomegaly and the presence of lymphoma in a patient with the highest level of sCD30 may suggest a soluble form of this marker as a prognostic tool in such diseases.

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# Molecular Studies in Patients with Severe Congenital Neutropenia Associated with Primary Immunodeficiencies

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**Background & Objective:** Primary immunodeficiency diseases are inherited disorders that predispose individuals to recurrent infections, autoimmunity, malignancies and hematological disorders. Some of them are associated with neutropenia, which ranging from isolated form of neutropenia such as severe congenital neutropenia to complex inherited disorders associating neutropenia. Severe congenital neutropenia (SCN) is a rare primary myelopoiesis disorder, characterized by persistent severe neutropenia. **Methods:** In this study, the patients with SCN who referred to the Children's Medical Center were enrolled. ELA2, HAX1 and G6PC3 genes were sequenced to identify the molecular basis of disease. **Results:** Serial complete blood counts indicated persistent neutropenia in all cases. Bone marrow aspiration of the patients demonstrated maturation arrest of myeloid series at promyelocyte-myelocyte stages. In nine patients with SCN, molecular studies revealed a mutation in one of the above-mentioned genes. Mutations in HAX1 gene were the most common, which were detected in six patients, followed by mutation sin G6PC3 gene (two cases) and ELA2 gene (one patient). All the patients treated by granulocyte colony-stimulating factor. **Conclusion:** Severe congenital neutropenia should be considered in children with early onset recurrent infections and neutropenia, while early diagnosis and appropriate treatment can prevent further complications. HAX1 deficiency seems to be the most common form of SCN in our regions, considering high rate of consanguinity in the country.

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# Evaluation of Antibody Response to Polysaccharide Vaccination in Common Variable Immunodeficiency (CVID)

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**Background & Objective:** Common variable immunodeficiency (CVID) is a heterogeneous group of disorders, characterized by hypogammaglobulinemia and increased susceptibility to recurrent infections, autoimmunity and malignancies. This study was performed to evaluate bactericidal antibody responses of patients to polysaccharide vaccine. **Methods:** In this study, serum bactericidal antibody (SBA) responses to serogroup C Neisseria meningitidis of 23 patients with CVID and 23 sex and age-matched controls were measured before and after three weeks and one year vaccination with the plain A/C meningococcal polysaccharide vaccine. **Results:** Serum bactericidal antibody geometric mean titers (GMTs) 3 weeks after vaccination were 6.9 for the patients and 12.2 for the controls. SBA GMTs after one year for the patients and controls were 4.7 and 7.5, respectively. These levels were all significantly higher than the GMTs measured pre-vaccination in both groups ($P< 0.01$). The fold-rise in GMT from pre-vaccination to one year post-vaccination was significantly higher in the control group compared to the patient group (5.41 vs. 2.96, P-value<0.01). Among 23 CVID patients, 8 had a poor response to vaccine (<4 fold-rise) 3 weeks after vaccination and low titers remained when measured one year later. Of the 15 CVID patients who had a normal response to vaccine (≥4 fold-rise) 3 weeks after vaccination, 6 cases failed to maintain protective SBA titers; whereas the remaining 9 had protective titers one year after vaccination. Only one of the 23 controls, who developed protective SBA titers after 3 weeks, lost their protective titers after one year. Amongst the patients, the presence of bronchiectasis and/or splenomegaly at enrolment was associated with poor SBA response to vaccine at 3 weeks and/or failure to maintain protective levels at one year. **Conclusion:** This study demonstrate that a number of CVID patients can produce protective antibody titers that can even persist for one year after vaccination, which lends strong support to the inclusion of polysaccharide vaccine in the immunization program for CVID patients.

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# Comparison of Lymphocyte Subsets in Cigarette Smoker & nonsmoker Iranian thermal burn Patients

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**Background & Objective:** The effects of Cigarette smoking on Lymphocyte subsets are provided. There isn't any information about the effect of cigarette smoking and burn on lymphocyte subsets in Cigarette smoker & nonsmoker that have thermal Burn. This study is done to compare Lymphocyte populations Iranian patients in cigarette smoker & non smoker with thermal burn. **Methods:** peripheral blood was collected in 49 male and female, 18-60 years old, with thermal injury (Third & seventh day post burn accident). Partec Flow cytometry system and triple color Flow cytometry reagents were used for analysis. All results were analyzed by SPSS. **Results:** There are 77.6% nonsmoker & 22.4% smoker. Comparisons of lymphocyte subsets include CD3, CD4, CD8 & CD19 markers in third day with seventh day were significant ($P< 0.05$) in nonsmoker patient. And other markers were non significant. In smokes comparison of lymphocyte subsets include CD8 marker in third day with seventh day is significant ($P< 0.05$). **Conclusion:** Significant changes in CD8 subpopulation in both group (Cigarette Smoker & nonsmoker) would have an important role in Cellular Immunity suppression. Non significant Changes in other lymphocyte subsets could be related to some factors which need more investigations.

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# Patients Prognostic Impact of CD27 Expression in Pediatric Acute Lymphoblastic Leukemia Patients

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**Background & Objective:** Acute lymphoblastic leukemia (ALL) is the most common form of leukemia in children. The possible prognostic significance of the expression of a variety of markers has been investigated in ALL. Objective: The present study was performed to find the prognostic importance of CD27 expression in B-ALL patients. **Methods:** Bone marrow or peripheral blood samples of 63 patients with ALL were assessed for the expression of T lymphoid, B lymphoid, myeloid and non-lineage-associated differentiation antigens by a two-step strategy using panels of monoclonal antibodies and direct Flow cytometry. Expression of CD27 in B-ALL group was studied in relation to immunophenotype, clinical and paraclinical findings. **Results:** CD27 expression was observed in 41.4% of ALL patients. The mean expression was obtained as 23.4 ± 23%. There was a significant positive correlation between favorable prognostic indices including higher platelet count and less extramedulary involvement with expression of CD27 ($P< 0.05$ and $P< 0.01$ respectively). **Conclusion:** These data suggests the usefulness of CD27 molecule in predicting the prognosis of ALL. The reason for such a role for CD27 molecule perhaps is related to its binding capability to CD70 molecule on the T cells resulting in activation of these cells and therefore promoting cellular immune responses against leukemic cells.

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## Serum Levels of CD26 and CD30 in Chronic Renal Failure and Hemodialytic

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**Background & Objective:** Various abnormalities of the immune system have been demonstrated in patients on hemodialysis. Imbalances in Th1 and Th2 responses have an important role in these complications. These cells produce predominantly some cytokine profiles and also express preferentially costimulatory CD26 and CD30 molecules. The aim of the present study was the determination of the levels of soluble CD26 and CD30 co-stimulatory molecules in sera of CRF and hemodialytic patients (HD). **Methods:** In this case-control study the serum levels of soluble CD26 and sCD30 were determined by a sandwich enzyme-linked immunosorbent assay in 60 CRF patients (30 patients with CRF and 30 end-stage renal disease under hemodialysis, and 60 healthy individuals. Renal function was evaluated by measuring serum levels of creatinin, albumin and urea. The one-way ANOVA used to analyze differences between study groups. **Results:** The serum levels of CD26 in the HD, CRF patients, and healthy controls were 275.4 ± 125.6, 402.9 ± 103.1, and 389.2 ± 117.04 ng/ml, respectively. The level of CD26 was significantly decreased in the HD group than in the CRF and control groups ($P < 0.005$). On the other hand, the serum levels CD30 in the HD, CRF patients, and healthy controls were 45.3 ± 13.7, 38.9 ± 14.5, 20.7 ± 10.5 U/ml, respectively. The CD30 levels were significantly higher in HD and CRF patients than controls ($P < 0.0001$). There is no significant difference between HD and CRF groups ($P \approx 0.2$). **Conclusion:** High serum levels of CD30 in line with low expression of CD26 indicate a Th2 polarization in immune responses of HD patients. It is possible that this Th2 dominated immune response may contribute to the abnormality of the immune system in HD patients.

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# Study of New B Cell Differentiation Stages in Acute Lymphoblastic Leukemia

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**Background & Objective:** Recently, it has been reported that the expression of CD44 and CD27 molecules correlates with the differentiation stage of B cell precursors and the following developmental sequence has been suggested: CD27 single positive (SP), CD27/CD44 double positive (DP), CD44SP and CD27/CD44 double negative (DN). The present study was performed to investigate these differentiation stages in B cell acute lymphoblastic leukemia (ALL) patients and find their prognostic importance. **Methods:** Bone marrow or peripheral blood samples of 58 patients diagnosed as B-ALL were studied for the expression of CD27 and CD44 using Flow cytometry analysis. **Results:** According to the expression of CD27 and CD44, four patterns were observed. CD27SP in 20.7% of patients, CD27CD44DP in 20.7%, CD44SP in 25.8% and CD27CD44DN in 32.8%. Expression patterns of CD27 and CD44 were studied in relation to prognostic factors including age, hemoglobin (Hb), white blood cells (WBC), platelet, and percentage of blasts in peripheral blood and bone marrow (BM), extramedullary involvement (EMI) and cytogenetics. Generally, patients with CD44SP cells showed a worse prognosis, whereas patients with CD27/CD44DP cells had a more favorable prognosis according to the relationship with clinical and paraclinical findings as follows. CD27/CD44DP pattern was correlated with higher Hb ($P< 0.05$), lower WBC count ($P< 0.05$) and lower percentage of blasts in the BM ($P< 0.05$). A higher platelet count was also detected in this group but it was not significant. CD44SP pattern was correlated to lower Hb, higher WBC count and BM blasts. There was a significant positive correlation between favorable prognostic indices and CD27SP patients. **Conclusion:** These data suggest the usefulness of both molecules in predicting the prognosis of ALL. This is the first report studding the correlation between different patterns of CD27 and CD44 expression and prognosis in ALL patients.

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# Immunophenotypes, Cytogenetics and Clinical Features of Acute Lymphoblastic Leukemia in Children

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**Background & Objective:** Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. Objective: The incidence and immunologic characteristics of various subsets of ALL in Iranian patients was studied. **Methods:** We studied 63 patients below the age of 16 years with newly diagnosed ALL. Bone marrow or peripheral blood samples were assessed for the expression of T lymphoid (CD2, CD3, CD4, CD5, CD7, CD8), B lymphoid (CD19, CD20, CyCD22, CyCD79a), myeloid (CD13, CD33, CD14, CD15, CD117) and non-lineage-associated (CD10, CD34, TdT, HLA-DR) differentiation antigens by a two-step strategy using panels of monoclonal antibodies and direct Flow cytometry. **Results:** Immunophenotyping of patients shows that 92% of cases were B-ALL, 8.6% T-ALL and 1.4% were mix lineage. Among the B-ALL patients 8.6% were Pro B-ALL, 53.5% were Early Pre B-ALL and 37.9% were Pre B-ALL. In this study cytogenetic and molecular abnormality detected in 36.2% patients with ALL (TEL/AML1 14.9%), (BCR/ABL 2.1%), (E2A-PBX1 4.3%), (MLL Rearrangement 4.3%), (Hyper Diploid 10.6%). Physical examination revealed extra medulary involvement in 53.8% of patients (hepatomegaly in 48.3%, splenomegaly in 21.4%, and lymphadenopathy in 13.8% and CNS involvements in 3.4% of patients. **Conclusion:** Our results showed that the distribution of ALL subsets in Iranian children is similar with the general distribution pattern in developed countries except for the low frequency of mature B-ALL phenotype.

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# Evaluation of Some Factors of Innate, Humoral and Cellular Immunity in Major Beta Thalassemia Patients

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**Background & Objective:** Thalassemias are the most common genetic disorders in the world. These disorders are common in the Middle East countries containing Iran. It seems that factors like splenectomy, iron overload, frequent contacts with antigens during blood transfusion & using chelating agents cause severe disturbances to immune system. This study is done to evaluate the immune system in thalassemic patients in Khoozestan province. **Methods:** This study was done on 40 major thalassemic patients. Patients who had the history of frequent bacterial & viral infections, splenectomized patients, patients using immunosuppressive drugs& patients with hepatitis & diabetes or other chronic diseases excluded. Control group contained 31 healthy persons. The last CBC, serum ferritin was taken from patient files. NBT test was done & CD4, CD8, CD5, CD20 lymphocytes percent achieved with Flow cytometry and IgG, IgM, & IgA levels achieved with nephlometry method on peripheral blood of the samples. **Results:** CD4, CD8 & CD5 lymphocytes percent & CD4/CD8 ratio had no significant difference between case & control groups.CD20 lymphocyte percent & IgG, IgM & IgA serum levels were significantly higher in case group. NBT test in all case & control groups was normal. **Conclusion:** It seems cellular immunity in beta thalassemia patients is the same as normal people. However, humoral immunity changed significantly because of increase in allo-antibodies & auto -antibodies due to frequent blood transfusion in these patients.

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# Lack of Association between Tumor Necrosis Factor-Alpha (TNF-α) -308G/A and Risk of Developing Alzheimer's Disease

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**Background & Objective:** Late-onset Alzheimer's disease (LOAD) is a neurodegenerative disorder and the most common form of dementia affecting people over 65 years old. Alzheimer's disease is a complex disease with multi-factorial etiology. Inflammation has been approved to have an important role in the pathogenesis of Alzheimer's disease (AD). TNF-α is a main pro-inflammatory cytokine that plays an essential role in initiation and regulation of inflammatory responses. Several studies have shown the probable association of -308G/A polymorphism at TNF-α gene's promoter with AD pathogenesis. This study was performed to determine whether this polymorphism contributes to the risk for late-onset Alzheimer's disease (LOAD) in Iranian population. **Methods:** One hundred and forty AD patients and 158 healthy controls were recruited in the study. Following extraction of genomic DNA, using PCR/RFLP methods the genotype and allele frequencies were determined in case and control subjects. **Results:** After statistical analysis, no significant difference in the allele and genotype frequencies due to this polymorphism between two groups was identified. Also after stratifying subjects by their ApoE-ε4 status, no significant association was observed. **Conclusion:** Our results suggest that TNF-α gene -308G/A is not a risk or protective factor for late-onset Alzheimer's disease in Iranian population. Although to confirm these results further study with a bigger sample size may be indicated.

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# Effects of Enamel Matrix Derivative on the Viability, Cytokine Secretion and Phagocytic Activity of Human Monocytes

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**Background & Objective:** The Enamel matrix derivative (EMD) contains amelogenin and other enamel matrix proteins, has been revealed to promote the formation of acellular cementum as well as tissue regeneration. There is some controversy about the effect of EMD on inflammation and resorption. The aim of this study was to examine the viability and pro-inflammatory cytokine [(tumor necrosis factor-alpha (TNF-α) and Interleukin-1 β (IL-1β)] production of monocytes treated with different concentrations of EMD and to assay the phagocytic function of monocytic cells exposed to EMD. **Methods:** Monocytes were isolated from peripheral blood mononuclear cells from healthy donors by adherence into plates and incubated with 50,100 and 200µg/ml EMD in the presence or absence of 10µg/ml LPS. After 12, 24, 48 and 72 hours incubation, cellular viability was evaluated through MTT assay and cytokine secretion were analyzed by enzyme-linked immunosorbent (ELISA) assay. Phagocytic activity of the monocytic cells exposed to EMD after different time periods of culture was assayed using PHAGOTESTTM kit based on the phagocytosis of FITC conjugated E-coli by treated cells. **Results:** A partial increase in cell viability was observed after 72hours with 200µg/ml concentration of EMD which was not statistically significant. There was no significant differences in production of TNF-alpha and IL-1β among samples with various concentrations (50,100 and 200µg/ml) of EMD and control (EMD=0) at 12, 24, 48 and 72 hours. Phagocytic activity of monocytic cells increased significantly after 72 hours compared to 12 hours. **Conclusion:** In the condition of this study, EMD does not promote releasing of two proinflammatory and resorbing cytokines TNF-α and IL-1β. Increasing the phagocytic activity of monocytes cells, EMD also might accelerate wound healing through clearing of inflammation site from the foreign bodies.

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# Evaluation of Resilon– vs. Gutta Percha-Induced IL-1β and TNF-α Production on a Human Monocyte/Macrophage Cell Line (THP-1)

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**Background & Objective:** Acceptable obturation of the root canal space has been pointed to as a principle part of successful endodontic treatment. It is well-documented that substances leach from root canal filling materials causing decreased cellular viability. The aim of this study was to evaluate the effect of Gutta percha and Resilon on viability and modulation of inflammatory responses on human monocytic cell line THP-1. **Methods:** Three-millimeter segments were cut from the tip of gutta percha and Resilon cones after disinfection by immersion into sodium hypochlorite solution and placed at the bottom of 96-well culture plates in direct contact with cells suspended in culture medium in the presence or absence of Lipopolysaccharide. LPS-stimulated cells were considered as positive controls. Cellular viability was assessed through MTT assay. IL-1-beta and TNF-alpha production by cells after exposure to test materials was assessed by quantitative real-time PCR and enzyme-linked immunosorbent assay. **Results:** Both Resilon and gutta percha are non-toxic to human monocyte cell line and do not elicit significant IL-1-beta and TNF-alpha production (either in terms of gene expression or protein synthesis) from these cells. **Conclusion:** Gutta percha was slightly more toxic and induced more pro-inflammatory cytokines compared to Resilon regardless of LPS treatment or time intervals tested, although such difference was not considered statistically significant. More extensive studies either in vivo or ex vivo should be conducted to thoroughly evaluate the effects of root canal materials on the process of inflammation in periradicular tissues.

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# Correlation between sCD14 Concentration in GCF and GCF- induced Apoptosis of Neutrophils in Patients with Chronic Periodontitis

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**Background & Objective:** Lipopolysaccharide (LPS) plays an important role in stimulating immune system and subsequent destruction of periodontium. The CD14 is one of the LPS receptors that are either bounded to cell membrane or circulating soluble form (sCD14). Different studies have shown that sCD14 plays a protective role in periodontal disease. So the aim of this study was to evaluate the correlation between sCD14 concentration in gingival crevicular fluid (GCF) and apoptosis of neutrophils by GCF samples. **Methods:** GCF samples from 30 healthy and 30 diseased sites of 30 patients with moderate to severe chronic periodontitis were obtained and the levels of sCD14 in the above mentioned samples were determined by ELISA. After separating neutrophils from the peripheral blood sample of a healthy individual, they were incubated with GCF samples, and then by staining with Annexin V, the percentage of apoptotic, necrotic and vital cells were determined. **Results:** The concentration of sCD14 in diseased sites was significantly higher than normal sites. Diseased sites with clinical attachment level (CAL) of more than 5 mm had lower levels of sCD14 than those with CAL of less than 5 mm. **Conclusion:** It is concluded that sCD14 probably has some protective role against periodontal disease.

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## Evaluate the Inflammatory Effects of Dental Alloys on Fibroblasts

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**Background & Objective:** Based on the previous studies it was shown that in vitro cytotoxicity of casting alloys relates to releasing of metal ions from alloys. So, the aim of this study was to determine the inflammatory effects of base metal alloys on mouse L-929 fibroblasts. **Methods:** For each of the dental alloys, rings with external diameter of 5.5, internal diameter of 3.5, height of 1.5 and thickness of 1 millimeter were prepared and place in the bottom of each well of 24 wells culture plates or their corrosive material were added to each well instead of the alloy rings. Then fibroblasts were added in each well and culture media consisted of RPMI-1640, Antibiotics (Penicillin and Streptomycin) and Fetal Bovine Serum (FBA) 10% was added to them. MTT test and ELISA were used for determining the cytotoxicity (after 1 hour, 24 hours and 1 week after treating) and concentration of IL-6 (24 hours after treating), respectively. **Results:** All of alloy has the most toxicity in first hour. Despite of Vera bond v, for the other alloys, cytotoxicity was reduced with time. There was no significant difference regarding IL-6 concentration between different alloys but there was significant difference between the effects of their corrosive materials on IL-6 production ($P< 0.05$) they ranks as below; Vera bond v2 < San kin < Super cast < Vera bond v. **Conclusion:** It is concluded that among the above four base metal alloys, super cast and Vera bond v has the most cytotoxicity. Vera bond v2 could be considered as the best alloy regarding to its less effects on cytotoxicity and IL-6 production.

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# Correlation between the Concentration of Pulpal Neuropeptides and Different Kinds of Dental Caries

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**Background & Objective:** Dental pulp has neural fibers which produce neuropeptides such as Substance P (SP) and Calcitonin Gene Related Peptide (CGRP). It was shown that the number of neural fibers is increased in the inflamed tissue surrounding an injured pulp. In these fibers, the amount of SP and CGRP are also increased, although it was also shown that CGRP has a possible role in repair. So the aim of this study was to investigate the possible relationship between the concentrations of SP and CGRP with different kinds of dental caries lesions. **Methods:** 81 teeth were collected with different kinds of caries. The teeth were divided with turbine and their pulpal tissues were removed and immediately frozen at -20 centigrade degree. Then different groups of caries lesions were selected by observing the radiographic views and tooth sections. Then tissue extracts were prepared by lysing buffer and were centrifuged for several times. ELISA method was used for measuring the concentration of SP and CGRPP in tissue extracts. **Results:** There was a significant difference between different carious lesions regarding the concentration of SP and CGRP and they were directly correlated with severity of the lesions. **Conclusion:** With progressing of carious lesion, the concentration of SP and CGRP would be increased. Of course the concentration of CGRP is more than SP and this reflects the regulatory effect of this neuropeptide.

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# Correlation between Salivary CD14 and Saliva-induced Neutrophil Apoptosis in Patients with Periodontal Diseases

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**Background & Objective:** The saliva and gingival crevicular fluid (GCF) of patients with chronic periodontitis could induce neutrophil apoptosis. In one study it was shown that salivary sCD14 has lower concentration in GCF of patients with periodontitis. So the aim of this study was to investigate the possible relationship between the concentration of salivary sCD14 and saliva-induced apoptosis of neutrophils in patients with peridontal diseases. **Methods:** Salivary samples were obtained from 30 patients with chronic periodontitis and 30 patients with gingivitis (as Case groups) and 30 healthy subjects (Control group). After separating neutrophils from peripheral blood of a healthy subject, it was mixed with salivary samples. Then the percentage of apoptotic cell was investigated and the concentration of sCD14 was measured by ELISA. **Results:** It was significant difference between case and control groups regarding sCD14 concentration but there is not any significant difference between gingivitis and periodontitis. The percentage of apoptotic cells was significantly higher in case groups ($P< 0.005$). There is not any significant correlation between sCD14 concentration and the percentage of apoptotic neutrophils. **Conclusion:** There is higher level of salivary CD14 in periodontal diseases (gingivitis and chronic periodontitis) and it might be due to the stimulation of immune system by periodontopathic bacterial products such as LPS.

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# Investigating the Effect of the First Phase of Periodontal Therapy on the Concentration of IL-17 & IL-23 in GCF of Patients with Severe Periodontitis

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**Background & Objective:** The purpose of the present research was to investigate the effect of the first phase of the periodontal therapy on the amount of existing IL-17 IL-23 in GCF of severe chronic periodontitis patients. **Methods:** In 22 patients with severe chronic periodontitis, GCF was gathered, and then the first phase of periodontal therapy (Scaling and Root Planning) was carried out. After 4weeks, taking GCF samples from the same parts was conducted. The same phase was carried and out on 24 healthy patients. ELISA was used in order to measure the concentrations of IL-17 and IL-23 in GCF samples. **Results:** There is a significant difference and significant statistical difference between the IL-17 and IL-23 concentration before and after the phase 1 periodontal therapy in the case group but there is not such a significant difference in the control group. **Conclusion:** IL-17 and IL-23 have a significant role in the pathogenesis of periodontal diseases.

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# Relationship between Gene Expression of IL-17A andIL-17F in Gingival Tissue and Concentration of IL-17A and RANKL in GCF of Patients with Periodontal Diseases

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**Background & Objective:** TH-17 is a new T-Cell subset producing IL-17 associated with many immune-related destructive tissue diseases. Periodontal disease is a chronic inflammation of the attachment structures of the teeth, triggered by potentially hazardous microorganisms and the consequent immune inflammatory responses.IL-17 has been implicated in various autoimmune, inflammatory and bone-destructive conditions. The exact role of IL-17A and IL-17F and RANKL in Periodontitis and gingivitis is still controversial. Therefore in this study we investigated the presence of IL-17A, IL-17F and RANKL in human periodontal disease. **Methods:** Gingival samples and GCF were collected from 20 healthy, 20 gingivitis and 20 periodontitis (chronic to acute) then used for the subsequent assays. The gene expression for IL-17A and IL-17F was measured by using quantitative real-time PCR and protein level of IL-17A and RANKL was measured by using ELISA. The difference of gene expression between gingivitis and periodontitis was analyzed by Mann-whiney U-test. Correlations between each gene expression were also analyzed. **Results:** The expression level of IL-17A was higher than IL-17F and a significant difference in expression IL-17A (not for IL-17F) between healthy, gingivitis and periodontitis was observed. The protein level of IL-17A between groups was significant, but for RANKL wasn't significant and no difference between the three groups. **Conclusion:** The elevated IL-17A in priodontitis and gingivitis in this study demonstrated that IL-17A is an important cytokine in pathogenesis of periodontal disease. Therefore TH-17 pathway play important role in inflammatory periodontal lesions.

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# The Relationship between Salivary RANKL and OPG Concentration and Periodontitis in Patients with Type 1 Diabetes Mellitus

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**Background & Objective:** The relationship between diabetes and periodontitis has been proven by several studies. Alteration of the receptor activator of nuclear factor-kappaB ligand (RANKL)/osteoprotegrin (OPG) system have been implicated in several metabolic diseases characterized by increased osteoclast differentiation and enhanced bone resorption. This study aimed to evaluate the relationship between salivary RANKL and OPG concentration and periodontitis in patient with type1 diabetes Mellitus. **Methods:** Unstimulated whole saliva samples were obtained from 27 type 1 diabetic patients of whom 12 had periodontitis and from 23 systemically healthy subjects of whom 12 had periodontitis. Clinical periodontal measurements were then recorded. Statistical analysis was performed by using Kruskal Wallis, Mann-Whitney U test and Spearman correlations. **Results:** Although lowest RANKL and OPG concentration and their relative ratio were observed in diabetic patients without periodontitis, there was not a significant difference in RANKL and OPG concentration and RANKL/OPG ratio between the four groups of study, but OPG concentration and RANKL/OPG ratio correlated positively with the duration of diabetes ($P< 0.05$). When comparing 2 groups of diabetic patients (with or without periodontitis), there has been a significant difference in OPG concentration ($P< 0.05$). There was also a strong positive correlation between CAL and RANKL concentration ($P< 0.01$) and RANKL/OPG ratio ($P< 0.05$) in diabetic patients with periodontitis. **Conclusion:** Type 1 diabetes can cause disturbances in RANKL/OPG ratio, therefore makes the patients susceptible to periodontal breakdown.

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# The Relationship between Salivary RANKL and OPG Concentration and Periodontitis in Patients with Type 2 Diabetes Mellitus

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**Background & Objective:** Alteration of the receptor activator of nuclear factor-kappaB ligand (RANKL)/osteoprotegrin (OPG) system have been implicated in several metabolic diseases characterized by increased osteoclast differentiation and enhanced bone resorption. So the aim of this study was to determine the correlation between the salivary receptor activator of nuclear factor-kappaB ligand (RANKL) and osteoprotegrin (OPG) with periodontitis in type 2 diabetes. **Methods:** Whole saliva samples were obtained from 28 patients with type 2 diabetes, of whom 15 had periodontitis and 13 were healthy. Among 23 non diabetic patients, 12 of them had periodontitis and 11 of them were healthy. Clinical periodontal measurements were done for 6 teeth in each patient. (2 incisors, 2 premolars and 2 molars) saliva was first freezed at -20 c degrees for 24 hours and then at -70 c till all the samples was gathered. RANKL and OPG concentrations were determined by ELISA. Statistical analysis was performed using Mann Whitney U test, Kruskal Wallis for multiple comparisons and Spearman correlation coefficient. **Results:** Although in diabetes with periodontitis group RANKL and OPG concentrations were lower than diabetic groups without periodontitis, there were no significant statistical differences between RANKL & OPG concentrations and RANKL/OPG ratio in diabetic groups, with and without periodontitis. The concentration of OPG and RANKL in healthy group was more than the other groups but the difference was not statistically significant. There was no significant correlation between FBS,2HPP,HbA1c,morning insulin dose ,evening insulin dose and diabetes type2.There was significant negative correlation between RANKL concentration and RANKL/OPG ratio ($P< 0.001$) and negative correlation between OPG concentration and RANKL/OPG ratio ($P< 0.01$) in diabetic groups, with and without periodontitis. **Conclusion:** The imbalance of RANKL or OPG in type 2 diabetes is not associated with periodontitis. As the treatment of diabetes lowers OPG concentration, considering the protective role of OPG in bone destruction and periodontitis, this might indicate that patients with type 2 diabetes are more susceptible to periodontitis. Further studies are warranted to prove it.

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# In vitro Cytotoxic Effect of Some Herbal Medicines on Human Breast Adenocarcinoma MCF-7 Cell Line

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**Background & Objective:** There is also evidence that extracts from some plants contain some pharmacologically active compounds and anti-cancer potential. Breast cancer is a common malignancy and major health problem for women in developed and developing countries and it is leading cause of cancer-related mortality in women. Although significant progress has been made in cancer prevention and treatment, the development of effective treatment schedule remains one of the most challenges in medical technology. Therefore, the search for effective and safe anticancer agents has become very important issue. **Methods:** Breast cancer (MCF-7) cell line was obtained from the Pasteur Institute of Iran and maintained in RPMI with 10% fetal bovine serum. The cells were grown and maintained in a humidified incubator at 37 °C and in 5% CO2 atmosphere. The cells were seeded in 96 well plates and grown, then treated with different concentrations of herbal medicines (Aloe Vera, Origanum, Saffron and Ginger) for 24, 48 and 72 hours, MTT assay was performed for each group. **Results:** As our results show that concentrations of 5, 2 and 1 mg/ml of origanum extract significantly decreased cell viability at 24, 48 and 72 h, dose- and time-dependently manner. In addition concentrations 5, 2, 1, 0.05 and 0.02 mg/ml of ginger extract significantly decreased cell viability at 24, 48 and 72 h. Cell viability significantly decreased with concentrations 5, 2 and 1 mg/ml of saffron extract. However, viability Cells that were treated with aloe vera extract have not shown any significant differences compared to control group. **Conclusion:** Regarding the results, some of the medicinal plants share more cytotoxic effect for special cell lines and this should be in the next studies.

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# Study of Cytotoxic Effect of Garlic Extract on Malignant and Non-Malignant Cell Lines

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**Background & Objective:** Cancer is a major health problem worldwide. Despite of many years of researches, the treatment of cancers are still a problem and effective treatment has not been reported up to now. Natural products are frequently used to prevention and treatment of many diseases, including cancer. Garlic (*Allium sativum L*., Alliaceae) has traditionally been used as a medicinal plant. In this study we have evaluated the effect of garlic on cell viability of malignant cell lines (melanoma cell line (Sk-mel3), gastric cell line (AGS), breast cell line (MCF-7), colon cell line (HT-29)) and compared it to non-malignant cell line L929. **Methods:** Malignant and non-malignant cell lines were obtained from the Pasteur Institute of Iran and maintained in RPMI with 10% fetal bovine serum. The cell line were grown and maintained in a humidified incubator at 37 °C and in 5% CO2 atmosphere. The cells were seeded in 96 well plates and grown, then treated with different concentrations of garlic for 24, 48 and 72 hours, MTT assay was performed for each group. **Results:** The Result show 5, 2, 1, 0.2 and 0.1 mg/ml of garlic extract significantly decreased cell viability of Sk-mel3 cells at 48 and 72h. Furthermore cell viability of AGS cells significantly decreased with Concentrations of 5, 2 and 1 mg/ml of garlic, at 24, 48 and 72h. In addition dose of 5 and 2 mg/ml of garlic significantly decreased cell viability of MCF-7 cells at 24, 48 and 72h, and 5, 2, 1 and 0.01 mg/ml of garlic extract cause significant induction on cell viability of HT-29 cell line, at 24 h. Regarding to the results, garlic has not shown any significant effect on L929 cells. **Conclusion:** In conclusion different doses of garlic extract cause cytotoxic effects on various cancer cell lines. Serenity more study should be planned to clarify effective components.

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# In vitro Effect of Antidepressant Drugs on Murine Macrophages and Lymphocytes

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**Background & Objective:** Fluvoxamine Maleate and Sertraline Hydrochloride which is typically used as antidepressants in the treatment of depression and anxiety disorders are selective serotonin reuptake inhibitors (SSRI). Considering that the serotonergic receptors are present on macrophages and lymphocytes, the aim of this research was to study the in vitro effect of these antidepressant drugs on cell viability of peritoneal macrophages and spleen lymphocytes because these cells play many crucial roles in both the innate and adaptive immune systems. **Methods:** The powder of drugs was dissolved and diluted in RPMI 1640 at final concentrations of 0.02 – 200 mg/L. Peritoneal macrophages and spleen lymphocytes were isolated from female BALB/c mice and cultured for 24 and 48 h respectively. Pharmaceutical solutions were added in different doses. PMA and fMLP were used as stimulators for macrophages. NO assay (Griess method) and MTT test were carried out on cultured cells. **Results:** Sertraline Hydrochloride at presence and absence of stimulators significantly increased NO production of macrophages at all concentrations (0.02-200 mg/L) ($p<0.03$). Fluvoxamine Maleate increased NO production at concentration of 200 mg/L whereas it significantly increased NO production of stimulated macrophages at all concentrations. The MTT assay showed that Fluvoxamine Maleate at concentrations of 20 and 200 mg/L significantly decreased macrophages viability while Sertraline Hydrochloride had no effect. The same results were obtained in the presence of stimulators. Sertraline Hydrochloride decreased viability of lymphocytes at concentration of 200 mg/L and Fluvoxamine Maleate had no effect on viability of lymphocytes. **Conclusion:** Although serotonin is a neurotransmitter, it has different functions as an immune modulator. Therefore the effect of Fluvoxamine Maleate and Sertraline Hydrochloride as SSRIs on NO production of macrophages and cell viability of lymphocytes and macrophages could be attributed to alteration in the amount of intracellular serotonin in these cells. Furthermore, one of the main side effects of antidepressant drugs is gastrointestinal disturbance; hence the effect of these drugs on gastrointestinal immune cells is proposed.

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# Compare Apoptosis in Renal Cell Carcinoma Cell line (ACHN) by Etanolic Extract of *Nigella Sativa* and Thymoquinone and Cisplatin

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**Background & Objective:** In herbal medicine, *Nigella sativa*, Thymoquinone (TQ) is used against cancers, infectious and inflammatory diseases. cisplatin is a drag for cancer. However, its effect on induction of apoptosis in renal cell carcinoma (ACHN). **Methods:** ACHN cells were treated with different concentration of *Nigella sativa* extract (0-2000 μg/ml) and Thymoquinone (5-60 μM) and cisplatin (0-3 μg/ml) for 24, 48 & 72h. Also, L929 cells were studied as normal controls. These Effects on the cell cycle were determined using Flow cytometry. Apoptosis induction was assayed Annexin V and Propidium Iodide (PI) by Flow cytometric analysis. Phosphatidylserine (PS) externalization is relatively increased in early process of apoptosis. It has high affinity for binding a protein called AnnexinV. In this method, PS binding to Annexin V conjugated fluorescein isothiocyanate (FITC). PI allows differentiation of necrotic and apoptotic cells. **Results:** Cytotoxicity effect of TQ *Nigella sativa* and cisplatin were time and dose dependent. TQ (60μM 24h), *Nigella sativa* (1250μg/ml 24h) and cisplatin (1.5μg/ml 24h) induced apoptosis and caused DNA fragmentation in ACHN cells, these concentration in TQ decreased the number of ACHN cells in S-phase and increased them in G1-phase, indicating cell cycle arrest at G1 but in cisplatin is not. **Conclusion:** This study showed that *Nigella sativa* extract and TQ induced early and late apoptosis and also necrosis in renal cell carcinoma without cytotoxicity on L929 cells and cisplatin induced late apoptosis whit necrosis on L929 cells. These results suggested that the tumor cytotoxic effect of thymoquinone on ACHN cells is mediated by process involving apoptosis, necrosis and cell cycle arrest.

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# Morphine Affects Reaction of BALB/c Mice to Infection with *Leishmania Major*

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**Background & Objective:** The aim of this study was to evaluate the effect of acute morphine injection on *Leishmania major* infection in sensitive (BALB/c) and resistant (C57BL/6) mice strains. **Methods:** For this purpose, a low dose (2mg/kg) and a high dose (10mg/kg) of morphine in two injections, the first concordant with parasite inoculation and the second 15 days later, were used. As controls, groups of mice received naloxone before injections of morphine and also a group of mice received saline. Local reaction to parasite inoculation was measured once a week. **Results:** In C57BL/6 mice, all tests and controls, local reaction at the site of parasite inoculation appeared as a small swelling which increased a little during the following five weeks, but decreased to the normal form afterwards. In contrast, in BALB/c mice (except mice receiving 10 mg/kg morphine) local reaction increased continuously and became several times as much as the previous size and some became necrotic. The worst reaction was seen in mice injected with 2 mg/kg morphine and mice receiving 10 mg/kg morphine + 20 mg/kg naloxone, and the least reaction was seen in mice injected with 10 mg/kg morphine. **Conclusion:** These results showed that acute morphine injection doesn't have a significant effect on the reaction of C57BL/6 mice to infection with *Leishmania major*, but has a biphasic dose-dependent effect on BALB/c mice reaction to infection with this parasite.

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# Down-Regulation of IL-2 in Human PBMCs by Isosorbide

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**Background & Objective:** Interleukin-2 (IL-2), a cytokine belonging to Th1 type, has a key role in cell mediated immunity and control of T-cell proliferation. Th1/Th2 cytokine balance influences on immunological condition and imbalance of the Th1/Th2 plays an important role in many pathological states. Isosorbide, as a nitric oxide donor, is one of the most widely used drugs in treatment of many cardiovascular diseases such as congestive heart failure and myocardial ischemia. Furthermore this drug was found out to have anti-inflammatory properties and also inhibitory effect on tumor growth and metastasis in vivo. In the present study we evaluated the isosorbide effect on the IL-2 production in human peripheral blood mononuclear cells (hPBMCs) in vitro. **Methods:** PBMCs obtained from healthy adult volunteers were cultured in complete RPMI medium. The cells were stimulated with phytoheamagglutinin (PHA) and then incubated with different concentrations of isosorbide (0.01-100 µg/ml) for 48 hours. The IL-2 concentrations were quantified by enzyme-linked immunosorbent assay (ELISA) in the cell culture supernatants. **Results:** Isosorbide significantly and dose-dependently decreased the level of IL-2 produced by the PHA-stimulated hPBMCs compared with untreated control cells. **Conclusion:** The results of this study showed that isosorbide down-regulates the production of IL-2 in hPBMCs. These data suggest the potential immuno-modulatory effects of isosorbide through inhibition of IL-2 production. Modulation of Th1/Th2 cytokine balance could be useful for immunotherapy of certain diseases. Our findings suggest that further studies are required to determine isosorbide effect on other Th1/Th2 cytokines.

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# 119Glucantime and Miltefosine Loaded Liposomes for Treatment of Cutaneous Leishmaniasis

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**Background & Objective:** Cutaneous leishmaniasis is caused by different species of Leishmania, and transmitted by the bite of infected sand flies. Available treatments have not provided a strong consistent result, and there is an urgent need for new efficacious treatment. The weak response of current chemotherapeutics is due to their deficient effects on stealth parasites inside macrophages, rapid clearance form site of action and systematic side effects in high doses. Liposome formulation of anti-leishmanial drugs could overcome these problems. **Methods:** Two famous anti-leishmanial drugs; Glucantime and Miltefosine were encapsulated individually in liposomes by a Freeze-drying Double Emulsion method. Liposomes' size and drug concentration were evaluated by Coulter Counter and HPLC, respectively. In addition, formulations were evaluated in vivo by 0.2cc subcutaneous injection of them into skin lesion caused by *L.major* in BALB/c mice twice a week for 35 days. **Results:** The final product is a dry powder with a long shelf life, which forms liposomes when introduced to water. Miltefosine and Glucantime loaded Liposomes were 123 and 138nm in size, respectively. The drug concentration was 124µg/ml for Glucantime and 293µg/ml for Miltefosine. Both formulations significantly decreased the lesion sizes compared to the control group (without treatment). In addition, both formulations reduced amastigote counts after treatment; however, only the Miltefosine effect on amastigote counts was significant ($P< 0.05$). **Conclusion:** Liposomes target drugs passively to the stealth parasites, and inhibit the rapid clearance of drugs from the lesion site. Therefore, liposomal formulations of anti-leishmanial drugs demonstrated a therapeutic effect in much lower doses than traditional doses. In fact, Macrophages clear liposomes rapidly by phagocytosis/endocytosis. Consecutively, lysosome degrades liposome structure and releases its content into the cytoplasm where Leishmania parasites inhabit.

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# 120Effects of Shark Cartilage on T Cells Stimulation Activity of Dendritic Cells

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**Background & Objective:** Shark cartilage is primarily known for its anti-cancer effects. Powerful immunostimulating activity is also reported for some of its components. Dendritic cells (DCs) comprise an essential component of the immune system. These cells, as antigen presenting cells (APCs) to naïve T cells, are crucial in the initiation and regulation of antigen specific immune responses. In this investigation we studied the effect of shark cartilage on T cell stimulation activity of DCs. **Methods:** Shark cartilage proteins were isolated and purified with guanidine hydrochloride, Amicon filters and gel filtration chromatography and their purity was checked by SDS-PAGE. We purified DCs from spleen of BALB/c mice using density gradient (Nycodenz) centrifugation and their adhesion to plastic dish. Purified proteins (14 and 45 KD components) from shark cartilage were added to DCs medium during over night culture. T lymphocytes were isolated from lymph nodes of C57BL/6 mice. These allogenic T cells were used in the MLR assay along with cartilage treated and untreated DCs. T cell proliferation was measured via MTT assay. **Results:** Our results showed that treated DCs with both 14 and 45KD fractions of shark cartilage induced significantly ($P< 0.05$) greater allogeneic responses than control DCs. **Conclusion:** Shark cartilage proteins induce stimulation and maturation of DCs and increase the T-cells stimulation potential of these important cells of the immune system, Therefore can be used in DC mediated T-cells stimulation and production of anti-tumor response.

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# 121Comparison of Apoptosis in Iranian Standard Strain of Leishmania with Anti-Neoplasia Drug (Miltefosine)

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**Background & Objective:** Cell death resembling metazoan apoptosis has been reported in several parasitic protozoans. Apoptosis greatly affects the host-parasite relationship, since the survival of the parasite inside the macrophage requires strict control of the population of the parasite. Miltefosine is originally developed for the treatment of cutaneous metastasis from mammary carcinomas, has proved to be an effective treatment for human leishmaniasis. In the present study we sought to determine the mode of action of miltefosine in Iranian standard strains of Leishmania promastigotes. **Methods:** Iranian standard strains of *Leishmania major* (MRHO/IR/75/ER) and *Leishmania infantum* (MCAN /IR/96/LONDON49) was cultured in NNN and RPMI1640 media. Cytotoxic effect of miltefosine was study with MTT assay. Promastigote stained with Annexin V/PI staining kit and was analyzed with FACS. Also DNA fragmentation test was set. Morphological changes of Promastigote were study in light microscope. **Results:** IC50 of Miltefosine calculated 7-8 µmol and 22 µmol for *L.infantum* and *L.major* respectively. In *L.infantum* after 24 hours, 22% were Annexin V positive but only 2% of control cells were Annexin V positive. In *Leishmania major* 60% were Annexin V positive. After 48 hours percent of apoptotic cells increased in *Leishmania infantum* (92% Annexin V positive) and in *Leishmania major* (80% Annexin V positive) whereas no change was detected in control group. Morphology of promastigotes after treatment with different dose of drug showed shrinkage and rounded cells. **Conclusion:** In this study we sought to determine the mode of action of miltefosine in Iranian standard strain of Leishmania. We have demonstrated that miltefosine causes apoptosis death in Leishmania but *L.infantum* is more sensitive than *L.major* to drug. Our data set the stage for future development of this class of drug for better treatment of leishmaniasis.

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## 122Immunosuppressive Effect of Silymarin on T Lymphocytes in vitro

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**Background & Objective:** Text Silymarin, a mixture of bioactive flavonolignans isolated from *Silybum marianum*, exhibits anti-carcinogenic, anti-inflammatory and cytoprotective effects. **Methods:** In this study, the in vitro immunomodulatory activity of silymarin was investigated using lymphocytes from C3H/HeN mice. Proliferation assay revealed that silymarin at 50 µM concentration significantly inhibited lymphocyte proliferation and induced cell cycle arrest at G0/G1 phase. **Results:** The results of ELISA and RT-PCR analyses indicated that 50 µM silymarin significantly inhibited IL-2 production, both at protein and mRNA levels. The inhibitory effect of silymarin on IL-2 production was also associated with a decrease in IL-2 receptor alpha (CD25) expression in anti-CD3 activated T cells. Immunofluorescence performed on the mouse hybridoma T-cell line (3DO) showed that silymarin 50 µM remarkably inhibited nuclear translocation of nuclear transcription factor kB (NF-kB), which is known to be responsible for IL-2 transcriptional activation. However, no significant change of Glucocorticoid-Induced Leucine Zipper (GILZ) mRNA expression was detected in anti-CD3 activated lymphocytes. **Conclusion:** This study provokes an interest in understanding the molecular mechanism of the immunomodulatory effects of silymarin on lymphocytes. Characterizing the molecular mechanism of such immunomodulatory effects may have a great potential in future treatment of autoimmune diseases.

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# 123The Effect of Different Doses of *Nigella Sativa* Extract on Lung Inflammation of Ovalbumin- Sensitized Guinea Pigs

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**Background & Objective:** Although there are many effective drugs for treating Asthma but sometimes side effects limited usage of them. So, the new drugs with the fewer side effects must be prescribed. As medicinal herbs have therapeutic effects without obvious side effects, nowadays the use of them is advised. *Nigella sativa* (Siah-Daneh) is an example of these herbs. In previous studies, the relaxant, anticholinergic (functional antagonism) antihistaminic effects of *Nigella sativa* have been demonstrated on guinea pig tracheal chains. In the present study, the effect of hydro-ethanolic extract of *Nigella sativa* on lung pathologic changes of ovalbumin-sensitized guinea pigs was examined. **Methods:** Three groups of sensitized guinea pigs to ovalbumin (OA), were given drinking water alone (group S), drinking water containing low concentration of *Nigella sativa* extract (S+LNS group) and drinking water containing high concentration of the plant extract (S+HNS group, n=8 for all groups). **Results:** The pathologic changes of the lung including infiltration of eosinophil and lymphocyte, local epithelial necrosis, the presence of edema, thickening of basement membrane, smooth muscle layer hypertrophy, mucosal secretion and the presence of mucosal plug in these groups were compared with those of control animals. Treatment of S group with the extract significantly improved pathological changes of the lung except for the presence of edema in the sensitized animals which treated with low dose of *Nigella sativa* extract ($P< 0.01$ to $P< 0.001$). **Conclusion:** These results showed a preventive effect of *Nigella sativa* extract on lung inflammation of sensitized guinea pigs. Non significant effect of two different concentrations of the extract and thymoquinone may indicate that the maximum preventive effect of both the plant extract and thymoquinone obtained at lower concentration used.

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# 124Expression of NMDA Receptor Subunits in Human Peripheral Blood Lymphocytes in Opioid Addiction

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**Background & Objective:** Glutamate receptors especially the NMDA-activated ones have a key role in development and maintenance of opioid addiction. It has been proposed that the neurotransmitter receptors expression in peripheral blood lymphocytes may be parallel to their expression state in the brain. This study was designed to evaluate the possibility of using the mRNA expression state of NR2A and NR3A subunits of NMDA receptors in human peripheral blood lymphocytes as a peripheral marker in opioid addiction studies. **Methods:** Four groups each comprising of 20 male individuals participated in the study: opioid addicts, methadone maintained patients, long-term abstinent former opioid addicts, and non-addicted control subjects. Real-time PCR method was used to investigate the mRNA expression level of NR2A and NR3A subunits of NMDA receptor in peripheral blood lymphocytes of all groups. **Results:** Our data indicated that mRNA expression of NR2A subunit of NMDA receptor in all three test groups was not statistically different from control subjects. However, the NR3A subunit expression was significantly down-regulated in abstinent subjects reaching 0.14 the amount of the control group. The expression of NR3A subunit was not significantly changed in addicted and methadone maintained individuals in comparison to control subjects. **Conclusion:** Deficiency in expression of NR3A subunit of NMDA glutamate receptor detected by a peripheral marker may be a risk factor making individuals vulnerable for opioid addiction.

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# 125Effect of Garlic Consumption on the Th1/Th2 Cytokine Patterns in Phytohemagglutinin (PHA) Activated Rat Spleen Lymphocytes

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**Background & Objective:** The balance and regulation of T helper1 (Th1) and Th2- type cytokine patterns are important in the effective immune response against different diseases. To clarify the effect of garlic (*Allium sativum L.*) consumption on the Th1/Th2 balance, we attempted to detect and compare the secretion of gamma interferon (IFN-γ) and interleukin-4 (IL-4), as two prototypes of Th1/Th2 cytokines, in serum and in supernatant of in vitro Phytohemagglutinin (PHA) activated rat spleen lymphocytes. **Methods:** 30 male rats were divided equally into two groups. Treatment group received garlic solution in water (600mg/kg/4mL) and other one received distilled water by gavage as control. After one month, serum and supernatant of PHA activated spleen lymphocytes were analyzed for IFN-γ and IL-4 by Enzyme-Linked Immunosorbent Assay (ELISA) test. In addition, thymus and spleen weights also were measured. **Results:** The results showed, garlic treatment decreased production of IFN-γ from101.73 ± 4.62 pg/ml to 74.64 ± 4.64 pg/ml and increased IL-4 from 26.75 ± 3.35 to 83.92 ± 6.56 significantly ($P< 0.001$) in supernatant of PHA induced spleen lymphocytes. The serum level of these cytokines was not detectable. The mean weight of thymuses in the garlic fed animals was also reduced to 0.368 ± 0.023 g from 0.456 ± 0.016 g in control group ($P< 0.005$). But there were no significant differences between spleen weights in two groups. **Conclusion: G**arlic treated rats developed Th2 type and humoral immune response.

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# 126The in vitro Effect of ACA1, an Herbal Preparation and Its Fractions on Macrophage Viability

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**Background & Objective:** Immunomodulators are the valuable compounds that affect immune responses. ACA1 is reported as an anti-cancer compound recently which is prepared based on traditional medicine. Regarding the potential of traditional Iranian Medicine (TIM) and side effects of chemical drugs on immune system in this study the in vitro immunomodulatory effect of ACA1 and its fractions (R100, R50, R30, R10, R5, F5) on macrophages viability were evaluated. **Methods:** Ten female BALB/c mice were chosen randomly and the pool cells were obtained from intrapretoneal macrophage. The cells were incubated with different doses of ACA1 and fractions (5, 1, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001, and 0.0005mg/ml) in a period of 24 h and macrophage viability evaluated by the use of MTT method. **Results:** Our results have been shown that macrophage proliferation and MTT reduction significantly increased using ACA1 extract and all fractions with doses of 0.1, 0.05, 0.01, 0.005, 0.001and 0.0005 mg/ml. In contrast ACA1 extract and its fractions cause cytotoxic effect with 5, 1, 0.5 mg/ml doses. **Conclusion:** High doses of ACA1 and its fractions result cytotoxicity and intermediate and low dose have proliferative effect on macrophages. Regarding to the cytotoxic effects of ACA-1 on cancer cell lines in the previous studies in intermediate and low doses it can be an opportunity to find new drug for cancer therapy.

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# 127The in vitro Effect of an Herbal Preparation, ACA1 and Its Fractions on Lymphocyte Viability

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**Background & Objective:** ACA1 is a preparation of an Iranian medicinal herb which would be used in Iranian Traditional Medicine (ITM).In vitro studies has been shown ACA1 cause a cytotoxic effect on cancerous cell lines. The purpose of this study is in vitro immunomodulatory evaluation of ACA1 and its fractions (R100, R50, R30, R10, and R5) on lymphocyte cell viability. **Methods:** Three female BALB/c mice were chosen randomly and pool cell were obtain from their spleens. The cells were incubated with different doses of ACA1 and fractions (5, 1, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001and 0.0005 mg/ml) in a period of 48 h and lymphocyte viability were evaluated by the use of MTT method. **Results:** Our data have been shown that lymphocyte proliferation and MTT reduction significantly increased using R5 and R10 fractions in all doses, and decrease in high doses of R100, R50 R30 and whole ACA1. **Conclusion:** The result of this study demonstrated that lower molecular weight fractions induce lymphocyte proliferation in a doses independent manner and high molecular weight fractions have cytotoxic effect in high doses.

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# 128The Effects of Ionizing Radiation on Humoral Immunity and Hematological Markers of Radiology Staff Members of Imam Khomeini Hospital, Tabriz, Iran

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**Background & Objective:** There are many reports about effects of ionizing radiation on immune system functions (Cell mediated or hum oral Immunity) of long term exposed workers. The aim of this study was investigation the effects of occupational exposure of low doses ionizing radiation in humoral immunity and hematology markers of radiology workers. **Methods:** Serum concentrations of total immunoglobulins (IgG, IgM, IgA) and complement fragments (C3, C4) and hematology markers (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT…) were analyzed in 45 radiology workers and 35 age and sex matched healthy control groups. Immunoglobulins (IgG, IgM, and IgA) and C3, C4 and hematology markers were measured by SRID technique (Single Radial Immunodiffusion) and Sysmex K1000 auto analyzer respectively. **Results:** Serum immunoglobulins (IgG, IgM, and IgA) and C3, C4 were measured in workers exposed to ionizing radiation (45 cases) and control groups (35 cases). In comparison, Levels of immunoglobulins (IgG, IgM, and IgA) and C3, C4 of studied group were significantly lower than of control group ($P< 0.005$), but exception of Hb there was no significantly differences of Hematology markers between two groups. **Conclusion:** Humoral immune response were determined as weaker in workers exposed to low levels of ionizing radiation compared with controls, indicating the importance of taking appropriate measures to protect radiology workers from exposure to ionizing radiation and for those workers to avoid smoking . Further studies are needed for determining the appropriateness of periodic check up of immune functions and the most efficient and cost–effective ways of monitoring in radiology workers for detecting early changes in the immune system function.

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## 129Macrophages Cell Viability following Silver Nanoparticles Exposure

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**Background & Objective:** Macrophages are the important cells in innate and cellular immunity. Silver nanoparticles, the resurgence in the use of silver-based antimicrobial agents, are becoming one of the progressively growing products in nanotechnology. Meanwhile, the potential side effects of these nanoparticles on the immune cells have not been studied thoroughly yet. In this experimental study, we assessed the effect of commercial colloidal nanosilver containing 4000-ppm nanosilver particles with the size range of 18–34 nm on viability of murine peritoneal macrophages. An attempt was made to study the cell viability of macrophages after exposure to different concentrations of nano-Ag. **Methods:** MTT reduction assay was used for evaluating cells viability. Cell viability assessment in all of the cultures was fulfilled by measuring the relative absorbance or optical density (OD). Measurement of optical density was performed at 540 nm. **Results:** A significant decrease in cell viability was observed for 1 ppm to 25 ppm of nano-Ag concentrations compared to the control group ($P< 0.01$) after 24 h of cell culture. Also, a significant decrease in the cell viability was observed for 2 ppm to 25 ppm of nano-Ag concentrations after 48 h and 72 h respectively ($P< 0.05$). **Conclusion:** Although, this study has shown the commercial colloidal nanosilver toxicity effect on peritoneal macrophages in vitro at high concentrations, but there are no implications of the health effects in humans. Undoubtedly, cautious measurements must be taken against usage of large concentrations of nano-Ag but it is highly recommended to carry out the in vivo investigation for human to confirm its use.

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# 130In vitro Antitumor and Antibacterial Activities and Synergistic of *Cuminum Cyminum* Essential Oil

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**Background & Objective:** Laboratory tests would be affected by different factors. Moreover medical plant ingredients have been applied for different nutrition, medical and industrial purposes. As main medicine uses are Infections and tumors. In this study we evaluated *Cuminum cyminum* essential oil effects on several common antibiotic functions and WEHI-164 tumor cell line. **Methods:** WEHI-164 mice fibrosarcoma cell line was cultured with different concentrations of *Cuminum cyminum* and cytotoxicity were evaluated by MTT assay. For countercurrent synergistic and antagonistic effects, standard bacteria cultured encounter to plant component contiguous to common antibiotics. **Results:** This oil component had different effects on examined antibiotics. It caused Gentamycin function in every 4 examined bacteria. MTT assay showed that *Cuminum cyminum* essential oil in 50 and 500 μg/ml concentrations significantly inhibit tumor cells growth ($P< 0.001$). **Conclusion:** This study showed that *Cuminum cyminum* essential oil could enhance effects of antibiotics which suggest its application especially in case of drug resistance. In other hand showed probable effect of extrinsic factors on antibiogram tests. Also this component in distinct concentrations had adequate inhibitory effects in tumors.

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# 131Association between the Phe206Leu Polymorphism of L-Selectin and Brucellosis

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**Background & Objective:** Brucellosis remains a major zoonosis worldwide; therefore better understanding of its immunology is a priority for the development of new therapeutic and vaccination strategies. Genetic factors appear to have an important role in the pathogenesis brucellosis. The impact of L-selectin polymorphisms on brucellosis has not been investigated. The aim of this study was to assess L-selectin Phe206Leu polymorphism in patients with active brucellosis and to analyze its possible relation with disease progression. **Methods:** A case-control association study was carried out on 619 subjects, including 374 patients with brucellosis and 245 age and sex- matched healthy controls. Genomic DNA was isolated and amplification of L-selectin genomic regions was performed by polymerase chain reaction-sequence specific primers (PCR-SSP) to distinguish the genotypes. **Results:** The frequencies of F206L polymorphism were studied. A significant difference in F206L polymorphism was found between patients with brucellosis and controls. The 206Leu allele was more frequent in patients than in healthy individuals (36.6% versus 28% P< 0.005). In addition, there was an association between the presence of the 206Leu allele and a relapse of brucellosis (OR 6.53, 95% CI 1.5 to 28.8, P< 0.01). **Conclusion:** The higher frequency of L-selectin genotypes in patients with brucellosis compared to control individuals as well as the association between the 206Leu allele and the occurrence of brucellosis relapse suggests that the Phe206Leu polymorphism could make individuals more vulnerable to brucellosis.

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## 132Frequency of Anti-HCV Antibody in Urban Area of Guilan Province

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**Background & Objective:** Hepatitis C virus was estimated to be responsible for 20% of acute viral hepatitis and 60-70% of chronic viral hepatitis. WHO estimated that about 170-200 million people (3% of the world's population) are infected with HCV, at least 21.3 millions of infected population live in Eastern Mediterranean countries. Prevalence of HCV among general population in Iran is supposed to be less than 1%. The prevalence of hepatitis C is very different in various countries and various regions. Unfortunately, more than 50% of HCV-infected persons are not aware of the disease and spread the infection. Anti-HCV antibody detection test is the first available test for HCV diagnosis. The goal of this study was to define anti-HCV Ab frequency in the population greater than 15 years in urban area of Guilan province, north of Iran. **Methods:** This study was done on the basis of multi-stage simple random sampling. Blood samples were collected from 1240 volunteers above 15 years old and were willing to donate blood samples and sign an informed consent. The serum was separated and freezed. The samples were tested by ELISA method and frequency was calculated. **Results:** The mean age of participants was 40 years and 47% of them were male. Only eight samples were anti-HCV antibody positive (0.6%). **Conclusion:** The prevalence of HCV was less than the previous report on the residents of a Guilan nursing home. This differences might be due to lower level of health in the latter individuals in contrast to our study but we should be aware about increasing rate of intravenous drug abuse may increase HCV prevalence.

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# 133Cloning, Expression, Purification and Production of Antibody against Heat-Labile B Subunit (LTB) Gene against *Enterotoxigenic Escherichia Coli* (ETEC)

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**Background & Objective:** *Enterotoxigenic Escherichia coli* (ETEC) is one of the most common cases of diarrhea among other bacterial agents with a high mortality rate world wide. It has been shown that immune response may be effective against this disease, therefore designing and producing vaccine against this disease is one of the purposes of World Health Organization (WHO). Vaccine candidate molecule(s) have to be safe and immunogenic and induce perform protective immunity against broad spectrum of ETEC strains. Most ETEC strains can produce LT. So, it can be proposed as a vaccine candidate molecule. The aim of this study is the expression of heat labile B subunit toxin (LTB) for investigation of its immunological property. **Methods:** In this study, LTB gene sequence was obtained from gene bank and appropriate primer was designed accordingly. Genomic PCR reaction was performed and its product was cloned into pBlueskriptII SK cloning vector and pET28a expression vector. LTB gene was expressed by IPTG induction and its expression was confirmed with immunobloting. Optimizing of 3 expression parameters (IPTG concentration, time and temperature of promoter induction) was investigated. Then, recombinant protein was purified with Ni-NTA column. For antibody production, purified LTB was injected (s.c) along with complete Freunds' adjuvant to mice. ELISA test was performed on mice sera for determination of antibody production. **Results:** High expression of a 15.5 KD protein was observed after IPTG induction and it's antigenicity was confirmed with anti-CTXB antibody. The optimum expression condition was 1mM IPTG concentration, for 3h and at 37°C. ELISA technique showed high titer of antibody production in immunized mice. **Conclusion:** This preliminary experiment showed that LTB could be expressed as recombinant form and the expressed molecule is a good immunogene, therefore it can be considered as one component of vaccine against ETEC.

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# 134Cytokine Networking of Tumor Bearing Mice in Response to Invasive Aspergillosis

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**Background & Objective:** Invasive Aspergillosis (IA) was increased in chronic immunosuppressive diseases, such as cancer. Because paucity of information about mechanisms by which aspergillus infection effect on immune regulation and cytokine networking of cancer patients, this study was made to investigate the effect of invasive aspergillosis on cytokines and Th1/Th2 pattern of tumor bearing mice (TBM). **Methods:** After implantation of spontaneous mouse mammary tumor (SMMT) in BALB/c mice, they were infected with *Aspergillus conidia* intravenously. For comparison, groups of mice were experimentally infected with *Aspergillus conidia* or implanted with tumor cells separately. Seven days after aspergillus infection, the serum levels of IL-10, IFN-γ, IL-4 and TNF-α were measured by ELISA. **Results:** Tumor bearing mice that challenged with *A. fumigatus conidia* showed a decrease in IFN-γ and increase in TNF-α level. But these data was not significant in compared with control groups (normal mice challenged with A. fumigatus and non infected cancer mice). Conversely, IL-10 and IL-4 were dominantly raised and mortality rate of I.A infected TBM animals was highly increased. It would be noted that in addition to stimulation of innate and Th2 cells mediated immune responses, I.A infection could change regulation cytokine production from CD4+ T helper cells and acquired immunity of cancer mice. **Conclusion:** We hypothesize that concomitance of I.A and cancer may change the microenvironment for local or systemic immune responses. Other complementary studies could help for supporting our hypothesis.

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# 135Cloning, Expression and Purification of *Mycobacterium Tuberculosis* ESAT-6 and HSP70359-610 as a Tuberculosis Vaccine Candidate in a Prokaryotic System

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**Background & Objective:** Tuberculosis (TB) remains as a major public health problem worldwide. The identification of immunodominant antigens of *Mycobacterium tuberculosis* (MTB) which were able to induced strong immune responses is goal of researcher for TB vaccines. **Methods:** According to previous reports, this study was designed to produce a novel *M. tuberculosis* fusion protein consisted of ESAT-6 (early secreted antigenic target-6 kDa), as a potent immunogenic protein from *M. tuberculosis*, fused to C-terminus of MTB.HSP70 (HSP70359-610), as a carrier and adjuvant. We fused the gene of ESAT-6 and HSP70359-610 in pQE30; a prokaryotic expression vector. This recombinant protein, with a histidine tag, was successfully over expressed in *Escherichia coli* M15. In order to obtain high yields of the recombinant fusion protein, inclusion bodies from bacterial cell lysates were solubilized and recombinant protein was purified by Ni–NTA affinity chromatography under denaturing conditions, followed by urea gradient dialysis. The immunogenicity of E6H70C fusion protein was demonstrated in indirect ELISA. **Results:** The results indicate that this fusion protein could induce the production of specific antibody in mice. **Conclusion:** Thereupon, this fusion protein would be considered as a novel fusion protein which would be used as a potential candidate for subunit vaccine against tuberculosis.

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# 136Studies on Immune-Dominancy of HCV-CTL Epitopes by Peptide Immunization in BALB/c Mice

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**Background & Objective:** Epitopic-peptides presenting either single or multiple CTL epitopes are considered as promising vaccine candidates against hepatitis C virus (HCV). However, the so called immune-dominancy of epitopes upon each other is still a not well characterized phenomena and a matter of challenge towards the epitopic-peptide vaccine development. To address this phenomenon, three groups of mice were immunized by either single minimal-CTL-epitopic peptides, their mixture or long synthetic peptides composed of these minimal-HCV-epitopes in tandem and CTL responses against H2D epitopes were compared. **Methods:** Two HCV-derived HLA-A2 and two H-2d-restricted epitopic peptides of core132-142(C1), E2405-414(E4), E2614-622(E6) and NS31405-1414(N) were synthesized either individually or as multiepitope long peptides in tandems of C1E4E6N, C1E6NE4, and NE4E6C1. BALB/c mice subcutaneously received 2 × 50 uM of either the minimal peptides (C1 or E4) or their combinations (C1+E4+E6+N) or the multiepitope peptides that were emulsified in human compatible adjuvant mixture of Montanide ISA720 + CpG1826. Promoted T-cell responses against the H-2d-restricted C1 and E4 epitopes in vaccinated mice were separately evaluated using IL-4 and IFN-γ-ELISpot assay. **Results:** While the mice that received individual C1 or E4 peptides promoted epitope-specific IFN-γ-secreting cells, the group immunized with the mixture of peptides (C1+E4+E6+N) mounted a high frequency of E4-specific T-cells but a low response against the C1 epitope, indicating immunodomination. Interestingly, application of multiepitope long peptides with different tandems led to the simultaneous induction of balanced immunity against both epitopes, though in a lower level, signifying the compensating effect of epitope processing on the immunodomination. Herein, no group indicated a considerable increase in the frequency of IL-4-secreting cells. **Conclusion:** The results of this study argues in favor of the determining outcome of epitope positioning within the multiepitope long peptides towards the prevention of immunodominance and propounds the multiepitope strategy as a way of compensation when encountering with the immunodominance phenomenon.

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## 137Comparative Studies on Mice Immunization with HCV Minimal-CTL Epitopic Peptides by Mixed or Polytope Formulations

**Background & Objective:** CD8+ T-cell (CTL) synthetic peptide vaccines hold great promise for prevention/therapy against hepatitis C virus (HCV). However, there is no comparative information available regarding their administration as mixture of minimal epitopic peptides or multiepiope single peptides compromised of individual epitopes as well as the requirement for inclusion of CD4+ helper epitopes. This study proceeds to the evaluation and comparison of the T-cell responses induced against the HCV CTL epitopes, when administered either in the context of multiepitope peptides or as mixed epitopic-peptides with or without the inclusion of a universal T-helper epitope (PADRE). **Methods:** Six HLA-A2 and H-2d-restricted epitopic peptides of core132-142(C1), core35-44(C3), E1363-371(E3), E2405-414(E4), E2614-622(E6) and NS31405-1414(N) that are categorized among the immunodominant or subdominant HCV epitopes were synthesized either individually or as multiepitope long peptides in tandems of C1E4E6N, C1E6NE4, NE4E6C1 and E4E3C3. BALB/c mice subcutaneously received 2 × 50 uM of either the minimal peptide combinations or the multiepitope peptides that were emulsified in human compatible adjuvant mixture of Montanide ISA720 + CpG1826. Promoted T-cell responses against H-2d-restricted epitopes in vaccinated mice were evaluated using IL-4 and IFN-gamma ELISpot assays. **Results:** Immunized groups mounted different levels of T-cell immunity against the BALB/c epitope of E4 administered in different contexts. Wile the inclusion of PADRE in multiepitope peptides didn't significantly increase the IFN-gamma secreting cells, the minimal epitope mixtures induced the highest level of effector cells capable of producing IFN- gamma, especially when mixed with PADRE. However, no group indicated a considerable increase in the frequency of IL-4-secreting cells. **Conclusion:** These results evidenced for the efficient induction of epitope-specific T-cells by minimal epitopic peptide immunization, though the multiepitope peptides requiring the internal processing could also promote the T-cell responses in a lower level. Moreover, the effect of PADRE seemed to depend on the formulation but generally was shown to be positive.

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# 138Comparison of Different Adjuvants for the Induction of Humoral Response against HCV ARFP/F Synthetic Peptides in BALB/c Mice

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**Background & Objective:** In vitro studies have described the synthesis of an alternative reading frame form of the hepatitis C virus (HCV) core protein (known as F protein or ARFP), which includes a domain encoded by the +1 frameshift mutation in core gene. Previously, we showed that two ARFP-derived B-cell peptides, denoted as p3 and p8, effectively induced the humoral response in BALB/c mice. Herein, we study the capacity of different adjuvant formulations to enhance the induction of humoral response against these immunogenic peptides. **Methods:** Different types of Montanide (ISA206, ISA50V, Ims1312Pr) as well as plourounic acid, imiquimod and incomplete Freunds adjuvants were separately mixed with p3/p8 peptides to prepare the immunogene mixtures. Antibody titers against p3 and p8 were analyzed using ELISA assay. **Results:** Results of ELISA assay indicated that high titers of specific antibodies recognizing the ARFP/F-related synthetic peptides were obtained when these peptides were delivered with Montanide ISA50V and incomplete Freund's adjuvant. However, Montanide Ims1312Pr and plourounic acid formulations were less effective towards the inducing of humoral immune response. **Conclusion:** This approach provided the conceptual and experimental framework for the determination of potent immunogenic adjuvants eliciting an enhanced and specific anti-ARFP/F humoral response with the aim of preparation of monoclonal antibody against ARFP/F protein.

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# 139Studying the Capacity of HCV ARFP/F Peptides to Induce Humoral Immune Responses in BALB/c Mice

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**Background & Objective:** Hepatitis C virus ARFP/F (core+1) protein, a newly discovered HCV gene product, is expressed by translational ribosomal frameshift in core open reading frame. While the biological properties of this protein still remain unknown, specific anti-ARFP/F antibodies and T-cell responses in HCV-infected patients have been reported by many independent laboratories. In this study, we designed four B-cell epitopic peptides of ARFP/F protein to evaluate their immunological properties and further utilize them for the preparation of monoclonal antibody (mAb) against core+1. **Methods:** An immunogene mixture containing four designed ARFP/F-derived synthetic peptides (P3, P4, P6, and P8, 20ug each), formulated in Montanide ISA720 + CpG adjuvants was subcutaneously injected to BALB/c mice and humoral immune response was evaluated using ELISA assay. **Results:** The adjuvant-mixed ARFP/F-related peptides were capable of eliciting potent humoral immune responses in BALB/c mice. However, differences were observed in the induced Ab titers against these peptides. Accordingly, p8 and p3 were known as the most effective immunogenes for eliciting B-cells in mice. **Conclusion:** Up to here the results of this study indicated the potency of designed ARFP/F-derived synthetic peptides as promising targets in HCV immunological studies and provided enough motivation for further attempts towards the production of mAb proficient of differentiating between core and core+1 for diagnostic aims.

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# 140Frequency of Hepatitis B Carriers and Evaluation of Vaccination Status and Immune Response in Health Care Workers in Kahnooj, Kerman

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**Background & Objective:** Hepatitis B virus (HBV) vaccination is recommended for all Health care workers (HCW) at risk of exposure to infection. However the efficacy of immunization some times dose not occur perfectly .Therefore we evaluated the state of HBV vaccination and surveyed immune response to HBV vaccine and other subjects related to this in HCW in Kahnuoj, Kerman. **Methods:** in this cross-sectional study we enrolled 191(120 women, 71 men) HCW from Hospital and Health care center in Kahnooj . we acquire their agreements and completed data forms included age, sex , history of vaccination , smoke , for each participant hen we collected blood samples and determined 3 items of HBV infection including HBs antigen(HBsAg ), HBs antibody (HBsAb), HBc antibody(HBcAb) using ELISA method . Analysis of data was performed with SPSS soft ware and $\chi 2$ and t tests. **Results:** HBs Ag were detected in 2 subjects (1%) and 4 participants (2%) were positive for HBcAb . Only 118(62%) of 191 HCW who enrolled completed full course of vaccination and 24(12.6%) subjects did not have any history of vaccination, 75(63.2%) subjects who had completed vaccination had perfect immunity (HBsAb > 100 mu/ml), 25(21.2%) subjects were immune partially (HBsAb 10-100 mu/ml) and 18(15.7%) subjects dose not have any response to vaccine (HBsAb <10 mu/ml). There were significant relation between age, sex and HBsAb titer $P< 0.001$ and there was not significant relation between smoke and immune response ($P< 0.05$). **Conclusion**: HCW vaccination program in Kahnooj did not have performed perfectly, only 51% of HCW have good immunity to HBV. We suggest to implement vaccination program and use of hepatitis B immunoglobulin be mandatory in needle pricked HCW in Kahnooj.

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## 141Performance of Western Blot in Hydatidosis Diagnosis

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**Background & Objective:** The aim of this study is to evaluate the contribution of the immunowesternblot for the diagnosis and follow-up of pre-and postsurgical hydatidosis. **Methods:** This study related to 67 serums of confirmed cases of hydatidosis, but they had a negative hydatic serology by the three traditional techniques (ELISA, Hemagglutination, Electrosynerese).12 patients with sera in pre and post operative were monitored for 2 years, providing a mean of 2 sera per patient. **Results:** For the 38 hepatic cases of hydatidosis, the test of immunowestern blot made it possible to rectify the diagnosis in 73, 68 %. The rate of positivity was 100 % for the cysts multivesiculaires of the type III, of 60 % for the young cysts of type (I, II) and of 50 % for the calcified cysts standard (IV, V).In the pulmonary cysts hydatic, Western blot made it possible to rectify the diagnosis in 62.5 % of the cases. Western blot was positive in 50 % of the cases for the cerebral localizations. For the cysts with multiple localization, Western blot was positive in 100% of the cases. Analysis of WB evolution in the 12 patients followed in pre and post surgical revealed a time of negativity of this technique was 8 months. **Conclusion:** The biological diagnosis of the hydatidosis is difficult, serologic interpretation is sometimes delicate in front of unmatched results. This study proved the value added of the technique Echinococcus western blot IgG by contribution to the other traditional techniques for the immunodiagnostic and the post-surgical monitoring of hydatidosis.

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# 142Comparison of Real time PCR, PCR-ELISA and Galctomannan Antigen in the Diagnosis of Invasive Aspergillosis in Hematologic Disorders

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**Background & Objective:** Invasive aspergillosis is a major opportunistic infection in hematology patients. Early diagnosis of invasive aspergillosis is essential to reduce lethality. Objectives: evaluate the contribution of Galactomannan antigen and molecular methods (real-time PCR assay and a PCR-ELISA) in both serum and bronchoalveolar lavage for diagnosis of invasive aspergillosis. **Methods:** BAL samples, if available, and serum were prospectively taken twice weekly from patients at risk of IA in the hematology wards of the Sfax University Hospital. The detection of Galactomannan antigen was performed by Platelia TM Aspergillus test. Using a nested case-control design, both PCR assays were performed on proven and probable IA cases and matched control samples. **Results:** Of the 163 patients included in the cohort, one proven, 31 probable, and 15 possible cases of IA were diagnosed on the basis of EORTC/MSG criteria. Real-time PCR, PCR-ELISA, and galactomannan antigen (GMA) assays performed on 459 serum samples found 93.8%, 96.9%, and 100% sensitivity, respectively. Specificity was 100%, 100%, and 91.5%, respectively. In 42 BAL samples, sensitivity was 64.3%, 71.4%, and 85.7%, and specificity was 96.4%, 96.4%, and 92.9%, respectively. **Conclusion:** While slightly less sensitive, the real time-PCR assay was highly specific and considerably faster and more workable than PCR-ELISA. Combining real-time PCR and GMA detection in both serum and BAL samples enhances routine laboratory IA diagnosis.

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# 143Apoptosis of Human Lymphocytes after Exposure to Fertile and Infertile Hydatid Fluid

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**Background & Objective:** Modulation of the immune response is an important strategy by which establishment and growth of hydatid cyst in the human body is warranted. Induction of apoptosis in the lymphocytes might be a considerable component in this immunomodulation. This study was designed to evaluate apoptotic impact of hydatid fluid (HF) on human lymphocytes. **Methods:** Human lymphocytes from a healthy person were separated and treated with hydatid fluid of fertile and infertile hydatid cysts. A control with no added hydatid fluid was used for comparison of apoptosis in the lymphocytes. After 6 hours of exposure, caspase-3 activity, the central enzyme of apoptosis cascade, was measured by fluorometric assay in the HF-treated lymphocytes and control. In addition, expression of Bax (a pro-apoptotic protein) and Bcl-2 (an anti-apoptotic protein) at mRNA level was assessed by RT-PCR after 12 hours of exposure. **Results:** Both the ratio of Bax/Bcl-2 mRNA expression and Caspase-3 activity were higher in the fertile HF-treated lymphocytes regarding to the infertile HF-treated lymphocytes. Comparing with cell control, apoptosis in the human lymphocytes was higher after exposure with fertile hydatid fluid. **Conclusion:** This study represents the apoptosis as a possible mechanism by which E. granulosus overwhelms host defenses.

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# 144Production of Different Transgenic Leishmania Strains Expressing GFP through Homologous Recombination Method

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**Background & Objective:** In cellular biology, the enhanced green fluorescent protein (GFP) has been used as reporter gene, cell marker and fusion tag. The researchers have also shown GFP ability to quantitatively monitor gene expression in different organisms. Herein, we report the use of Leishmania expression system (LEXSY) for high and stable levels of GFP production in different Leishmania strains such as *L. tarentolae*, *L. major* and *L. infant*. The GFP-transfected Leishmania strains can be used for in vitro screening of anti-leishmanial drugs and also understanding the biology of the host-parasite interactions at the cellular level. **Methods:** Directional cloning of GFP into pLEXSY-neo2 was performed and confirmed by PCR and restriction analysis of recombinant plasmid (pLEXSY-GFP). The recombinant plasmid was prepared in large scale with high purity. Promastigotes of different Leishmania strains were transfected by electroporation using 5 microgram of linearized pLEXSY-GFP. The growth of cells highly resistant to neomycin (G418) was observed after 15-20 days. Clones were selected on Noble agar plates and further propagated in liquid culture medium (M199). Confirmation of genomic integration was done by diagnostic PCR using genomic DNA of transgenic strains as template. GFP expression in live Leishmania was evaluated and confirmed by fluorescence microscopy, Flow cytometry, in vitro bone marrow–derived macrophages as well as RT-PCR. **Results:** The linearized expression cassette containing the encoding region for GFP (pLEXY-GFP) was integrated into the chromosomal ssu locus of three Leishmania strains through homologous recombination. Our data showed that GFP transgenes can be abundantly and stably expressed in promastigote and amastigote stages of *L. tarentolae*, *L. major* and *L. infant* in the absence of G418. **Conclusion:** Our studies indicated that the promastigotes and amastigotes expressing GFP from the integrated plasmid could be detected directly without the need for additional preparation. The utilization of this DNA cassette will be appropriate for studies of long-term expression of transgenes during infection detection.

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# 145Reduced Infectivity of *Leishmania Major* Heterozygote Mutants of Signal Peptidase Type I in Macrophages

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**Background & Objective**: Leishmania species are responsible for a wide spectrum of disease in human and it is. One of the major parasitic diseases targeted by the WHO. Safe and effective vaccine is the major goal to control the disease. Various secreted proteins contain signal peptides which are used as targeting component for the membrane. These peptides are cleaved by signal peptidase (SPase). It is a proven fact that SPase type I is essential for survival and growth in prokaryotic cells. Our aim in this study is to evaluate the role of SPase type I in *Leishmania major*, the causative agent of cutaneous leishmaniasis. **Methods:** By homologous recombination strategy, we were able to disrupt one allele of SPase I gene in *L.major* and heterozygote line was obtained. In order to define its role in infectivity, three types of cells were infected in vitro with heterozygote mutant and wild type *L. major* as positive control. In this study, bone marrow-derived and peritoneal macrophages were isolated from BALB/c mice in addition to mouse macrophage cell line RAW 264.7 were used. After macrophage infection, the potential of parasites infectivity was evaluated with either Hoechst 33258 or Diff-Quick solution and visualized by light or fluorescence microscope. **Results:** In all infected macrophages, the level of infectivity of heterozygote mutants is significantly reduced in compare to wild type strain of *L. major*. The heterozygote parasites were weak to invade, to attach or even to replicate and/or survive inside of macrophages in the form of amastigote. **Conclusion:** The obtained data showed for the first time that *L. major* SPase type I is an essential gene for parasite, and has an important role in infectivity and survival of the parasite inside the macrophages.

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# 146Th1/Th2 Cytokine Pattern in Human Amoebic Colitis

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**Background Objective:** Amoebiasis caused by *Entamoeba histolytica* is still mentioned as one of the major health problems in developing countries. Since the immune response during human amoebiasis has not been profoundly studied we tried to evaluate cytokine production in patients suffering from amoebic colitis. **Methods:** A case-control association study was carried out on 62 subjects, including 31 patients with amoebic colitis and 31 age, sex and geographic region-matched healthy controls. Serum levels of IL-12, IFN-γ, IL-13 and IL-5 were measured by solid-phase sandwich enzyme linked immunosorbant assay. **Results:** Serum levels of IFN-γ, IL-12, IL-13 and IL-5 were higher in the patients with amoebic colitis than healthy controls, but these differences statistically significant only for IL-5 ($P< 0.05$) and IL-13 ($P< 0.05$). Stratification of patients according to gender revealed a significant elevated of IL-13 in men than women ($P< 0.05$). **Conclusion:** It is proposed that in human amoebic colitis, developing Th2 response which presents with increasing in IL-5 and IL-13 in the early stage of disease, acts as a double blade sward.

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# *147Lactobacillus Acidophilus* and *Lactobacillus Casei*, on BALB/c Mice with Systemic Infection of *Candida Albicans*

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**Background & Objective:** Candidiasis is one of the most important and widespread opportunistic fungal disease in human. Probiotics are bacteria with beneficial effect and many study showed their immunomodulatory effects on many infections. In this report we study the immunomodulatory effect of two probiotics on BALB/c mice with systemic infection of *C.albicans*. **Methods:** female BALB/c mice (6-8 weeks old) were treated orally for a month with 2.4×108 of different probiotic daily. Control groups received PBS. Then mice were systematically infected with 2×106 *Candida albicans* via tail vein and then orally administration was continued for 2 weeks. Then cytokine assay of spleen lymphocytes was performed and the production of cytokines IL-12, IL-4, TGF-β and IFN-γ was determined by ELISA. Also, their survival was monitored daily up to all mice died. **Results:** our results showed there is no survival benefit from probiotic administration. In cytokine assay there is no differences between IFN-γ production between the groups and the control. However, in all the groups the amount of TGF-β and IL-4 increased significantly as compared with control groups. IL-12 increased significantly in mice which received *L.acidophillus* only. **Conclusion:** These results showed that in mice model system with the schedule and dosage we used probiotics can be used as prophylactic but they are not effective for treated of *Candida albicans* systemic infection.

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# 148Proteome Study of Sera from Chronic Hepatitis, Cirrhosis and Hepatocellular Carcinoma Patients Related to Hepatitis B Virus by 2-Dimentional Electrophoresis

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**Background & Objective:** Hepatocellular carcinoma (HCC) is the fifth most common cause of cancer and the third cancer- killer worldwide. More than 50% of HCC cases are related to hepatitis B virus (HBV) infection. HBV cause a wide spectrum of clinical manifestations ranging from healthy carrier state to acute and chronic hepatitis, liver cirrhosis, and HCC. The 5-year survival of HCC patients is only 5% that in part related to the diagnosis of HCC at an advance stage, because of poor sensitivity and specificity of available diagnostic tools. So, early diagnosis remains the key to effective therapy for survival improvement of the patients. The aim of this study was to identify proteins that are differentially expressed in HCC patients compare to cirrhosis and chronic active hepatitis related to HBV infection by two dimensional polyacrylamid gel electrophoresis (2D- PAGE). **Methods:** Sera from chronic active hepatitis, cirrhosis and HCC patients related to HBV were subjected to -2D- PAGE. After silver staining, gels were scanned with GS-800 scanner and analyzed by prodigy software for finding differentially expressed proteins. **Results:** We found that chronic hepatitis, cirrhosis and HCC patients had different serum protein patterns. Thirty eight protein spots were differentially expressed between chronic hepatitis and cirrhosis patients. Twenty nine protein spots were different between HCC and chronic patients and 32 spots were differentially expressed between cirrhosis and HCC patients more than 2 fold. These spots had molecular masses (Mr) between 15 - 60 kDa and isoelectric point (pI) from 4-7. **Conclusion:** Our data show that sera from different stages of hepatitis patients have different protein patterns. Characterizations of these differentially expressed proteins by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) is underway.

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# 149Route of *Leishmania Tropica* Infection Modulates Pathogenicity, Immune Response and the Induced Protection against *Leishmania Major* in BALB/c Mice

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**Background & Objective:** *Leishmania tropica* is the causative agent of human cutaneous and viscerotropic leishmaniasis. We have already proposed BALB/c mouse as an experimental model for these human diseases. Clarification of the details of this interesting experimental model can show in what extent this model can represent the related human diseases. Route of infection can be an important variable in this experimental model. Aim of this study was to explore the effect of the route of *L. tropica* infection on the immunologic parameters and the disease outcome in BALB/c mice. **Methods:** BALB/c mice were infected by *L. tropica* by two different routes: subcutaneously into the footpad and intradermaly into the ear dermis. Mice were challenged by *L. major* in the contra lateral footpad after establishment of *L. tropica* infection. The immune response was evaluated at two intervals: one week and one month after challenge. Lesion development was monitored throughout the experiment. Single cell suspensions were prepared from draining lymph nodes of mice. Cells were stimulated by phorbol myristate acetate (PMA). The production of gamma interferon (IFN-γ) and interleukin 10 (IL-10) were determined by intracellular cytokine assay using Flow cytometry. Cellular expression of CD4 and CD25 were studied by Flow cytometry. **Results:** Infection through subcutaneous route in comparison to the intradermal route induces higher levels of IFN-g production, lower levels of $CD4^+$ lymphocytes, and more protection against *L. major* challenges in BALB/c mice. **Conclusion:** Intradermal infection of *L. tropica*, in comparison to subcutaneous route, induces significantly different immune responses in BALB/c mice. Since the intradermal route of infection in the ear dermis seems to be more close to the natural route of infection, we propose the route of *L. tropica* infection in BALB/c mice as an important variable that should be taken into consideration in establishment of an appropriate experimental model for human *L. tropica* infections.

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# 150Evaluation of Immune Response against Recombinant Proteins HSP70 and GP63 of Leishmania Parasite in vitro

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**Background & Objective:** HSP70 and GP63 are presented by all Leishmania species studied so far, and are major immunogene in infections caused by the parasite. The aim of the present study was to amplify, clone and express HSP70 and GP63; and evaluate immune response against them in vitro. **Methods:** *L.infantum* HSP70 and *L.major* GP63 were cloned and expressed using *E.coli* Rosetta and purified by HiTrap Chelating column. C-terminal of GP63 was cloned and expressed too. Peripheral blood mononuclear cells of three groups of human (recovered from cutaneous leishmaniasis, healthy with positive Leishmanin skin test, healthy with negative skin test, respectively) were stimulated with PHA, HSP70, GP63, GP63-HSP70, C-GP63, C-GP63-HSP70 to evaluate their lymphoproliferation and cytokine production (IL-10 and IFN-γ) response. **Results:** We couldn't find any statistical significant differences between three human groups regarding lymphoproliferation and cytokine production after stimulation with different proteins. **Conclusion:** Based on the above mentioned result we can conclude that in vitro evaluation is not a suitable method to find efficacy of the antigens. Then we suggest the usage of these two antigens in an in vivo study using animal models.

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# 151Genetic Susceptibility Factors in Brucellosis

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**Background & Objective:** Brucellosis is a zoonotic disease caused by the genus Brucella. . It is now clear that infection with Brucella spp promote cell-mediated immune reactions. Physicians have long been aware of the markedly different immune responses of seemingly similar individuals to the same inflammatory or infectious agents. The role of individual genetic differences as an explanation for these observations has been the subject of much speculation. Several studies have identified some polymorphisms in cytokine gene regulatory regions that correlate with inter-individual variations of cytokine production in immune response against pathogens. Hence, we tried to find any probable association of genetic factors with susceptibility to the disease. **Methods:** Hundred and ninety-six patients with brucellosis and 81 healthy farmers (controls) who owned infected animals and consumed their contaminated dairy products were included in this study. IL-1B, IL-4, IL-6, IL-8, IL-10, IL-12, IL-15, IL-18, IFN-γ, TNF-β, TLR-4 and CD14 genotyping were carried out for all the subjects using PCR-RFLP and allele specific PCR (AS-PCR). **Results:** Genotypes and/or alleles frequencies of IL-10 (-592A/C and -819T/C, P=0.034), IL-8 (-251A/T, P< 0.01), IL-4 (-590C/T, P< 0.05), IL-12 (+1188 A/C, P< 0.05), IL-18 (-137G/+113T/+127C, P< 0.00005), IL-18 (codon35/3C, P< 0.005), IL-18 (-656G/-607C, P< 0.05), TNF-β (+252 A/G, P< 0.0005), IFN-γ (+874 A/T, P< 0.05), were significantly different between patients and the controls. **Conclusion:** Based on our findings, we can consider IL-10 (-592 C and -819C), IL-8 (-251A), IL-4 (-590T), IL-12 (+118C), IL-18 (137G/+113T/+127C/-656G/-607C/codon 35/3C), TNF-β (+252G), IFN-γ (+874A) as genetic susceptibility factors for brucellosis.

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# 152Finding Latent *Mycobacterium Tuberculosis* Infection in Health Care Workers Using ESAT-6 Interferon-Gamma Assay

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**Background & Objectives:** Latent tuberculosis infection is considered as a serious health problem around the world. Among population, health care workers are at high risk of such infections. To determine the frequency of IFN-gamma producing T cells against Mycobacterium ESAT-6 antigen and comparing that to PPD reactivity in vitro and in vivo in Shiraz University medical centers. **Methods:** Fifty lab workers from microbiology laboratories and radiology departments, in addition to 30 healthy individuals were recruited in this study. Standard PPD skin test was performed and reactivity of blood T cells against recombinant ESAT-6 and PPD were analyzed in IFN-gamma ELISPOT assay. Positive reactions and frequency of responsive cells were calculated and compared. **Results:** Participants in this study had work experience of 1-33 years (Mean 11.2±9.5). Only 6 of them (12%) showed skin reaction zone greater than 10mm. There was no correlation of this reactivity and years of work experience among microbiology technicians. ELISPOT assay showed presence of up to 106 PPD reactive T cells in a million PBMC of peripheral blood in 14 microbiology technicians (56%); however this reactivity in radiology personnel was one in tenth (8%). Analyzing data from ESAT-6 reactivity revealed only one technician of microbiology (4%) with reaction greater than cutoff, while neither of radiology personnel showed this reaction. Comparison of PPD and ESAT-6 data showed no correlation. **Conclusion:** Health lab workers always are in front line of exposure to infections and tuberculosis is one of the most dangerous. Latent infection of tuberculosis in these people not only can cause health problem for them but also can spread the infection. Results of this study showed the specificity and efficiency of interferon gamma to detect such infection.

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# 153Comparison of Cell Death, Circulating Levels of Tumor Necrosis Factor–α and Tumor Necrosis Factor Type-I Receptor in Iranian Patients with Sepsis and Normal Controls

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**Background & Objective:** The present study was designed to comparison of cell death, circulating levels of tumor necrosis factor-α and tumor necrosis factor type-I receptor in Iranian patients with sepsis and normal controls. **Methods:** Twenty-two patients with sepsis were included in this study. After blood draws, the serum circulating level of TNF and TNFR1 measured with ELISA kit. The PBMCs isolated from blood samples and proportion of apoptotic cells measured by Flow cytometry at the time of blood draws (0 time) and after 24-h incubation. PBMCs incubated at 37°C in medium (spontaneous apoptosis) and in the present of rTNF that capable of inducing apoptosis in activated T cells expressing the TNF family of receptors. **Results:** PBMCs obtained from the patients had significantly higher ($P< 0.001$) proportion of apoptotic cells than PBMCs of controls at 0 time, indicated that a higher fraction of PBMCs was undergoing apoptosis in vivo in patients than controls. After 24-h incubation, Spontaneous ex vivo apoptosis of PBMCs was nearly as high as that of TNF induced apoptosis, indicating that activated T cells were preprogrammed in vivo to die. The circulating level of both TNF and TNFR1 from patients had significantly higher ($P< 0.001$) than controls and this increase is proportional ($r=0.908$), indicating that TNFR1 may have been a protective effect in the early stage of sepsis. **Conclusion:** Sepsis is complicated malformations that induce activation of several cascades, including pro and anti inflammatory systems, coagulation and complement cascades, and it is predictable that several ways of cell death involve in pathogenesis of sepsis.

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# 154Evaluation of T Cell Immune Responses in Multi-Drug Resistant Tuberculosis Patients to *Mycobacterium Tuberculosis* Total Lipid Antigens

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**Background & Objective:** *Mycobacterium tuberculosis* lipid antigens produce significant T cell responses in healthy tuberculin reactor (PPD-positive) individuals. The present research aimed at studying the proliferation responses and IFN-γ/IL-4 cytokine production of the MDRTB patients T cells stimulated by Mycobacterium tuberculosis lipid antigens. **Methods:** Total lipid antigens were extracted from *Mycobacterium tuberculosis* H37Rv. Peripheral Blood Mononuclear Cells (PBMCs), $CD4^+$ and $CD8^+$ T cells were stimulated by immature dendritic cells primed by the lipid antigens. Proliferation responses by MTT assay and cytokine production by ELISA were evaluated. **Results:** Proliferation responses of the stimulated PBMCs and $CD4^+$T cells by lipid antigens in the MDRTB patients were lower than in the $PPD^+$ subjects. $CD8^+$T cells proliferation responses of the MDRTB patients against lipid antigens were similar to those of the $PPD^+$ subjects. Production of IFN-γ in the stimulated $CD4^+$T cells by the total sonicate and lipid antigens in the MDRTB patients was lower than in the $PPD^+$ donors and IL-4 concentrations in the patients significantly were elevated ($P< 0.05$). IL-4 production by $CD8^+$T cells stimulated by lipid antigens was not considerable. **Conclusion:** *M. tuberculosis* lipid antigens stimulated proliferation responses and IFN-γ/IL-4 production in the T cells that in the MDRTB patients were suppressed relative to the $PPD^+$ healthy subjects.

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# 155Escape Mutations within HIV Nef-Epitopes Generate Smaller but Functional New CD8+ T Cell Repertoire

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**Background & Objective:** One of the findings in HIV infection is the loss of HIV-specific $CD8^+$T cell activity. **Methods:** Breadth of $CD8^+$T cells, two treatment naïve study subjects, who developed high or low set points, were followed from the acute phase of infection to the chronic phase in a longitudinal analysis of Nef-specific responses using 10-color ICS multiparametric Flow cytometry. **Results:** Firstly, we observed a time dependent decrease in the breadth and magnitude of CD8 IFN-γ responses for autologous HIV sequences in the highly viremic subject while an increase in the breadth of the HIV-specific response in the low viremic subject. Loss of the IFN-γ response to autologous epitopes was not exclusively dependent on the occurrence of mutations; however, mutations had a profound impact on immunodominant epitopes. The lack of functional responses was not associated with the complete loss of Nef-specific CD8 T cells as detected by tetramers staining during chronic phase. However, longitudinal follow up of epitopes revealed the gain and loss of responses to two overlapping epitopes affected by the same mutation. The intensity of IFN-g response to the WT epitope in the acute phase was generally higher than IFN-g responses to the mutated epitope at the chronic phase. Moreover, despite the comparable frequencies of $CD8^+$T cells targeting WT and newly mutated epitopes (as detected by tetramer), the gained response was profoundly dysfunctional. Moreover, TCR repertoire analysis showed not only a high degree of cross reactivity between the subdominant WT and mutated epitopes but also revealed the emergence of new restricted oliglonal clonotypes. **Conclusion:** These data indicate that the loss of HIV-specific $CD8^+$T-cell activity is mainly due to progressively impaired function of HIV-specific $CD8^+$ T cells. Therefore, the primary T cell repertoire generated against HIV during primary infection is not totally deleted but functionally arrested and represents candidate clones to be rescued by vaccination.

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# 156Distinct Function and Maturation of CD8+ T Cell Responses to Consensus and Autologus Nef Peptides under High and Low Viremia

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**Background & Objective:** Hanisms that lead to the lack of an efficient HIV immune response is the accumulation of mutations in CTL epitopes under viremia persistence, which also results in the emergence of HIV quasi-species at the viral population level. **Methods:** To investigate the function and phenotype of Nef-specific $CD8^+$T cells during the early and chronic phases of the infection, we compared longitudinally Nef derived consensus and autologous overlapping peptides by using ICS in 10-color multiparametric Flow cytometry. We selected two naïve viremic HIV individuals who were recruited in the acute phase of infection and developed different plasma virus loads. **Results:** Autologous HIV Nef peptides induced polyfunctional and phenotypically distinctive $CD8^+$T cell responses in the primary phase when compared to consensus peptides ($P< 0.0003$), however, these differences faded in the chronic phase. Based on the major difference in the magnitude of IFN gamma in these individuals we explored the degree of polyfunctionality of Nef-specific $CD8^+$T cells under high and low viremia by calculating the ratio of polyfunctional ($GrB^+CD107a^+$) to monofunctional ($GrB^-$ $CD107a^-$) IFN gamma producing $CD8^+$T cells. A higher proportion of cells were polyfunctional in low viremic compared to high viremic patient during the chronic phase. Moreover, a higher ratio of central memory ($CD45RA^-$ $CCR7^+$ $CD27^+$), transitional memory ($CD45RA^-$ $CCR7^-$ $CD27^+$) and terminally differentiated ($CD45RA^+$ $CCR7^-$ $CD27^-$) to effector memory ($CD45RA^-$ $CCR7^-$ $CD27^-$) was detected among IFN gamma producing $CD8^+$T cells in the low viremic patient. Interestingly, while in the high viremic patient the ratio of central and transitional memory to effector memory cells decreased over time, the ratio of terminally differentiated to effector memory cells increased. This was accompanied by decrease in the ratio of polyfunctional cells in this patient. **Conclusion:** Our data shows that skewed maturation and dysfunction of Nef-specific $CD8^+$T cells is accelerated under high viremia.

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# 157Effect of Protein and DNA Components of *Toxoplasma Gondii* on the Ability of Dendritic cells to Production IL-12 and Proliferation of T Cells

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**Background & Objective:** Dendritic cells are professional antigen presenting cells and the main regulators of different type of immune responses. According to their microenvironmental conditions, these cells can induce different types of helper T cell activity. Th1 response is the most suitable response against tumors. The aim of the present study was to investigate the effect of protein and DNA components of *Toxoplasma gondii* on maturation of dendritic cells and their efficiency in IL-12 production and proliferation of T cells. **Methods:** For DC generation, Bone marrow cells were cultured in the presence of GM-CSF and IL-4 for 5 days. Tumor lysate and protein or DNA components of *Toxoplasma gondii* were added to the culture media and incubated for another 2 days. LPS was added as control for DC maturation. Proliferation of T cells was determined by MLR and IL-12 production was measured by ELISA kit. Maturation of dendritic cell was determined by Flow cytometry. **Results:** DCs treatment with protein components of *Toxoplasma gondii* caused a significant increase in IL-12 production and proliferation of T cells ($P< 0.001$). **Conclusion:** Different compositions of microbial body like protein and DNA components of *Toxoplasma gondii* can cause augmentation of antigen presentation capacity of DC and their IL-12 production capability. Among these components the protein was more effective as compared to DNA.

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# 158High Sensitivity C-Reactive Protein (hs-CRP) in *Helicobacter Pylori* Infected Peptic Ulcer Patients and Its Association with Bacterial CagA Virulence Factor

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**Background & Objective:** CRP is a marker of inflammation and it has been reported that the CagA+ *H. pylori* strains induce more severe gastric inflammatory reactions. The aim of this study was to compare the serum concentrations of hs-CRP in *H.pylori*-infected peptic ulcer (PU) patients, *H.pylori*-infected asymptomatic (AS) carriers and healthy control group and its association with bacterial virulence factor CagA. **Methods:** Totally 60 *H. pylori*-infected PU patients (30 patients were positive for anti-CagA antibody and 30 patients were negative for anti-CagA antibody), 53 *H. pylori*-infected AS carriers (25 subjects were positive for anti-CagA antibody and 28 subjects were negative for anti-CagA antibody) and 22 healthy *H. pylori*-negative subjects (as a control group) were enrolled to study. A serum concentration of hs-CRP was measured by using ELISA method. **Results:** The mean serum levels of hs-CRP in total PU patients (124.9 μg/dl ± 32.4) was significantly higher than those observed in total AS subjects (18.6 μg/dl ± 2.6, P< 0.001) and healthy uninfected control group (10.7 μg/dl ± 2.9, P< 0.0001). Moreover, the mean serum levels of hs-CRP in AS group was significantly higher than that observed in uninfected control group (P< 0.04). No significant difference was observed between the mean serum levels of hs-CRP of PU patients with positive test for anti-CagA antibody (132.6 μg/dl ± 49.4) and PU patients with negative test for anti-CagA antibody (117.1 μg/dl ± 42.9). Moreover, the mean serum levels of hs-CRP similarly expressed in AS subjects with positive test for anti-CagA (18.4 μg/dl ± 3.1) and those were negative for anti-CagA antibody (18.9 μg/dl ± 4.1). **Conclusion:** These results showed that the mean serum concentration of hs-CRP was higher in PU patients and *H. pylori*-infected AS carriers in comparison with healthy control group. Although, the *H. pylori* infection associated with higher serum levels of hs-CRP, however, the serum concentrations of this inflammatory parameter did not influenced by the expression of bacterial CagA virulence factor.

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# 159Determination of Initial Neonatal Septicemia with Use of IL-10 and CRP Measurement as Diagnostic Markers

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**Background & Objective:** Septicemia is one of the most important infant period disease which is an accompanied by lots of problems and mortality. Quick detection of this disease can help to reduce of mortality in infants. Our purpose at this study is quick detection through measuring immunologic marker including the IL-6, IL-10, γ IFN and CRP. **Methods:** 20 affected or suspected infants to septicemia at age range of 0-1 years old before drug treatment have gotten under the control of infant infections supper expertness. Sampling has done after accruable clinical examinations. The level of CRP and cytokines (IL-10 , IFN-γ) determined by ELISA method. **Results:** Mean and standard deviation of interleukin-10 in patients was 25.5 ± 21 that in comparison with normal individual (15.2 ± 11) significantly differences ($P< 0.05$) While, mean and standard deviation of γIFN significantly no different from control group ($P< 0.05$). CRP of patients was significantly different from control ($P< 0.05$). **Conclusion:** In this study, septicemia patients had high level of CRP and IL-10 cytokine that it can result of immune response shift to Th2 lymphocyte. In addition, significant difference of CRP with control group is an acute inflammatory in disease background. The result of this research shows that CRP and IL-10 measurement can suitable marker at 24 hour of birth for quick detection of septicemia.

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# 160Evaluation of Serum Beta 2- Microglobulin (β2-MG) Levels in HBsAg Positive and HIV Positive Donors

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**Background & Objective:** Beta 2- microglobulin is a nonpolymorphyic, single- chain protein with 99 amino acids. This protein is increased in autoimmune disease infections and some hematological malignancies which reflects lymphocyte activation and in the hepatitis infection the viral antigen presentation on the hepatocyte in the presence of class 1-HLA antigen plays a role in the elimination of the virus. The aim of this study was to evaluate β2-MG in HIV positive and HbsAg positive blood donors in comparison with negative donors. **Methods:** In this study serum level of 50 HIV-infected subjects Referred to our Lab, include 8 (16%) females and 42 (84%) males in age between 20- 53 years , and 50 healthy16 (32%) female and 34 (68%) male in age between 18- 56 years Subjects were determined by ELISA Kit. Beta 2-micro globulin was measured in serum drawn from 45 HbsAg positive blood donors Include 5(11.1%) female and 40 (89.9%) male in age between17-56 years, and 50 HbsAg negative blood donors include 16(32%) female and 34(68%) male in the same age. We detected serum β2-MG by Enzyme immunoassay (ELA). **Results:** In this study B2MG level increased in 12 (24%) patients HIV positive and B2MG level was higher in HIV-positive subjects than Healthy subjects (P< 0.0005). Our study showed β2 MG level in increased in 7(15.6%) HbsAg positive donor that was significant differences with healthy control (P< 0.0005). **Conclusion:** These findings suggest that B2MG measurement may have prognostic value for HIV infected populations in developing countries. We conclude that B2MG is an extremely useful marker in initial stratification and follow up of patient with HIV positive. It seems that serum β2MG is a good marker for HbsAg replication. The role of β2MG in monitoring of response therapy needs to be more evaluated.

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# 161The Use of *Leishmania Major* Recombinant Superoxide Dismutase B1 for Serodiagnosis of Leishmaniasis

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**Background & Objective:** Leishmaniasis- a neglected public health problem- is a group of diseases affecting an estimated 12 million people worldwide. Objective: In the present study, recombinant *Leishmania major* superoxide dismutase B1 (rLmSODB1) has been utilized as a potential antigen for the serodiagnosis of human cutaneous (CL) and visceral leishmaniasis (VL) in the endemic regions at the south of Iran. Additionally, the sensitivity and specificity of ELISA-based serodiagnosis using rLmSODB1 and the soluble Leishmania antigen (SLA) were compared. **Methods:** For the first time, rLmSODB1 has been cloned successfully and used for ELISA-based serodiagnosis. Sera from 31 CL and 24 VL cases were included in this study. Additional studies were also done for the evaluation of cross-reactivity using sera from 41 endemic controls including normal endemic donors (n=20), systemic lupus erythematosus patients (n=5), rheumatoid arthritis patients (n=5), and patients with tuberculosis (n=11). **Results:** Analysis indicated that rLmSODB1 was recognized by 62.5% and 13.3% of sera from patients with VL and CL, which showed a sensitivity of 72.7% and 53.6%, respectively, while 95.8% of VL and 30% of CL sera reacted with SLA, revealing sensitivity equal to 96% and 58.8%, respectively. Additionally, from 41 sera either collected from healthy subjects or patients affected with other diseases, 97.5% were negative with SLA or rLmSODB1 (specificity 97.6%). **Conclusion:** These results show that rLmSODB1 almost do not react with sera from patients with tuberculosis and autoimmune diseases and may be considered as a candidate antigen for the specific immunodiagnosis of visceral leishmaniasis.

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# 162Prevalence of Serologic Markers Hepatitis B Viruses in Special Patients in Mazandaran

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**Background and objective:** Hepatitis is a common disease placed in liver of human and proliferates. Produced substances by this viruse pour in to blood. Doing sensitive blood tests define virus proliferation in a body. This virus is a serious danger to transfer in special patients (Thalassemia, Dialysis, hemophilia) and totally in patients who receive blood repeatedly. The aim of this study is definition of different HBV serologic markers in special patients in the way of serology in Mazandaran. **Methods:** From the total of 94 serum samples in special patients clienteles to clinics of Mazandaran in the first 6 month of 1388, samples were collected and identified by the way of ELISA and were evaluated by using SPSS Statistical software. **Results:** Evaluating HBV serologic markers in these special patients about the number of Blab title was (57.4%), but HBeAb (12.6%), HBC IgM (13.8%), HBC total were (10.5%) respectively. According to accomplished test the errant of HBsAb in comparison of another test had a meaningful difference ($P< 0.05$). The result of anti-HIV is negative, but anti HCV is positive. **Conclusion:** The findings of this study showed that the most antibody titer related to HBsAb and considering all these patients receive vaccine, in negative people it was caused by no repetition the vaccine reminder and because these people are in dangerous group, should be concerned.

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# 163Evaluation of Anti-Tetanus Immunity in Hemodialysis Patients

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**Background & Objective:** The incidence of infectious diseases is increased in patients with chronic renal failure. Chronic renal failure severely influences the immune functions of the host. The aim of this study was to evaluate the antitetanus immunity level in southern Iranian patients with end stage renal disease undergoing hemodialysis and to find its association with sex, age, blood hemoglobin and serum albumin, duration of dialysis. **Methods:** This cross sectional study was carried out on a total of 52 patients, who were on hemodialysis and matched with 52 healthy individuals without any underlying renal disease as a control group. Individuals in the both groups receiving antitetanus toxoid vaccine or immunoglobulins a year prior to the study were excluded. The serum antitetanus IgG antibody levels were measured by an ELISA method. **Results:** Tetanus protected individuals in the patients and the control groups were 34.6% and 63.30% respectively. Of the evaluating factors just hemodialysis duration found to affect on tetanus immunity. Conclusion: Tetanus protected individuals in the patients group were significantly less than tetanus protected individuals in the control group ($P< 0.05$). Hemodialysis duration has significant effect on antitetanus immunity level.

# 164Seroprevalence of Cytomegalovirus Infection in Haemodialysis Patients in Jahrom, 2008

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**Background & Objective:** Patients admitted in dialysis centers are at greatest risk to achieve blood-borne infections. One of the most frequent is the cytomegalovirus (CMV) infection, acquired from transfused blood products. The aim of our study was to determine the prevalence of the incidence of CMV infection by measuring IgG and IgM anti-CMV in hemodialysis patients, Jahrom, Iran. **Methods:** This study was carried out on all (43) hemodialysis patients of Jahrom as patients group and 43 sex and age matched healthy subjects as control group, on May 2008. The sera of the patients were tested by enzyme linked Immunosorbent assay (ELISA) method for determining anti-CMV antibodies. **Results:** The prevalence of anti-CMV IgG in the patients group and the control group was 90.69% and 81.39% respectively ($P< 0.05$) and the prevalence of anti-CMV IgM in the patients group and the control group was 18.60% and 2.32% respectively ($P< 0.05$). **Conclusion:** Out of 43 studied hemodialysis patients, 8 patients (18.60%) had active cytomegalovirus infection that was higher than prevalence of active infection in the control group ($P< 0.05$).

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## 165Seroprevalence of Hepatitis E Virus among β-Thalassemic Patients

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**Background & Objective:** The hepatitis E virus (HEV) has a global distribution and is known to have caused large waterborne epidemics of icteric hepatitis. Transmission is generally via the fecal-oral route. Some reports have suggested parenteral transmission of HEV. There are not prevalence data of HEV among β-thalassemic patients in Iran. The aim of this study was to determine the prevalence of anti-HEV antibodies in β-thalassemic patients in west-southern of Iran. **Methods:** This descriptive and cross-sectional study was conducted in March of 2008. We tested 110 β-thalassemic patients attending the thalassemic unit in the city of Jahrom, west-southern part of Iran, for anti-HEV IgG and IgM using enzyme-linked immunosorbent assay (ELISA). **Results:** The overall seroprevalence of hepatitis E was 7.4% (95% CI: 4.6%-10.6%). No significant association was found between anti-HEV positivity and age, sex, positivity for hepatitis B or C virus infection. **Conclusion:** We observed high anti-HEV antibody prevalence; there was no association between HEV and blood borne infections (HBV, HCV and HIV) in our β-thalassemic patients.

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# 166Effect of Testing for IgG Avidity in the Diagnosis of *Toxoplasma Gondii* Infection in Pregnant Women

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**Background & Objective:** The usefulness of testing for IgG avidity in association with *Toxoplasma gondii* was evaluated. **Methods:** In this study, 125 serum samples taken from 125 pregnant women in the first trimester were chosen retrospectively, because either the IgM or differential agglutination (AC/HS) test in the Toxoplasma serologic profile suggested or was equivocal for a recently acquired infection. **Results:** Of 93 (74.4%) serum samples with either positive or equivocal results in the IgM ELISA, 52 (55.9%) had high-avidity antibodies, which suggests that the infection probably was acquired before gestation. Of 87 (69.6%) serum samples with an acute or equivocal result in the AC/HS test, 35 (40.2%) had high-avidity antibodies. Forty women were given spiramycin, to prevent congenital transmission, and 7 (17.5%) had high-avidity antibodies. **Conclusion:** These findings highlight the value of testing a single serum sample obtained in the first trimester of pregnancy for IgG avidity.

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# 167Evaluation of *Toxoplasma Gondii* IgM and IgG Antibodies among Pregnant Women by ELISA in Tabriz, Iran

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**Background & Objective:** Toxoplasmosis is a disease caused by the organism *Toxoplasma gondii*. Common sources of this organism include cats or birds, and undercooked meat, lamb, or venison. droppings or kitty litter than contains cat droppings is a major source of infection. Toxoplasmosis is a serious concern for pregnant women, and patients receiving immunosuppressive treatments. Congenital transmission may occur when a pregnant mother acquires *T. gondii* infection for the first time in her life during pregnancy. Detection of anti-Toxoplasma immunoglobulin M (IgM) and IgG is essential for the diagnosis of Toxoplasma infection in pregnant women. The current study is one of the prime investigations to evaluate the prevalence rate of *T. gondii* among pregnant women in Tabriz and to consider some of the environmental and personal factors that may contribute to infection. **Methods:** Serum samples of 197 pregnant women aged 17 to 45 years attending the Imam Khomeini Hospital in Tabriz were tested for anti-Toxoplasma IgG and IgM antibodies using ELISA. Serological results, reflecting *T. gondii* prevalence rate, were statistically analyzed and linked to epidemiological data collected through a standard questionnaire. **Results:** The seroprevalence of anti-Toxoplasma IgG was 29.4% (58 out of 197), whereas IgM seropositivity was 5.6%. The highest IgG and IgM seroprevalence were among participants aged 35 to 43 years (48.8% and 12% respectively). No statistically significant relation was observed between *T. Gondi* seroprevalence and the other variable factors studied. **Conclusion:** The current study indicates that there is a considerable rate of Toxoplasma infection among pregnant women in Tabriz and support the concern that Iranian women may be vulnerable to that infection. Moreover, it shows the need to provide health education to pregnant women in order to prevent primary infection during pregnancy.

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# 168Interleukin-1R Receptor Antagonist and Interleukin-1β Genes Polymorphisms Associated with Hepatocellular Carcinoma in Hepatitis B Virus Infection

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**Background & Objective:** To investigate the relationships between polymorphisms of IL-1RN and IL-1β promoter region -511C/T and susceptibility to HBV infection in Khorasan population. **Methods:** Genomic DNA from 186 patients with chronic HBV infection, 118 carrier and 101 healthy individuals were genotyped using PCR and PCR- RFLP. The PCR products were digested by restriction endonuclease AvaI for IL-1β (-511) .The association between these polymorphisms and status of the disease were evaluated by Stata 7 software. **Results:** The 8 kinds of polymorphism (1/1, 1/2, 1/3, 1/4, 2/2, 4/4, 3/3 and 2/3) were found in this study. The frequencies of IL-1RN genotypes in patients with chronic hepatitis B were 60.75% , 26.88%,4.84%,2.15%,0.03%,0.005%,0.005% and 005% and carrier were 52.5%, 41.52%, 1.48,0,5.08% and zero for other while in healthy group were 58.41%, 24.75%, 4.95%, .99%, 6.9%, 0,0.99%and 0.99%. The results showed that there were significant differences in the frequencies of genotype1 between groups($P< 0.0001$, OR=3.25,CI=2.36-4.48 and OR=0.27,CI-0.188-0.389),while the frequencies of genotype2 in patients chronic hepatitis B and carrier were higher than in controls ($P< 0.05$ ,OR=1.57,CI=1.059-2.34 and $P \approx 0.3002$,OR=.78,CI=0.503-1.216 respectively). The frequencies of IL-1β (-511) genotypes CC, CT and TT in patients with chronic hepatitis B were 21.57%, 49.02% and 26.41%, and carrier were 23.38%, 40.26% and 36.36% while in healthy group were 33.68 %, 47.67% and 18.65% . The results showed that there were no significant differences in alleles and genotypes frequencies of IL-1β between patients with chronic hepatitis B and carrier. We observed significant differences in genotype frequencies IL-1β (-511) between HCC and healthy control ($P< 0.005$, OR = 1.5, 95%CI = 1.157- 2.167) also significant differences in genotype frequencies IL-1B (-511) between carrier compared with healthy group ($P< 0.005$, OR = 1.75, 95%CI=1.18-2.61). **Conclusion:** Our data may suggest that IL-1RN and IL-1β (-511) genes polymorphisms are associated with outcome of HBV infection.

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# 169Comparison of the Effects of *S . Aureus* and the *E. Coli'* s Toxin on the Induction of Apoptosis in Neutrophils

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**Background & Objective:** Neutrophil plays an important role as a first line of defense against bacterial invasions. In the other hand certain bacterial species uses some strategies in order to escape from neutrophils. One of them is to induce programmed death (apoptosis) in neutrophil. Many different bacteria and bacterial products have been recognized as agents that can act in this way and either induce or inhibit cell death. **Methods:** For this purpose peripheral blood sample was drawn from a healthy volunteer. Then PMNs were separated by Dextran solution. Then, isolated polymorphonuclear leukocytes (PMNs) were treated with *Staphylococcus aureus* bacterium and *Escherichia coil's* toxin. After 1 hour and 2 hours of incubation, PMNs were stained with Giemsa for evaluating apoptosis, for this purpose the fusion of PMN's nucleus lobe was considered as a sign of apoptosis. For further confirmation, PMNs also were tested by nitroblue tetrazolium (NBT) test to reveal their natural ability to engulf pathogens. **Results:** It was shown that, *Staphylococcus aureus* bacterium has a strong effect on apoptosis induction, whereas *Escherichia coil's* toxin can inhibit programmed death. NBT also shows that, PMNs hold their ability for phagocytosis after 1 hour of incubation. **Conclusion:** It seems that the lippolysaccharide (LPS) of *E.coli* can inhibit apoptosis but in contrary, *S.aureus* probably is a potent inducer of apoptosis so can escape from the phagocytosis by neutrophils because phagocytosis is the main process for killing the gram positive bacteria.

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# 170Specific Human Recombinant Antibodies to PP150 of Human Cytomegalovirus for Detection of HCMV Infections

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**Background & Objective:** Recent advances in antibody engineering have made possible the production of human recombinant antibody fragments in phage display vectors in the form of single chain fragment variable (scFv). It is possible to select scFv against a desire antigen using panning process. Here we describe the production and selection of human scFv to the immunogenic region of the PP150 of human cytomegalovirus from a phage antibody display library for their use in the detection of CMV infection. **Methods:** Antibody engineering technology was applied to lymphocyte mRNA of an immune donor and a scFv library was constructed and selected against major immunodominant epitope of phosphoprotein 150 of HCMV using panning process. The selected scFvs were screened using ELISA and indirect Immunoflourescent assay. **Results:** A large library was constructed which was detected by the clones differentiated by BstN1 fingerprinting. One scFv accounted for 25% of clones after panning. Three of the panned scFv bound to the pp150 epitope in ELISA. The ability of the scFvs to bind the infected cells were shown in the IF assay. **Conclusion:** Production of human recombinant antibodies can be used for antibody selection against a number of microorganisms. This technique has been used for production of antibodies in detection, prophylaxis and treatment of infectious disease. The highly specific anti-PP150 scFvs selected in this study show potential for the development of a new generation of detecting reagents.

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# 171Comparing the Effect of Different Classes of CpG-ODN on Human Neutrophil Survival and Activation

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**Background & Objective:** Polymorphonuclear neutrophils (PMNs) are key components of the inflammatory response contributing to the development of pathogen-specific immune responses. Following infection with *Leishmania major*, neutrophils are recruited within hours to the site of parasite inoculation. Leishmania is able to survive in PMNs, after 2-4 days, even infected cells become apoptotic and are phagocytosed by macrophages. Uptake of apoptotic bodies harboring parasite do not cause macrophages to generate pro-inflammatory responses. This study set out with the aim of expanding of the life span of PMNs and subsequently, increasing the potency of them to eliminate intracellular parasite before becoming apoptotic. According to evidences, oligonucleotides containing unmethylated CpG motifs prolong neutrophil survival by delaying apoptosis. Furthermore, they trigger and modulate cytokine responses in human leukocytes. **Methods:** PMNs were isolated from heparinized blood of healthy donors through dextran sedimentation and density gradient. The cells were stimulated with different concentration of GM-CSF for 90 min and then 2 µg/ml of CpG-ODN type B (7909) was added. Then the cells were stained with Propidium Iodide and Annexin V to evaluate the amount of apoptotic population using Flow cytometry. The concentration of TNF-α produced by CpG-ODN treated and untreated neutrophils was assessed by ELISA. **Results:** According to obtained results, the apoptosis-delaying action of CpG-ODN was not observed; in addition; CpG-ODN did not cause PMNs to produce significant level of TNF-α. **Conclusion:** At this stage, we are comparing the effect of other CpG-ODN such as type A which is recognize by TLR-9 dependent pathway on PMN of infected individuals with *L. major* at different stages of cutaneous leishmaniasis.

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# 172Differential Expression of Toll- Like Receptor (TLR) Families by Cell Lines of Diverse Origins

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**Background & Objective:** Toll like Receptors (TLRs) comprise a family of 13 molecules acting as the first line of innate immune defense by specifically recognizing pathogen associated molecular patterns. Recent evidence shows that some types of tumors may benefit from TLR signaling pathways that trigger enhanced proliferation, resistance to apoptosis, metastasis and escape from immune surveillance. The aim of the present study was to investigate expression of TLR 1-10 in 19 tumor cell lines of different tumor origins. **Methods:** Cell lines originated from diverse tumors including cancers of ovary, breast, prostate, colon, skin, brain, hematopoietic system, cervix, pancreas, lung, kidney and bladder were propagated in optimal culture conditions. Cells were harvested in logarithmic phase and RNA extraction and cDNA synthesis were carried out according to the standard protocols. Semi-quantitative RT-PCR was run for each cell line using a panel of 10 pairs of specific primers for TLR 1-10. Beta actin amplification was used as internal control. Density of each amplicon was quantified by Alpha ease processing program. **Results:** Interestingly our results clearly showed that tumor cell lines of different origin differentially express different families of TLRs with varying densities. Whereas cell lines from ovarian cancer including OVCAR-3 and Caov-4 and such monocytic cell lines as THP-1 predominantly over expressed almost all TLR families, some lines from other origins like lymphoblastic (EHEB) and fibroblastic (HFFFPI6) cell lines failed to express most members of TLRs. TLR 6 and 10 were expressed by all cell lines, while only three cell lines expressed TLR7. **Conclusion:** It seems that differential expression of TLRs by tumors may be related to their different behavior in terms of invasion and metastases and neoplastic process may use TLR signaling pathway to promote cancer progression. Our results highlight the importance of TLRs as potential target for cancer immunotherapy.

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# 173The Effect of *Urtica Dioica* on Peritoneal Macrophages Cell Viability of BALB/c Mice

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**Background & Objective:** *Urtica dioica* L. has been used in Iranian Traditional medicine. It has been reported recently as an anti-inflammatory herb. Macrophages have the most central and essential functions in the innate immune system, and have multiple roles in inflammation. In this study the effect of *Urtica dioica* extract were conducted on macrophage cell viability and nitric oxide (NO) production. **Methods:** This research performed on 30 male BALB/c mice with 8 weeks average age. Mice were divided into 6 groups; one as control and others take 10, 50, 100, 200 and 500 mg/kg/day of the extract. After two weeks orally prescription of the extract, peritoneal macrophages obtained and Nitric oxide and MTT measurement performed on samples. **Results:** Data obtained from this research show NO production was increased and viability of macrophages was decreased after *Urtica dioica* extract treatment. The mean of NO concentration in control group was 0.197 and for 10, 50, 100, 200 and 500 mg/kg/day of *Urtica dioica* were 0.177, 0.151, 0.144, o.141 and 120 respectively. **Conclusion:** The results of this study emphasize an immunomodulatory effect for *Urtica dioica*. More research regarding the cytokines and other inflammatory mediators in animal and human models is recommended.

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# 174Black Seed *(Nigella Sativa)* Augments the Phagocytic Activities of Peripheral Blood Monocytes of Guinea Pig

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**Background & Objective:** Black seed (*Nigella sativa*) is a plant that has been broadly used for treating infectious and noninfectious disease in traditional medicine. It is substantially reported that various parts of this plant (e.g. seed) could augment innate and acquired immune responses. In the present work we studied the effects of orally administered black seed on phagocytic activities of guinea pig peripheral blood monocytes. **Methods:** Twenty one young male guinea pigs were arranged in three groups, the first group was fed normally (control) and two others were given 6.25% and 12.5% black seed nutrient for 3 weeks. At evenly periods, blood samples were taken and peripheral blood monocytes were separated using Ficoll/hypaque gradient and then phagocytic activities of plastic adherent monocytes were assayed by nitro blue tetrazolium (NBT) as well as yeast and fluorescent latex bead uptake tests. **Results:** Our result showed that black seed administration increased monocytes phagocytic activities in comparison to controls significantly ($P< 0.05$). The highest response was seen in the end of first and third week for 6.25% and 12.5% of black seed nutrient respectively. **Conclusion:** Our findings confirmed that oral administration of black seed will potentiate innate immune responses. So it can be taken as prophylactic nutrient additive to prevent infectious diseases.

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# 175Evaluation of the Effect of 45KDa Protein Molecule Isolated from Aged Garlic Extract on Stimulation and Activation of Dendritic Cells

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**Background & Objective:** Garlic is known as a potent spice and a medicine with broad therapeutic properties ranging from antibacterial to anticancer, and anticoagulant. One major protein has been isolated and purified; it is the. In this study we were evaluated effectiveness the 45kDa protein molecule isolated from aged garlic on stimulation and activation Dendritic cells (DCs). **Methods:** Aged garlic extract was prepared by the method used by Mantis et al. Proteins were purified from aged garlic extract by Ammonium sulfate and purified the 45kDa protein molecule by Ultrafilteration of Amicon, and gel filtration. SDS-PAGE was used for determination the molecular weight and HPLC was used for determination purity of isolated fraction. DCs were isolated from spleen of BALB/c mice by Nycodenz centrifugation and their adhesiveness to plastic dish. The 45 kDa protein isolated from aged garlic extract was added to DCs medium at over night culture and determinate percent expression of CD40, CD86 and MHCII by Flow cytometric analysis. Also, proliferation of T-cells was measured by allogenic MLR test. **Results:** 45kDa fraction–pulsed DCs and negative control test (DCs without pulsed for antigen) were similar for percent expression of CD40, CD86 and MHCII. 45kDa fraction pulsed-DCs and DCs without pulsed for antigen had similar induction of the cell proliferation. **Conclusion:** Previous study showed that 45kDa protein isolated from garlic extract did not significantly augment the antibody titer and DTH response in comparison to the group receiving SRBC alone. In this study we showed that 45kDa protein isolated from aged garlic extract did not effect on stimulation and activation DCs. 45kDa protein showed that have Immunosuppressive effect on DCs.

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# 176Dendritic Cell Induced Specific T Cell Responses Following Their in vivo Treatment by 1, 25 Dihydroxycholecalciferol

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**Background & Objective:** Dendritic cells (DCs) as the managers of the immune response have a crucial role in forming the direction and nature of the immune response. Some compounds such as 1,25dihydroxycholecalciferol affect the function of DCs and can be used to shift the immune functions toward favorite direction. The aim of this study was to investigate the in vivo effects of 1, 25 Dihydroxycholecalciferol on DCS potential to induce the specific T cells response and the cytokines profile. **Methods:** 1,25dihidroxycholecolciferol was regularly injected intraperitonealy to C57BL/6 mice. DCs were separated from spleens of calciferol treated and non-treated mice using magnetic beads. The separated cells were pulsed by MOG (Myelin Oligodendrocyte Glycoprotein) and injected subcutaneously into front footpad of syngeneic mice. After 5 days, the lymphocytes from regional lymph nodes were separated and used for lymphocyte transformation test (LTT) and determination of the IFNγ/IL-4 ratio on behalf of the Th1/Th2 ratio by ELISA technique. **Results:** Statistical analysis of the obtained results showed that the specific T cell stimulation potential of treated DCs as well as the induced IFNγ/IL-4 ratio was downregulated in calcitriol treated group compared to non-treated cells ($P< 0.05$). **Conclusion:** It seems that 1, 25 dihydroxycholecolciferol can regulate the DCs functions in vivo. This modulation effect appears as decrease in their T cell stimulation potential and shifting the cytokine profile to Th2.

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# 177In vivo Effects of Calcitriol on Phenotypic and Functional Properties of Dendritic Cells

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**Background & Objective:** Nowadays regarding the crucial role of dendritic cells (DCs) in management of immune response, one useful challenge is to adjust the function of immune system towards favorite and suitable directions employing these cells. The compounds which have the ability to induce immunomodulatory effects on DCs, may be applicable in treatment of autoimmune diseases. The aim of this study was to investigate the in vivo effects of calcitriol (active form of vitamin D3) on DCs. **Methods:** 0.1 microgram calcitriol was injected intraperitonealy into C57BL/6 mice every other day within 3 weeks and spleen DCs were extracted by magnetic beads. The phenotypical and functional properties of DCs were studied by Flow cytometry and mixed lymphocyte reaction (MLR) respectively. **Results:** The expression of CD86 and MHCII as maturation markers and costimulatory molecules, were significantly decreased while CD11b expression as marker of Th2 pathway inducer DCs were significantly increased. Allogeneic T cell stimulation in MLR was also significantly inhibited in comparison with control groups. **Conclusion:** It seems that, in vivo calcitriol administration inhibits maturation and activation of DCs.

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# 178Differential Expression of Interferon Regulatory Factor 1 by Native and Truncated HCV Core Proteins

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**Background & Objective:** Hepatitis C virus (HCV) proteins are known to interfere at several levels with both innate and adaptive responses of the host. A key target in these effects is the interferon (IFN) signaling pathway. While the effects of nonstructural proteins are well established, the role of structural proteins remains controversial. HCV core protein (HCVcp) -One of the structural proteins- is a multifunctional protein and consists of distinct domains. We investigated the effect of native and different domains of HCVcp on the expression of interferon regulatory factor 1 (IRF-1), a secondary transcription factor of the IFN system responsible for inducing several key antiviral and immunomodulatory genes by Real Time PCR. **Methods:** pcDNA (+) 3.1 vectors harboring different native and truncated HCV core proteins (1-175, 1-122 and 123-175aa) were constructed and transiently transfected into HepG2 cells via electroporation. Expression analysis was assessed via western blotting (WB) and flourescence microscopy (IFM). After treatment of transfected HepG2 cells with IFN-α, quantitative Real Time PCR analysis were performed in duplicate using SYBR green with primer sets to examine the effect of proteins on IRF-1 expression. Fold induction was then calculated by the Δct method using GAPDH mRNA level to normalize values. Untransfected cells and IFN-α untreated cells were used as controls. **Results:** Construction of appropriate expression vectors for HCVcp was confirmed by restriction analysis and DNA sequencing. WB and IFM analysis confirmed proper expression of the core proteins inside the transfected hepatic cells. Real Time PCR Results indicated that IRF-1 is differentially regulated by native and truncated HCV core proteins compared to controls at the transcriptional level. **Conclusion:** These data suggest that HCV core-induced IRF-1 repression may play a pivotal role in establishing persistent infection by dampening an effective immune response.

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# 179Non- Specific Resistance Immune Status in Ukrainian Children Related to Contaminating Region after Chernobyl Accident

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**Background & Objective:** The aim of this study was determined change of innate immune status in Ukrainian children after 23 years from Chernobyl accident. **Methods:** We studied 95 participants: 75 rural patients aged 4 to 18 who lived in a contaminated area exposed to natural environmental radiation that categorized in three groups and 20 healthy urban participants from Kiev aged 5 to 15 as control group. Peripheral blood leukocytes from Buffy coats were analyzed for Natural Killer cell, serum concentration of circulation immune complex measured by the polyethylene glycol method. Phagocytes activity performed by using latex article and phagocytic index calculated was considered significant ($P< 0.05$). **Results:** Percent of natural killer cells in groups II and III increased significantly in comparison to control group ($P< 0.05$). Concentration of Circulating Immune Complexes increased significantly in all study groups, comparison to control group ($P< 0.001$). Phagocytes activity and phagocytic index decreased significantly in all study groups comparison to control group ($P< 0.001$). **Conclusion:** The non specific resistance immune status of study groups that located in region contamination radioactive has changed .Our data have shown, this changed may be related to internal radiation activity depended to 137Cesium.

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# 180Whey Augments the Cytotoxic Effect of Human NK Cells on K562 Cell Line as a Target Cell

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**Background & Objective:** Whey is one of the byproducts of cheese producing factories, which contains some of biologically important materials such as proteins, amino acids, minerals, etc. There are repeatedly reports on anti cancer and immunomodulatory effects of whey, so, in the present study the effects of whey on NK cells cytotoxic activity were addressed. **Methods:** At the first step, the viability of PBMCs and K562 cells was determined in the presence of 0%, 5%, 10% and 20% of whey using trypan blue exclusion test. At the next step, the effect of whey at above concentrations on NK cells cytotoxic activity against K562 cell line were determined using Annexin V/PI fluorescent dyes and Flow cytometry. **Results:** We found that there are no significant differences in viability of PBMCs in the presence or absence of the whey, however, the viability of K562 cells was decreased in the presence of whey significantly. The cytotoxic effect of NK cells on K562 target cells was significantly increased in the presence of whey (5% and 10% in particular). **Conclusion:** Our findings indicated that whey may have anti cancer effects directly or via up regulation of NK cell activity against tumor cells indirectly, however, more details need to be explored in the future.

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# 181Study on the HLA-G Polymorphism in the Normal Population

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**Background & Objective:** HLA-G is a nonclassical HLA class I molecule initially described as being selectively expressed at the maternal-fetal interface on cytotrophoblast cells. HLA-G polymorphism is reduced with only 9 different HLA-G protein variants encoded by 28 alleles, of which 23 correspond to substitutions in the coding sequence. To date, 28 HLA-G alleles have been officially recognized, presenting sequence variations in coding and none coding regions, primarily located in exons 2, 3 and 4. **Methods:** 100 samples from healthy unrelated Iranian individuals, as donor candidates for Bone Marrow Transplantation, (BMT), referred to Iranian Blood Transfusion Organization (Tehran, Iran) were randomly selected. DNA was extracted and purified from the whole blood samples collected in 5% EDTA using salting-out technique and HLA-G typing was carried out with polymerase chain reaction followed by restriction fragment length polymorphism. **Results:** The obtained results indicated that in Iranian population the frequency of the HLA-G alleles is G*01011(8.16%), G*01012 (67.32%), G*01041(61.2%), G*01043 (4.03%) respectively. **Conclusion:** The final data analysis showed that in Iranian population G*01012 have the highest frequency of 67.32%.

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# 182Modulation Production of CXCL-8 through the Inflammasome Signaling by Human Bronchial Epithelial Cells

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**Background & Objective:** Chronic obstructive pulmonary disease and emphysema (COPD) is characterized by chronic airway inflammation. Cigarette smoke (CS) has been considered a major player in the pathogenesis of COPD. The inflamed airways of COPD patients contain several inflammatory cells including neutrophils, macrophages, T lymphocytes, and dendritic cells (DCs). The relative contributions of these various inflammatory cells to airway injury and remodeling are not well documented. Myeloid and plasmacytoid dendritic cells (mDCs, pDC) are crucial immune cells detecting microorganisms and linking innate and adaptive immunity. mDC are antigen presenting cells and pDC are intermediate cells. They produce large amounts of IFN-α after stimulation with CpG motifs and are also antigen presenting cells. The antiviral effect exerted by IFN-α is due to the induction of IFN response genes. In current study we investigated the effect of cigarette smoke extract (CSE) on the mouse bone marrow derived- myeloid dendritic cells (mDC) and human pDC. **Methods:** We assessed CSE-induced changes in cDC function in the mixed lymphocyte reaction (MLR) examining CD4+ and CD8+ T cell proliferation. **Results:** CSE induces the release of the chemokines CCL3 and CXCL2 (but not cytokines), via the generation of reactive oxygen species (ROS) in mDC. In a mixed-leukocyte reaction assay, CS-primed DCs potentiate $CD8^+$T cell proliferation via CCL3. In contrast, proliferation of $CD4^+$T cells is suppressed via an unknown mechanism. The CS-induced release of CCL3 and CXCL2 by DCs may contribute to the influx of $CD8^+$T cells and neutrophils into the airways, respectively. In pDCs, we observed that CSE augmented the production of IL-8 and suppressed the release of TNF-α, IL-6 and IFN-α. Moreover, CSE suppressed PI3K/Akt signaling in pDC. **Conclusion:** our data indicate that CSE has both the potential to diminish anti-viral immunity by downregulating the release of IFN-α and other pro-inflammatory cytokines while, at the same time, augmenting the pathogenesis of COPD via an IL-8 induced recruitment of neutrophils.

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# 183Immunity and Fasting Ramadan

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**Background & Objective:** Fasting during Ramadan represent one of the five Pillars of the Islamic religion. So it is important to know the effect of this kind of fasting on people's life especially on their health. The aim of this study was investigate the effect of Ramadan fasting (R.F) on immunity: neutrophil's function contains phagocytosis, opsonization, respiratory burst and circulating immune complex (CIC) level. **Methods:** The effects of Ramadan fasting on neutrophil's phagocytosis, opsonization, measured by percentage and index and quality of respiratory burst with reduction of Nitro Blue Tetrazolium (NBT) and quantity with chemiluminescence, and also circulating immune complex with poly-ethylene-glycol method. First study contains 13 Muslim using phagocytosis, opsonization percentage and Index, and NBT percentage. Second have 28 men for CIC level; third have 24 normal male Muslim adults for neutrophil's respiratory burst, using chemiluminescence method. And, fourth study contains 21 normal young fasting Muslim individuals using standardized chemiluminescence and poly ethylene glycol method respectively. **Results:** All of the experiments measured before and after Ramadan for same subjects. There was no significant effect for NBT reduction, phagocytosis, opsonization percentage and Index before and after R.F. In the same as, there was no significant difference between the CIC before and after Ramadan. In another result also, there were no significant effects of fasting on blood neutrophil function during (R.F).Results of fourth study shown that changes of the immunological parameters were not significant and levels remained in the range of normal. **Conclusion:** According to the results, fasting during Ramadan does not have bad and hazardous effect on immune system of healthy people. Moreover there was a good correlation between following immunological parameters: (CIC & chemluminescence in 21 subjects), (phagocytosis, opsonization percentage and Index).

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# 184Effect of Ginger Extract on Nitric Oxide Production in Peritoneal Macrophages

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**Background & Objective:** Ginger (*Zingiber officinale*) is one of the more commonly used herbal supplements. Although often consumed as spice, Ginger has been used in traditional medicine for treatment of inflammatory pathological status. In this research the effect of Ginger was assessed on NO release by macrophages as an important cell in innate and adaptive immune system. **Methods**: Ginger extract was prepared in multiple concentrations (0.01, 0.1, 0.5 and 1 mg/ml). Mice peritoneal macrophages were separated and cultured with various concentrations of Ginger extract, and 50 U/ml of IFN-γ. MTT test was performed to evaluate the viability of the cells and nitrite levels were measured using the Griess reaction, which is an indirect assay for NO production. **Results**: Various concentrations of Ginger extract suppressed in vitro NO production in mice peritoneal macrophages significantly ($P< 0.001$), while had no effect on cell viability ($P> 0.05$). **Conclusion**: While ginger extract have a suppressive effect on NO production by peritoneal macrophages, therefore besides having anti-inflammatory and anti-oxidant properties, it is suggested that ginger can be noticed as an anti-tumor agent having no adverse effect.

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# 185Evaluation the Immunostimulatory Effect of 14kDa Protein Isolated from Aged Garlic Extract on Mouse Dendritic Cells

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**Background & Objective:** A wide range of biological activities of garlic in vitro and in vivo have been verified. One major protein has been isolated and purified; it is the 14kDa protein. Dendritic cells (DCs) are potent antigen presenting cells (APCs) that possess the ability to stimulate naïve T-cells. In this study the effect of 14kDa protein isolated from aged garlic extract on stimulation and maturation of dendritic cells was investigated. **Methods:** Aged garlic extract was prepared by the method used by Mantis et al. Proteins were purified from aged garlic extract by biochemical method and purified the 14-kDa protein by gel filtration Sephadex G50, their purity was checked by SDS-PAGE. DCs were isolated from spleen of BALB/c mice by Nycodenz centrifugation and their adhesiveness to plastic dish. 14kDa protein from aged garlic extract was added to DCs medium at over night culture and determinate percent expression of CD40, CD86 and MHCII by Flow cytometric analysis. Also, proliferation of T-cells was measured by allogenic MLR test. **Results:** 14kDa fraction isolated from aged garlic induction a high expression CD40 molecule but it was not effect on CD86 and MHCII molecules. 14kDa fraction pulsed-DCs and DCs without pulsed for antigen had similar induction of the cell proliferation. **Conclusion:** In this study we showed that there was an unexpected upregulation of CD40 on DCs after incubation with 14kDa protein compared with control group. CD40 is the earliest up-regulated costimulatory molecule and was used to verify activation of the DC populations. Interaction between CD80/CD86 on DCs and CD28 on T cells is necessary for T cell activation; however there are no significant increases in the surface expression of MHC class II and CD86 on14kDa fraction pulsed-DCs and DCs without pulsed for antigen.

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# 186In vitro Effect of Zanjan Pasteurized Yoghurt Derived Lactic Acid Bacteria on TNF-α Secretion by Peripheral Blood Mononuclear Cells of Ulcerative Colitis Patients

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**Background & Objective:** There is evidence for the immunomodulation disorders in the response to intestinal flora in inflammatory bowel disease. Yoghurt is a fermented milk product made with a starter culture consisting of different lactic acid bacteria (LAB) species which could be colonized in intestine after yoghurt consumption. However, the role of LAB in the etiopathogenesis of ulcerative colitis has not been fully clarified. In order to determine how the immune system responds to these bacteria in-vitro this study was planned. **Methods:** Zanjan pasteurized yoghurt was cultivated on MRS broth for 48h and centrifuged the bacteria at stationary phase. Then the bacteria were suspended in PBS and killed with UV light. Peripheral blood mononuclear cells (PBMCs) of 17 ulcerative colitis patients were separated from heparinized blood by Ficoll-Hypaque density gradient centrifugation and were co-cultured (2X106 cell/ml) with different concentrations (100:1, 50:1, 25:1, 12:1 bacteria: Cell ratios) of killed bacteria (or PBS as control) in RPMI-1640 plus 10% FCS at 37 ◦C and 5% CO2 condition for 48 and 72h. TNF- α was measured in supernatant of PBMCs by ELISA. **Results:** The Yoghurt derived bacteria strongly and significantly induced TNF-α in a manner of dose and time dependent (72h: 2750+/-780, 2300+/-550, 1950+/-430, 1250+/-360; 48h: 2150+/-520, 1680+/-385, 1320+/-255, 950+/-210 pg/ml at different bacteria cells ratios) in comparison with control (72: 320+/-235; 48h: 225+/-145 pg/ml). **Conclusion:** These data show that Zanjan pasteurized yoghurt derived bacteria may trigger the pro-inflammatory immune response of ulcerative colitis patients.

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# 187Efficient Generation of Human Th17 Cells from |Naive CD4+ Helper T Cells

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**Background & Objective:** The most of information about these cells are derived from experimental mouse models and in comparison there is a little knowledge about specific roles or differentiation pathways of human Th17 cells. The purpose was to compare different cytokine combinations in two distinct culture media to find the most efficient condition for development of human Th17 cells. **Methods:** Naive $CD4^+$ T cells were cultured in plates coated by anti-CD3 and anti-CD28 antibodies in X-VIVO 15 serum free medium or RPMI 1640 with 10% FBS in presence of different cytokines. Cytokine contents of supernatant culture media were measured by ELISA kits and transcripts were quantified by qRT-PCR using of QuantiFast SYBR Green PCR kit. **Results:** We used seven different cytokine combinations in two separate media. The evaluation of RORC2 and IL-17 gene expression demonstrated that the most of cytokine combinations used were effective in induction of expression of these genes specially when used in X-VIVO 15 serum free medium. In addition, we found a significant correlation between IL-17 gene expression with RORC2 mRNA levels ($P< 0.01$). Expression of IL-22 gene was highly increased at presence of a combination of IL-1β with IL-6 in X-VIVO 15 serum free medium while such a combination has a lower positive effect when used in RPMI with serum. No correlation was found between expression of this gene and RORC2 ($P \approx 0.98$). All the cytokine combinations either with X-VIVO or RPMI with serum were found to inductive for IL-23R gene expression except than TGF-β with IL-1β when used in X-VIVO 15. Significant correlation was found between IL-23R gene expression and RORC2 m RNA level ($P< 0.01$). **Conclusion:** During the past years, independent studies had controversial results. According to our data, the combinations of TGF-β with IL-6 and IL-23; or IL-1β with IL-6 or IL-23 all in X-VIVO 15 serum free medium are the most efficient way to generate human Th17 cells.

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# 188Comparison of Different Separation Methods for Monocyte Isolation from Peripheral Blood

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**Background & Objective:** Monocytes are largely used in immunological research particularly for the generation of dendritic cells. Several methods aiming monocyte isolation from peripheral blood already exist such as adhesion procedure which has some disadvantages and immunoselections which are too expensive. Considering the difficulties in monocyte detachment from plastic surfaces and the need for a simple and inexpensive method for monocyte isolation, we evaluated different Percoll density gradient protocols in comparison with adherence method. **Methods:** Enriched PBMCs on a Ficoll density gradient were incubated 2 hours in 37 º C in 6-well plates. Non-adherent cells were removed and adherent cells detached by 10 mM cold PBS-EDTA. Remaining Ficoll enriched PBMCs divided to three groups for following procedures: 1) monocyte isolation on a special Percoll gradient generated with ultracentrifugation; 2) using density gradient centrifugation with hyper-osmotic Percoll followed by low-density iso-osmotic Percoll; 3) centrifugation of PBMCs on Slight hyper-osmolar Percoll including PBS-Citrate. Cell viability was assessed through trypan blue staining and purity of monocytes was analyzed by evaluation of CD14 expression using Flow cytometery. **Results:** Considering a 10-20 % recovery by adherence method, we tried to improve adhesion of cells using Poly-L-lysine pre coated plates but no significant result was obtained. Separation of monocytes based on Percoll gradient centrifugation procedures mentioned above resulted in 34%, 38% and 50% monocyte recovery respectively. **Conclusion:** Although we obtained 50% recovery of monocytes via slightly hyper-osmolar Percoll-PBS-Citrate which was significantly higher than adherence protocol, there is still more effort needed to obtain higher purity.

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# 189Optimization of Gene Transfection in Mouse Myeloma Cell Lines Using Different Transfection Reagents

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**Background & Objective:** Purification and isolation of cellular target proteins for monoclonal antibody (mAb) production is a difficult and time-consuming process. Immunization of mice with murine cell lines stably transfected with genes coding for xenogenic target molecules is an alternative method for mouse immunization and mAb production. Here we present data on transfection efficiency of some commercial reagents used for transfection of murine myeloma cell lines. **Methods:** Mouse myeloma cell lines (SP2/0, NS0, NS1, Ag8, and P3U1) were transfected with pEGFP-N1 vector using Lipofectamine 2000, jetPEI and LyoVec in different combinations. The transfection permissible HEK293-FT cell line was used as a control in transfection procedure. Transfected cells, expressing the enhanced green fluorescent protein (EGFP), were analyzed by Flow cytometry 48 hrs after transfection. **Results:** Flow cytometry analysis showed transfection efficiency of 71%, 57% and 22% for HEK293-FT, 5.5%, 3.4% and 1% for SP2/0, 46%, 40.1% and 9.6% for NS0, 8.2%, 6% and 5.5% for NS1, 22%, 49.2% and 5.5% for Ag8 and 6.3%, 21.5% and 4.6% for P3U1 cell lines after transfection with Lipofectamine 2000, jetPEI and LyoVec reagents, respectively. Our data indicates that NS0 and Ag8 are efficiently transfected by Lipofectamine 2000 and jetPEI reagents. **Conclusion:** We propose Ag8 and NS0 cell lines as suitable host cells for efficient expression of heterologous target genes which can be used for mouse immunization and mAb production.

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# 190Production and Characterization of Monoclonal Antibodies to Human FCRL1 Molecule

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**Objective:** FC receptor like 1 (FCRL1) belongs to the newly identified family of FCRL molecules which are constitutively expressed in human B cells. FCRL1 is unique in having 2 ITAM-like motifs in its intracellular domain with a potential capability to serve as an activating co-receptor. Due to the exclusive expression pattern of FCRL1 in B cells, it has recently been proposed as a target for immunotherapy of B cell malignancies. Here we present data on production and characterization of mouse monoclonal antibodies (mAb) to FCRL1 using synthetic peptides. **Methods:** BALB/c mice were immunized with KLH-conjugated synthetic peptides from the extracellular domain of FCRL1. Spleen cells of hyperimmunized mice were fused with a mouse myeloma cell line using PEG. Producer hybridoma cells were screened with the immunizing FCRL1 peptides by ELISA and cloned by limiting dilution assay. Positive clones were further characterized using recombinant FCRL1, 2 and 4 proteins by Western blot. The extracellular domains of human FCRL1, 2 and 4 genes were cloned and subsequently expressed in *E.coli* to determine the specificity of anti-FCRL1 mAbs. **Results:** Three hybridoma clones producing mAbs specific for FCRL1 were selected. These mAbs reacted only with recombinant FCRL1, but not FCRL2 or FCRL4 proteins both by ELISA and Western blot techniques. **Conclusion:** FCRL1 peptide-based antibody production might be a suitable approach for specific targeting and therapeutic interventions in B-cell malignancies.

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# 191Isolation of Ag- Ab Complex for Detection of Hepatitis C Core Antigen Using ELISA

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**Background & Objective:** Previous studies showed during first day's infection with hepatitis type C virus, core antigen will appear more quickly than antibody against the virus. Therefore detection of core antigen is one of the most useful indices to rapid diagnostic of the infection. These antibodies can prevent the binding of antigen to antibodies trapped in test by means of forming a complex with antigen and coating the antigenic epitome. Effect of treatment serum samples containing antibody with different solutions in different times. **Methods:** Regarding the comparison of light absorbance of samples with positive PCR test and negative antigen tests, adjacency to glycine buffer (pH= 2) for one hour in 37$^{o}$C resulted in more Titration of antibodies present in the serums which were treated by acid showed a considerable decreasing compared to serum sample without this treatment. Nonetheless, no such titration decreasing was observed in samples were treated to triton X-100. Antibody titration had a substantial decreasing after treatment with acidic solutions in samples with negative antigen test and positive PCR test. For excluding the probable interference effect of antibody presence against the Core antigen, the positive antibody serum samples were treated by different solutions, including 1.5M glycine buffer with pH=2, 0.5 N HCl, and triton X-100 with 0.1% concentration. **Results:** Increasing in light absorbance than treatment with normal chloridric acid in 37oC. On the other hand, this indicates that increasing in light absorbance in the samples with positive antibody test after treatment with acidic solution for testing the antigen is a specific phenomenon caused by separation of antibody from antigen and subsequent identification of Core antigen via this evaluation technique. Sensitivity of the test before and after treatment of serum with 1.5M glycine solution was 57.3 and 88 respectively. **Conclusion:** Increase in test sensitivity subsequent to adjacency to 1.5M glycine buffer indicates that the elimination of interference of antibodies against Core antigen present in serum.

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# 192Comparative Influence of γ-Irradiation and Mitomycin C Treatment of Feeder Cells on Efficiency of B Lymphocyte Transformation by Epstein-Barr Virus

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**Background & Objective:** The utility of feeder cells to support Epstein-Barr virus (EBV) transformed human lymphoblasts has been previously shown. Feeder cells provide support to EBV-treated B lymphocytes through cell-cell contact as well as secretory growth factors. Mitomycin C (MMC) and γ-irradiation treatments are commonly used to prepare nonproliferating feeder cells. In this study we compared the influence of γ-irradiation- and MMC treatment on the supportive effect of feeder cells on efficiency of B lymphocyte transformation by EBV. **Methods:** A human fetal foreskin fibroblast cell line was treated with either 10 ug/ml mitomycin C for 2h or 3 h or with γ-irradiation (6000 rad) and used as feeder cells. Peripheral blood mononuclear cells of 4 adult normal individuals were infected with EBV and seeded at different cell densities on treated feeder cells. Following 3 weeks incubation in a CO2 incubator at 37 C, the frequency of transformation was determined by enumeration of wells containing growing transformed cells. **Results:** Our data indicates that MMC-3h treated feeder cells were more efficient than MMC-2h to support the expansion of EBV infected B lymphocytes ($P< 0.01$). Furthermore, the frequency of EBV transformation was significantly higher using γ-irradiated feeder cells compared to MMC-2h ($P< 0.0001$) or MMC-3h ($P< 0.0001$) treated cells. **Conclusion:** γ-Irradiation is more effective than MMC for feeder cells preparation, to support the expansion of EBV infected human B lymphocytes. This difference could be attributed to the higher suppressive effect of MMC on cellular metabolic activity as well as gradual release of MMC from treated feeder cells in the culture supernatant which could have a negative effect on cocultured cells.

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# 193In vitro Production of Anti LPS Antibody by Peritoneal and Spleen B-Lymphocyte of BALB/c Mice

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**Background & Objective:** CD5B1 lymphocytes are the major cell subpopulation for defense found in many organs including peritoneum and splenic follicles. They can produce natural antibodies with poly specific reaction with an important ligand: Lipopolysaccaride (LPS). The aim of this study was isolation and purification of this unique population from cellular content of peritoneum, spleen and blood and determining their functional activity in producing IgM antibody in response to stimulants in cell culture under experimental conditions. **Methods:** With direct heart puncture, splenic puncture, and peritoneal lavage from inbred BALB/c mice, cells were collected and purified through Ficoll density gradient and nylon wool column for purification of B lymphocytes. In complete tissue culture medium (Bwool), cells were harvested and divided into 2 Groups, experimental and control. LPS stimulation was performed on the experimental group for different durations: 24, 48 and 72 hours. Finally culture supernatants were assayed for IgM concentration with ELISA Technique. The proliferation rate was defined by MTT assessment. Immunophenotyping studies for confirmation of cellular purity were carried out by CD3 and CD5 markers. **Results:** Lymphocytes from spleen and peritoneum organs had significantly higher levels of IgM secretory activity in 24 hours, as compared to the control groups. In Immuno-phenotyping studies, purified B lymphocytes from peritoneum showed highest levels of CD5 marker. **Conclusion:** The findings of this research indicate that Cells collected from splenic puncture and peritoneal fluid are excellent source for IgM antibody with polyspecific properties against LPS in the laboratory and extremely useful for research purposes.

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# 194Interleukin-1 Receptor Antagonist Gene Polymorphism and Susceptibility to Bipolar Disorder

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**Background & Objective:** Bipolar disorder (BD) is a chronic severe mood disorder that has been consistently demonstrated to have a strong inherited component. IL-1RA protein has been widely investigated and found to be associated with different human neurodegenerative disease. The aim of this study was to investigate IL-1RN genotype and its associations with different phases of BD disease in a group of Iranian subjects. **Methods:** Totally, 135 patients meeting DSM-IV-TR criteria for bipolar disorder and 182 controls matched to the cases on age, sex and geographic population were admitted to the study. The severity of disorder was assessed by using HDRS and YMRS for depression and mania phases, respectively. IL-1RN polymorphism was analyzed by amplifying the polymorphic region using SSCP- polymerase chain reaction. **Results:** IL-1RN1/2 heterozygote genotype was more prevalent in BD patients than controls (45.2% vs. 30.8%, P< 0.05). Further stratification of the BD patients into acute and chronic disease subgroups revealed a strong association between IL-1RN1/2 heterozygous and chronicity of disease (OR 3.8; 95% CI (1.8-7.9); P< 0.005) and also in subjects with chronic depression phase (OR 8.4; 95% CI (2.5-27.8); P< 0.0005). **Conclusion:** These findings suggest that the IL-1RN (86bp)n polymorphism might be a genetic susceptibility risk factor for bipolar disorder in an Iranian population.

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## 195Correlation between Anxiety and Salivary Cortisol and IgA

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**Background & Objective:** Psychological stress can induce some changes in the immune system. It is assumed that psychological stress increases the production of some defense factors. So the aim of this study was to determine the relationship between the rate of anxiety and cortisol and sIgA in saliva of dental students of Shahid Beheshti University of medical sciences. **Methods:** In this descriptive study 1 ml of unstipulated saliva was taken from 80 students between 7:30 and 7:45 am. ELISA was used for measuring the concentration of sIgA and cortisol in saliva and the level of anxiety was measured by Zung method. **Results:** We did not find any significant relationship between concentration of sIgA and anxiety; and concentration of salivary cortisol and anxiety. **Conclusion:** It is suggested that chronic stress or anxiety probably does not have any profound effect on mucosal defense of oral cavity. Of course more studies are needed in order to prove this hypothesis.

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# 196Association of Psychiatric Disorders and Serum Levels of IL-4 and IFN-γ in Sulfur Mustard Exposed Patients

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**Background & Objective:** It has been suggested that exposure to chemical warfare has severe and long-lasting detrimental effects on mental health and that these effects cannot be merely as a result of exposure to conventional warfare. Several studies have shown the relationship between Th1 or Th2 cytokines and psychiatric disorders. The aim of the present study was to determine the association of depression, anxiety and stress with serum levels of IFN-γ (Th1) and IL-4 (Th2) cytokines in Sulfur mustard (SM) exposed subjects 20 years after exposure. **Methods:** In a historical cohort study, Sardasht-Iran Cohort Study (SICS), 372 SM exposed participants were studied 20 years after exposure and were compared with 128 control participants who merely had exposure to conventional warfare. The Depression Anxiety Stress Scales (DASS) questionnaire was used to evaluate Depression, anxiety and stress. The serum concentrations of IL-4 and IFN-γ were measured by ELISA technique. **Results:** Mean (SD) scores for DASS-Depression, DASS-Anxiety and DASS-Stress scales was significantly higher in SM exposed group as compared to those in the control group (24.2 ± 8.2 vs. 18.0±7.5, P< 0.001; 24.5 ± 9.0 vs. 19.5 ± 7.9, P< 0.001; 27.1±7.8 vs. 20.4 ± 7.4 respectively). In SM exposed subjects the scores in the three domains showed significant positive correlation with serum levels of IL-4 (depression: r=0.125, P< 0.05; anxiety: r=0.134, P< 0.05; stress: r=0.144, P< 0.05). In both study groups there was no correlation between the scores in the three domains and serum levels of IFN-γ. **Conclusion:** SM exposed subjects had higher levels of depression, anxiety and stress. Level of depression, anxiety and stress in SM exposed subjects was correlated with higher serum levels of IL-4 but no correlation was seen in control group. It can be due to the exposure to SM and inflammatory status in these patients.

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## 197Neuropeptides and Induction of Neutrophil Death

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**Background & Objective:** Neutrophil apoptosis is an important event in the resolution of inflammation. The role of substance P (SP) and calcitonin gene related peptide (CGRP) in neutrophil apoptosis has not been previously investigated, so the aim of this study was to assess the effect of these neuropeptides on the neutrophil death. **Methods:** Neutrophils were collected from heparinized blood from a healthy individual. Then the cells were incubated with two different doses of SP (0.42 and 0.62 pg/ml) and CGRP (0.01 and 0.11 pg/ml). Annexin V fluos staining was used for detecting the apoptotic and necrotic neutrophils under fluorescence microscope. Results: Both SP and CGRP significantly induce apoptosis in neutrophils in a dose dependent manner ($P< 0.05$). **Conclusion:** It is concluded that both substance P and CGRP could induce apoptosis instead of necrosis in the neutrophils, thus could modulate inflammatory response by the nervous system.

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# 198Serum Leptin Level in Depression

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**Background & Objective:** It is well documented that leptin is a circulating hormone that plays a key role in regulating food intake and body weight via its actions on specific hypothalamic nuclei. However leptin receptors are widely expressed in the CNS, in regions not generally associated with energy homeostasis, such as the hippocampus, cortex and cerebellum. Moreover, evidence is accumulating that leptin has widespread actions in the brain. In particular, recent studies have demonstrated that leptin markedly influences the excitability of hippocampal neurons, via its ability to activate large conductance $Ca2^{+}$-activated $K^{+}$ (BK) channels, and also to promote long-term depression (LTD) of excitatory synaptic transmission. Regarding some controversies about the exact role of leptin in depression, the aim of this study was to evaluate serum leptin and cortisol levels and assess its correlation with depression. **Methods:** 38 depressed patients and 20 control subjects were enrolled. The level of depressive symptoms was measured with SCL-90-R. Serum leptin and cortisol levels were measured by ELISA. **Results:** We found significant lower concentration of Leptin in depressed patients ($P< 0.05$) but not significant difference regarding cortisol level. **Conclusion:** It seems that decrease in leptin level might play important pathophysiological role in these psychiatric disorders.

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## 199Serum Leptin and Cortisol Level in Anxiety

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**Background & Objective:** Leptin is known as a peptide hormone derived from adipose tissue, regulates food intake and controls weight. Serum leptin levels may be elevated in critically ill patients and in cases of physical stress. Our aim was to evaluate serum leptin and cortisol levels and the relationship between serum leptin and cortisol levels and anxiety. **Methods:** 30 patients with anxiety (consisted of 17 female and 13 male) and 20 control subjects (consisted of 12 females and 8 males) were enrolled. The level of anxiety symptoms was measured with SCL-90-R. Serum leptin and cortisol levels were measured by ELISA. **Results:** We found higher concentration of Leptin in patients with anxiety and higher cortisol level in anxiety patients than normal control. We could not find any significant correlation between leptin and cortisol level in patients with anxiety. **Conclusion:** Therefore, in addition to cortisol, leptin may be a valid neuroendocrinologic marker for the stress response during anxiety. Further studies are needed to examine the correlation between leptin levels and different components of stress response in patients with anxiety.

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# 200The Study of Th17 and Regulatory T Cells Population in Endometriotic Patients

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**Background & Objective:** Endometriosis is a chronic condition characterized by the growth of endometrial tissue outside the uterine cavity affecting at least 10-15% of reproductive-aged women. It is the most common cause of pelvic pain and occurs in 13%-33% of women with infertility. It has been suggested that, immune cells such as IL-17 producing T cells (Th17 cells) and regulatory T cells (Tregs) are involved in the pathogenesis of endometriosis. IL-17A is a pro-inflammatory cytokine secreted from Th17 cells which its level is elevated in a various range of inflammatory conditions in humans. Tregs have an anti-inflammatory role and maintain tolerance to self antigens. These regulatory cells are able to suppress the proliferation of autologous lymphocytes that kill ectopic cells. In the present study, we investigated the percentages of Th17-expressing $CD4^+$ and $CD4^+$ $CD25^+$ $FoxP3^+$ Treg cells in PBMC of 10 endometriotic women in comparison with normal ones by Flow cytometric analysis. **Methods:** For characterization of Th17 Cells, PBMC were stained with conjugated monoclonal antibodies specific for the CD3, CD4 and IL-17A protein and Treg cells were stained with conjugated monoclonal antibodies specific for CD4, CD25 and FoxP3. Flow cytometric analysis was performed using PARTEC system with FLOWMAX software. **Results:** In this study Th17 and Treg cells showed a statistically significant increase in blood of women with endometriosis in comparison with normal ones. **Conclusion:** The main role of Tregs in the maintenance of immune cell homeostasis and immunologic tolerance are to suppress auto-reactive T cells. In endometriosis, T cell-like populations, capable of targeting the autologous endometriotic transplant, may exist in the blood and peritoneal fluid. Increased Tregs can suppress these T cell-like populations and cause undesired ‘immunological tolerance’ of endometriotic lesions and sustain these lesions. One of the most relevant reasons of damage and inflammation in endometriosis is the increase of TH17 cells, producing pro-inflammatory cytokine such as IL-17, IL-6 and TNF-α, in the blood of patients suffering from endometriosisis.

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# 201Proteome Differences in Unexplained Recurrent Pregnancy Loss (URPL) Compared to Normal Placenta

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**Background & Objective:** Recurrent pregnancy loss (RPL) is defined as at least two sequential abortion before the 20th week of gestation. In approximately 40% of RPL cases the etiological factors which causes abortion are unknown and so named, unexplained RPL (URPL). Placenta is a pregnancy unique tissue and proper formation of the placenta is a key phenomenal for success a pregnancy or occurrence of UPRL. Therefore, the aim of the present study is comparison of the human placental proteome between URPL and normal first trimester placentas. **Methods:** Total placental proteins were extracted and two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) technique was used to compare the proteome of five URPL and five gestational matched normal placentas. After staining, the gels were scanned and the spots intensities were determined using Image Master 2D Platinum Software and compared between URPL and normal cases. Statistically differentially expressed spots were excised from the gels and identified by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF/TOF) technique after in gel digestion. **Results:** Though 19 spots showed statistically different expression ($P< 0.05$), 12 out of them were successfully identified. Among them only two proteins were down-regulated (Calumenin, Enolase 1) while the remaining ten spots (Actin gamma 1 propeptide, Cathepsin D prepropeptide, HSPgp96, Tubtlin bet, Tubulin alpha 6, Glutathione S-transferase, vitamin D binding protein, Prohibitin, Actin beta, Apolipoprotein A-I) showed increased expression in URPL cases in comparison with normal first trimester placentas. **Conclusion:** In conclusion, the data of the present study indicated that the alteration in expression of proteins involves in endothelial dysfunction might play an important role in the pathogenesis of URPL.

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# 202Proteome Differences of Placenta between Pre-Eclampsia and Normal Pregnancy

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**Background & Objective:** Placenta is a tissue unique to pregnancy and despite its major role in pregnancy, little is known about the proteome changes within placenta during pregnancy-related diseases such as pre-eclampsia (PE). Therefore, the aim of this study is the analysis of proteome differences between pre-eclamptic and normal full-term placentas. **Methods:** Five normal and five severe pre-eclamptic placentas were included in this study. Total placental proteins were extracted and subjected to two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). After staining, the gels were scanned and the protein spots were analyzed using Image Master 2D Platinum Software. Non-parametric Mann–Whitney test was used for analysis of the mean intensity differences of the spots between normal and pre-eclamptic placentas. Statistically differentially expressed spots were excised from the gels and identified by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF/TOF) technique after in gel digestion. **Results:** Statistical analysis indicated that 17 spots were differently expressed in pre-eclamptic compared with normal placentas ($P< 0.05$). Using MALDI TOF/TOF technique 11 out of 17 spots were identified. Among them, four proteins (chloride intracellular channel 3, apolipoprotein A-I, transthyretin (TTR) and protein disulphide isomerase) were up-regulated while seven (peroxiredoxin 2, peroxiredoxin 3, Hsc 70, Cu/Znsuperoxide dismutase (SOD-1), actin gamma 1 propeptide, chain A of enoyl-coenzyme A hydratase and HSP gp96) showed decreased expression in PE in comparison with normal placentas. **Conclusion:** Down-regulation of proteins with anti-oxidant activities (peroxiredoxin 2 and peroxiredoxin 3) and altered expression of stress-response proteins (Hsc 70, Hsp gp96 and protein disulphide isomerase) might play an important role in the pathogenesis of PE.

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## 203Regulatory Function of Menstrual Blood-Derived Stromal Stem Cells

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**Background & Objective:** Menstrual blood stromal stem cells have been demonstrated to exhibit stem cell properties such as the capability of self-renewal and multipotency, allowing for multilineage differentiation. Also these cells have different immunomodulatory effects. In this study we examined the immunoregulatory function of menstrual blood stromal stem cells. **Methods:** Menstrual blood was collected from 5 apparently healthy donors after menstrual blood flow initiated. Mononuclear cells derived from menstrual blood were separated by Ficoll-Hypaque. Cells were subsequently cultured in DMEM medium supplemented with fetal bovine serum. Adherent cells were allowed to propagate and used as stem cells. Flow cytometric Immunophenotyping was performed by a panel of monoclonal antibodies including CD44, CD45, CD34, CD9, CD29, CD10, CD38, CD105, CD73, CD133, STRO-1 and Oct-4. For functional analysis, peripheral blood mononuclear cells (PBMC) were co-cultured with menstrual blood-derived stromal stem cells and after 4 days were collected and added to allogeneic PBMCs. XTT assays were carried out to evaluate the cell proliferation. Also, the induction of regulatory T cells ($CD4^+CD25^+Foxp3^+$) was investigated by Flow cytometry. **Results:** Menstrual blood-derived stromal cells showed unique surface and intracellular markers of mesenchymal stem cells with some differences. The proliferation assays revealed that menstrual blood stromal stem cells decreased the proliferative capacity of allogeneic PBMCs. Additionally; the induction of regulatory T cells was shown to be one of the underlying mechanisms responsible for the inhibitory effect of menstrual blood stem cells. **Conclusion:** Here we show the immunoregulatory capacity of menstrual blood stromal stem cells which can be viewed as a prospect for the treatment of autoimmune diseases.

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# 204The Establishment of a Sandwich ELISA Based on a Homemade Polyclonal Antibody for Earliest Detection of Pregnancy Associated Glycoprotein

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**Background & Objective:** One of the most important management components involved in dairy industry is reproduction program, the corner stone of which is pregnancy diagnosis techniques. Achieving a fast, accurate and cost benefit pregnancy detection test has always been a predicament in Veterinary Science. Over the last two decades numerous attempts have been made to establish pregnancy diagnosis techniques based on serological markers. Pregnancy Associated Glycoprotein (PAG), an approximately 70 kDa protein secreted from trophoectodermal cells, is a new pregnancy indicator. This study describes development of a novel sandwich ELISA test by the use of homemade polyclonal antibody, to determine the earliest detection time of this protein. **Methods:** The polyclonal antibody was raised against a designed peptide representing a conserved sequence located at the C terminal part of PAG. Purity and reactivity of the polyclonal antibody was examined by ELISA and Sodium Dodecyl Sulfate- Poly Acrylamide gel electrophoresis (SDS-PAGE). One hundred and ten Blood samples were collected from Iranian Holstein Heifers in the period of 14 to 28 days after artificial insemination (AI). Samples were transferred at 4 0C to the laboratory; then, sera were separated and preserved for further examinations. The pregnancy status of inseminated heifers was clarified 35 days after insemination by rectal palpation; moreover, the statistical analysis was performed between clinical results and the optical densities obtained from direct sandwich ELISA of the sera. **Results:** Analysis of variance showed a statistically significant correlation between pregnancy status and ELISA optical densities. The earliest time of significant difference between pregnant and non-pregnant heifers was the period of 17 to 22 days after insemination. **Conclusion:** The ELISA revealed that pregnancy associated protein could be detected 14 days after insemination; however, the significant difference between pregnant and non pregnant heifers was mainly observed in 17-22 days.

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# 205Polyclonal Antibody Production against a Pregnancy Associated Glycoprotein Peptide: Application for Purification and Characterization of These Proteins from Placental Tissues

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**Background & Objective:** Pregnancy-associated glycoprotein (PAG) was discovered when trying to develop an early pregnancy test in cattle. Although 26 isoforms of PAG proteins have been identified in different species till now, many more are expected to come up in the near future. The objectives of the present study were to produce a polyclonal antibody against a designed peptide representing a conserved sequence located at the C terminal part of the protein, and to establish an immunoaffinity chromatography method for purification of these molecules from Iranian Holstein cattle cotyledonary tissues for the first time. **Methods:** The polyclonal antibody was produced by injecting peptide-KLH conjugate to a rabbit. The purity and reactivity of the polyclonal antibody was examined by ELISA and SDS-PSGE; then, it was conjugated to CNBr activated sepharose 4B beads to build up an immunoaffinity chromatography column. Placental samples were collected form a slaughter house in the urban of Tehran. Fetal ages were estimated by Crown rump length method. PAGs were extracted, precipitated, separated by DEAE sepharose Ion exchange chromatography and immunoaffinity chromatography and characterized by the use of SDS-PREGPAGE and Western blot. **Results:** The purification process was able to successfully isolate the Pregnancy Associated Glycoprotein from the placental tissues. Molecular weight ranges from 66 to 88 kDa; moreover, at different stages of pregnancy diverse isoforms are expressed. **Conclusion:** This study for the first time describes the isolation of PAG molecules from Iranian Holstein Dairy cattle placental samples. The use of immune affinity chromatography based on a homemade polyclonal antibody, in purification procedure resulted in a high degree of enrichment of the PAG molecules.

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# 206Purification and Characterization of Blood Circulating Forms of Bovine Pregnancy Associated Glycoprotein from Iranian Holstein Diary Cattle during Pregnancy

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**Background & Objective:** Pregnancy Associated Glycoprotein (PAG), also known as Pregnancy Specific Protein B (PSPB) was first discovered by trying to develop an early pregnancy test in cattle. PAGs are divided into three groups in terms of sequence identity, temporal expression and tissue expression pattern determined by in situ hybridization this study describes the purification and exploring the molecular properties of blood circulation forms of this family by the use of a homemade polyclonal antibody raised against a conserved sequence at the C terminal part of the protein. **Methods:** The polyclonal antibody was produced by injecting peptide-KLH conjugate to a rabbit. The purity and reactivity of the polyclonal antibody was examined by ELISA and SDS-PSGE; then, it was conjugated to CNBr activated sepharose 4B beads to build up an immunoaffinity chromatography column. Bovine Pregnancy Associated Glycoprotein was isolated form blood samples captured from Days35 to 252 after Artificial Insemination (7 days intervals) and their concentrations were determined. The isolated Pregnancy Associated Glycoprotein was characterized by SDS-PAGE and Western blotting. **Results:** The purification process was able to successfully isolate the bovine Pregnancy Associated Glycoprotein from the blood samples. Molecular weight ranges from 21 to 87 kDa; moreover, at different stages of pregnancy diverse isoforms of protein are expressed. A significant correlation was found between pregnancy progression and concentration of Pregnancy Associated Glycoprotein in blood samples. **Conclusion:** This study for the first time describes the isolation of PAG from Iranian Holstein dairy cattle blood samples. Whereas the use of immunoaffinity chromatography, based on a homemade polyclonal antibody, in purification procedure resulted in a high degree of these molecules isolation, serological tests such as radio immune assay or ELISA on the bases of this antibody can be used as an early pregnancy diagnosis technique.

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# 207The Role of NK Cells in Recurrent Spontaneous Abortion

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**Background & Objective:** At the time of implantation the endometrial is populated by abundant maternal leukocytes, the majority of which are large granular lymphocytes (LGL) whose cytoplasmic granules contain cytolitic molecules. These cells are unusual in that while they stain strongly for the natural killer (NK) marker CD56, most do not express other classical NK markers such as CD16 and CD3. The aim of this study was to investigate the percentage and absolute number of natural killer (NK) cells in women with recurrent spontaneous abortion (RSA) of unknown etiology. **Methods:** 24 Women with a history of recurrent pregnancy losses with unknown etiology were included in this study. We compared the percentage of peripheral blood NK cells by Flow cytometry in these patients with a group of fertile patients who had no history of pregnancy loss. Lymphocytes from peripheral blood were isolated by Ficoll-Hypaque density centrifugation. Lymphocytes were stained using Phycoerythrin anti CD56 and Fluorescent isothiocyanate (FITC)-anti CD16 and CYQ-CD3 monoclonal antibodies for identification of NK cells and was used anti CD56(PE) and anti CD69(FITC) for detection of activated NK cells. We used BD FACS calibure Flow cytometry for data analysis. **Results:** On the basis of the obtained results, absolute number of $CD16^{+}56^{+}$ cells showed significant increases in recurrent spontaneous abortion (RSA) in comparison with control group. Also absolute number of $CD16^{+}56^{bright}$ cells had significant increase in RSA. There was no significant difference of CD16+56dim cells between RSA and control group. Also, results showed significant increase in the absolute number of $CD56^{+}/CD69^{+}$ cells in RSA. **Conclusion:** The results suggested that the higher percentage of NK cells in peripheral blood of RSA patients compared to control group may indicate the same increase in number and cytotoxicity of uterine NK cells .So we can relate the percentage of NK cells in peripheral blood to the risk of recurrent pregnancy loss.

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# 208Proportional Change of $CD4^+CD25^+$T Regulatory Cells in Peripheral Blood in Unexplained Recurrent Spontaneous Abortion Patients by Flow cytometry

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**Background & Objective:** During human pregnancy, Th1/Th2 and CD4+CD25+Tregulatory cells paradigm is adapted to balance T cell immunostimulation and immunoregulation. CD4+CD25+Treg cells play a critical role in peripheral tolerance, transplantation tolerance and maternal tolerance to the fetus. The deficiency in proper recognition of fetal alloantigen by the maternal immune system is associated with recurrent pregnancy failure. Here, we investigate the proportional changes of $CD4^+CD25^+$Tcells in peripheral blood in unexplained recurrent spontaneous abortion (URSA) and normal pregnant women. **Methods:** Twenty-four women, who had at least three miscarriages of unexplained etiology, comprised the URSA group. The diagnosis of 'unexplained' abortion was made from the following guidelines to exclude any verifiable causes. Abnormalities of uterus and cervix, karyotypes of abortion couples, hyperprolactinemia, PCOD, diabetes, hyperthyroidism, hypothyroidism diseases were excluded. Serum samples were analyzed for FSH, LH, E2, PRL, T, TSH, thyroglobulin antibody, serum glucose, insulin, thrombin, lupus anticoagulant, anticardiolipinantibodies, antinuclear antibodies, thrombophilia.We compared the concentration of $CD4^+CD25^+$ Treg cells in peripheral blood of these patients until 21 normal pregnant women with any history of URSA. The procedure was done as below: Venous blood (100 µL) was dispensed into two tubes, and isolated PBLs lymphocytes, then FITC-conjugated anti-CD4 and PE-conjugated anti-CD25 antibodies were added to one tube. After samples incubation and centrifuged and washed twice with phosphate-buffered saline, and the cells were analyzed by a FACS Callibur system (Becton Dickinson) using Cell Quest software. **Result:** In PBL, the proportion of $CD4^+CD25^{bright}$ T cells in URSA patients were ($P< 0.001$) lower than those in control women. The proportions of $CD4^+CD25^{dim}$ Treg cells in URSA patients were ($P< 0.006$) higher than those in control women. Meanwhile, the proportion of $CD25^{bright}$ cells in the $CD4^+$ T-cell was statistically significantly lower in URSA patients than in control women. The proportion of $CD4^+CD25^-$ cells was higher in URSA patients than in control women. **Conclusion:** Inhibition of the immunostimulation of conventional Tcells can change proportional of $CD4^+CD25^+$Tcells in peripheral blood. This suggests that human $CD4^+$CD25brightTcells play a major role in tolerating conceptus antigens and cytokine meanwhile might contribute to the maintenance of pregnancy. Inadequate $CD4^+CD25^+$ Tcells or their functional deficiency may link with miscarriage, therefore $CD4^+CD25^+$ Tcells serve as a novel biomarker for monitoring in URSA patients.

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# 209The Association of Humoral Immunity and Iron Parameters in the Third Trimester of Pregnancy

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**Background & Objective:** To determine the relationship between humoral immunity, complement system and cytokines with iron parameters in third trimester of pregnancy. **Methods:** Participants were randomly selected from pregnant women referred to the obstetrics ward of a Dr. Shariati Hospital in Bandar Abbas, Iran, in their third trimester of pregnancy. Blood samples were obtained and hematologic and immunologic factors were analyzed. **Results:** From 92 participants, 21 had IDA (Iron deficiency anemia). In our study no association between the hemoglobin, ferritin, TIBC and levels of immunologic factors. we noticed that higher level of serum iron are correlated with higher level of C3 , C4 and IgG1 .In our study , C4 level were marginally lower in iron deficient pregnant women. **Conclusion:** Although limited relationship between c3,c4,IgG1 and iron parameters in pregnant women has been seen but there is no significant changes in immunological factors between normal and IDA pregnant women. Although uncompare with extend immunological changes have been observed in children suffering IDA, our study showed that IDA has little effect on humoral immunity system of pregnant women but our result can predict increased potential infection risk with pyogenic bacteria in pregnant woman with IDA.

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# 210The Changes of T Helper Associated Antibodies and Cytokines in Patients with Gestational Diabetes Mellitus

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**Background & Objective:** Diabetes mellitus (DM) is a group of metabolic disorders with hyperglycemia phonotype such as DM I, DM II, secondary causes and gestational diabetes mellitus. The etiology of gestational diabetes mellitus is unknown. Recent studies address chronic activity of immune system against infections (not autoimmunity) as an important cause of etiology of gestational diabetes mellitus that recognizes. This study aimed to compare T helper1&2 associated antibodies and cytokines in patient with gestational diabetes mellitus with normal pregnant women. **Methods:** A case-control study was performed on 45 female with GDM (case) and 45 healthy pregnant women in Bandar Abbas , Iran , from 2008-2009. The exclusion criteria were any infectious disease and autoimmune disorder such as SLE and RA. All of them were examined and they were asked about past medical history. Taken them 10cc of their blood and send to laboratory and were measured the IgE ،IgG1 ،IgG2، IgG3 ، IgG4 ، IL-10 ، IL-12 ، TGF-$\beta$1 ، IFN-$\gamma$ in their serum. T test and Kolmogorov-Smirnov test were used for data analysis. **Results:** This study reports that Th1 and Th2 associated antibodies and cytokines have not significant relationship between case and control groups. **Conclusion:** The changes of Th1 and Th2 associated antibodies and cytokines is not associated with gestational diabetes mellitus and could not be considered as a predictor in gestational diabetes mellitus recognition.

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# 211Th1 and Th2 Cytokine Production by Peripheral Blood Mononuclear Cells in Recurrent Spontaneous Abortion in Comparison with Normal Situation

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**Background & Objective:** Recent attention has focused on elucidating the immunobiological roles of Th1 and Th2 cytokines in abnormal human pregnancy following the accumulation reports of complex cytokine activity within uteroplacental tissues. This prospective study was designed to elucidate whether a failure of cytokine shift (Th2→Th1) pre-dated pregnancy loss and was therefore likely to be an etiological factor in recurrent spontaneous abortion (RSA). **Methods:** Cytokine production by stimulated peripheral blood mononuclear cells from 40 pregnant women suffered RPL in early pregnancy, with no defect in their Kariotype, was compared with 30 gestationally age-matched pregnant controls. Th1 (TNF-α, IFN-γ) and Th2 (IL-4, IL-6, IL-10) cytokines were assessed using ELISA method. Progesterone and hCG hormones were also measured by radioimmunoassay in serum of the samples. **Results:** Significant difference in IL-4, IL-6, IL-10 and TNF-α secretion were observed in RSA patients who subsequently miscarried with those who successfully completed the pregnancy. No significant difference in IFN-γ secretion was observed between the two groups. There was relationship between progesterone secretion and Th2 cytokines in the groups. **Conclusion:** Recurrent aborters who proceed to have successful pregnancy are more Th2-biased than abortion –prone women who abort. However, recurrent aborters who undergo other spontaneous abortion have a strong Th1 bias than aborters who have normal pregnancy. Progesterone hormone may has a role in regulation of Th2 type cytokines.

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# 212Analysis Serum Level of TGF-β1 and IL-18 in Post Menopausal Osteoporotic and Non-Osteoporotic Women

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**Background & Objective:** Osteoporosis that is encountered frequently in postmenopausal women may cause an increased incidence of vertebral and iliac fractures that are associated with excess morbidity. Bone cells produce multiple growth factors and cytokines that have effects on bone metabolism. Transforming growth factor-beta has been postulated to play a role in controlling bone density by the regulation the fine balance between bone matrix deposition by osteoblasts and its resorption by osteoclasts. Background & Objective: The aim of this study was to determine serum level of osteoclast activating cytokine, interleukin-18 and TGF-β1 in postmenopausal osteoporotic and non osteoporotic women. **Methods:** Subjects were 65 postmenopausal women with osteoporosis (T score<-2.5 in the lumbar spine or femoral neck) and 69 postmenopausal normal women. (T score 0-1) Exclusion criteria were known atherosclerosis, diabetes, morbid obesity, familial hyperlipidemia and severe systemic disease. We measured serum level of TGF-β1 and IL-18 by quantitative sandwich ELISA (Enzyme Linked Immunosorbent Assay). **Results:** TGF-β1 was significantly higher in the osteoporotic women than in non osteoporotic women (23.84 ± 18.21 vs. 15.77 ± 16.80 ng/ml; P< 0.01). But there wasn't correlation between serum level of IL-18 and bone mass density. There was a positive correlation between body mass index (BMI) and serum level of TGF-β1 (P< 0.04). **Conclusion:** Our study suggests that TGF-β1 may play a key role in osteoporotic women and appear to have a good predictive value for osteoporosis.

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# 213Study of the Relation between Soluble HLA-G with Success Rate in ICSI

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**Background & Objective:** Pregnancy is a successful transplantation. The factors evading rejection of the fetus are poorly understood. The success rate and maintaining the embryo in intracytoplasmic sperm injection (ICSI) procedure may also depend on the same factors. The molecules of HLA-G are nonclassical major histocompatibility complex class I antigens that have been attracted attention in relation to pregnancy. In order to find the relation of soluble HLA-G (sHLA-G) with the success of pregnancy, the serum levels of sHLA-G was measured in women before and after ICSI and was also compared to the serum levels of normal pregnant women. **Methods:** Serum samples of 131 women under ICSI (test group) were collected before and 14 days after embryo transfer and serum samples of 24 normal pregnant women (control group) were collected in the first trimester. Soluble HLA-G1 and G5 isoforms and total sHLA-G were assayed with a sandwich ELISA. **Results:** No significant differences in clinical parameters (age, infertility duration, treatment regimen) were observed between pregnant and nonpregnant women under ICSI procedure. The levels of sHLA-G1 and G5 and optical density (OD) of total sHLA-G prior and after ICSI in pregnant group were respectively 47.4 ± 62.8 U/ml, OD: 1.47 ± 0.58 prior and 59.6 ± 69.5 U/ml, OD: 1.38 ± 0.57 after ICSI. In nonpregnant group these were respectively 35.19 ± 54.3 U/ml, OD: 1.37 ± 0.45 prior and 39.7 ± 57.2 U/ml, OD: 1.31 ± 0.46 after ICSI. The same factors in control group were correspondingly 53.16 ± 47.92 U/ml and OD: 1.29 ± 0.49. No significant differences were found between pregnant and nonpregnant groups and corresponding control group. The results of 39 pregnant and 92 nonpregnant women in the test group showed no significant increase or decrease in the serum levels of sHLA-G1 and G5 isoforms and OD of total sHLA-G after embryo transfer. **Conclusion:** No relation was found between sHLA-G and the success of pregnancy in women under ICSI procedure.

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# 214Serum Level and Polymorphisms of Promoter Region of Transforming Growth Factor-Beta (TGF-β1) Gene in Pre-Eclampsia and Control Group

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**Background & Objective:** Pre-eclampsia (PE) is pregnancy associated disorder with hypertension and proteinuria that cause neonatal and maternal morbidity and mortality. The current hypothesis regarding the etiology of PE is focused on deviation of immune responses and Type1/Type2 cytokines disequilibrium. Type2 cytokines appear to contribute to the maintenance of pregnancy by controlling the immune and endocrine systems. TGF-β1 plays important roles in immunoregulation, immune deviation, placental development, hypertension and renal function. Presence of this cytokine in semen and pregnancy associated site (tissues) prompt us to evaluate association of this cytokine with PE ethiopathology. **Methods:** In this investigation the polymorphisms of the TGF-β1 gene at promoter region positions -800 (G/A) and -509 (C/T) that affect expression of this cytokine were studied in 142 PE and 140 normal pregnant female subjects by PCR-RFLP. Also TGF-β1 serum level was determined by ELISA method. **Results**: It was shown that at position -800 (G/A) polymorphism, genotype distribution and allele frequencies between PE patients (GG 73.9%; GA 21.1%; AA 4.93%) and normal control (GG 70%; GA 28.6%; AA 1.4%) showed no significant differences. but AA genotype of -800 (G/A) position were higher in PE patients than control group. In the case of the -509 (C/T) polymorphism, 28.2% of patients, 25% of normal controls, were homozygote CC. While 41.5% of cases and 44.3% of normal controls, were heterozygote CT, 30.3% of PE and 30.7% of normal controls, were homozygote TT respectively. Statistical analysis showed no significant differences of allele and genotype distributions frequencies between PE cases and normal controls at position-509 (C/T). But CC genotype at this position was higher in PE patients than control group. Mean TGF-β1 serum levels were 62.14 and 45.7 ng/ml in PE patients and control group respectively. **Conclusion:** The promoter region polymorphisms of TGF-β1 may not be associated with PE, but serum levels of this cytokine may contribute in the etiopathology of PE by different mechanisms. Future studies need to clarify the association of TGF-β1with PE.

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# 215Quercetin Modulate Expression of Different Regulatory Genes Involving in Tumor Invasion, Angiogenesis and Apoptosis in Human Prostate Adenocarcinoma Cell Line

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**Background & Objective:** Quercetin, the most abundant dietary flavonoid, is inversely associated with the risk of various types of malignancies. Moreover, it has been manifested that consumption of quercetin is inversely associated with PCa risk. The purpose of the current study is to evaluate the effects of quercetin on human prostate adenocarcinoma PC-3 cells. **Methods:** Lactate dehydrogenase release, microculture tetrazolium test and quantitative real-time RT-PCR array were employed to appraise the influences of quercetin on cell cytotoxicity, cell proliferation and expression of various genes in PC-3 cell line. **Results:** Quercetin inhibited cell growth and proliferation and modulated the expression of genes involved in tumor invasion, angiogenesis and apoptosis. In addition, no cytotoxicity of quercetin on PC-3 cells was observed. **Conclusion:** These issues introduce quercetin as an efficacious agent in order to be used in the future nutritional transcriptomic investigations and multi-target therapy.

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# 216Comparison of Plasma Vascular Endothelial Growth Factor and Complement C3a in Patients with Colorectal Cancer before and after Treatment in Southern Iran

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**Background & Objective:** Colorectal cancer is a major cause of world wide morbidity & mortality and is the second most common cause of cancer death. Colorectal cancer is often diagnosed at a late stage with poor prognosis. Vascular endothelial growth factor (VEGF) is a neo-angiogenesis with great importance for tumor growth, which has a direct effect on vascular endothelial cell proliferation and migration.C3a is also diagnostic factor in determining colon cancer. The aim of the study was to measure the VEGF and C3a level in patients with colorectal cancer. **Methods:** One hundred and nine patients with colorectal cancer, including 64 Men and 45 women. (At an average age of 54 years) were enrolled into the study. VEGF and C3a level of 109 patients with colorectal cancer were determined using ELISA method. Only 55 Patients with elevated serum VEGF and C3a were followed up after 3 months, because of death of the rest. **Results:** Our results demonstrate that VEGF is a suitable diagnostic tumor marker in patients with colorectal cancer. A combination of the serum tumor markers C3a and VEGF can significantly increase the pre-operative diagnostic. VEGF and C3a serum level slowed significantly difference pre- and post – treatment [(mean 385.7 pg/ml, 262.2 pg/ml; 2.2 ng/ml, 1.8 ng/ml), ($P< 0.001$) ($P< 0.005$)]. **Conclusion:** Both VEGF and C3a are useful markers to predict future metastasis, survival, and response to the treatment.

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# 217Soluble HLA-G7 Expression in Gastric Adenocarcinoma

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**Background & Objective:** HLA-G is a human nonclassical major histocompatibility complex antigen that is expressed as seven isoforms, including four membrane bound (HLA-G1 to HLA-G4) and three soluble (HLA-G5 to HLA-G7) forms. Membrane bound and soluble isoforms of HLA-G show tolerogenic properties that are appropriate for tumor escape from immune survillance. **Methods:** In this study, 30 gastric adenocarcinoma patients administered in Imam Khomeini Hospital, Tehran, were included. Soluble HLA-G7 transcript expression in leucocytes of these patients was evaluated by RT-PCR method. Before therapy, lymphocytes of patients were separated by Ficoll method. RNA was extracted by TRIZOL. Reverse transcriptase was used for cDNA synthesize. Then HLA-G7 expression was evaluated by PCR method. **Results:** This study showed that HLA-G7 expression in gastric adenocarcinoma patients was increased in compared to normal subjects ($P< 0.05$). **Conclusion:** By considering to this result and similar studies on other isoforms of HLA-G in various cancers, it is promising that HLA-G7 may be used as tumor biomarker in body fluids, a target for antitumor therapy, distinction marker between benign and malignant tumors and prognosis of clinical outcomes.

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# 218Cell Growth and Proliferation of Human Hepatocellular Carcinoma Cell Line, HepG- Inhibited by Silibinin through Downregulation of Hec1 and Inhibition of Extracellular Signal-Regulated Kinase 1/2 Phosphorylation

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**Background & Objective:** The aim of the current study is to evaluate the effect of silibinin on human hepatocellular carcinoma HepG-2 cells. **Methods:** Microculture tetrazolium test (MTT assay), Lactate dehydrogenase (LDH) release, Gelatin zymography, Griess reaction, Cell-based the extracelluar signal-regulated kinase (ERK) 1/2 phosphorylation assay and quantitative real-time RT-PCR were employed to appraise the effect of silibinin on cell proliferation, cytotoxicity, metastatic potential, nitric oxide (NO) production, ERK 1/2 phosphorylation and activation in HepG-2 cells. **Results:** Silibinin inhibited cell proliferation, matrix metalloproteinase 2 enzymatic activity, NO production and ERK 1/2 phosphorylation in a dose-dependent manner without exerting any cytotoxicity effect. In addition, an expressive increase in mRNA levels of Raf kinase inhibitor protein (RKIP), sprouty-related protein 1 with EVH-1 domain (Spred-1), sprouty-related protein with EVH-1 domain 2 (Spred-2) coupled with a significant reduction in transcriptional levels of highly expressed in cancer (Hec1) and MMP-2 were observed. **Conclusion:** Altogether, these issues show for the first time that silibinin treatment could inhibit cell proliferation and invasive potential of HepG-2 cells through inhibition of ERK 1/2 cascade both directly (through suppression of ERK 1/2 hosphorylation) and indirectly (through up-regulation of RKIP, Spred-1 and Spred-2). In addition, cell growth and proliferation may be inhibited by silibinin through downregulation of Hec1.

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# 219The Effect of SIM5 and Its Fractions on BCL1 Cell Line and Normal Lymphocytes of Mice in vitro

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**Background & Objective:** According to the high incidence of leukemia in the world and various side effects of available treatment methods, finding of an effective and low danger new treatment of these malignancies are unavoidable. Preparation of SIM5 has the herbal basic and our previous studies have shown inhibition effect of SIM5 on different cell lines. The present study investigated the effect of SIM5 and its five fractions on murine BCL1 cell line (resembles a subset of human patients with CLL) and normal lymphocytes. **Methods:** Alcoholic extract and R100-R5 fractions of SIM5 were prepared and diluted in RPMI 1640 at final concentrations of 2 – 0.001 mg/ml. BCL1 and mice spleen lymphocytes were cultured 20 ×104/well and incubated with extract and fractions for 48 h. Cell viability of normal lymphocytes and BCL1 were measured with MTT test. **Results:** 2, 1, 0.5, 0.2 mg/ml concentrations of alcoholic extract decreased cell viability of BCL1 without any toxic effect on normal cells. 2, 1, 0.5 mg/ml of all fractions (except R100) and 0.2, 0.1 mg/ml doses of significantly diminished BCL1 viability. The lowest IC50 was obtained using R10. **Conclusion:** According to the points that SIM5 is non-toxic and eatable preparation, is able to inhibit significantly BCL1 cell line growth, and has no toxic effect on normal cell, after appropriate experimental trials, could be designed for clinical usage.

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# 220FC Receptor-Like (FCRL) 1-5 Gene Expression Profiling in Burkitt`s Lymphoma Cell Lines

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**Background & Objective:** FC receptor-like (FCRL) 1-5 molecules, owing to exclusive expression in B-cell lineage and possession of ITAM/ITIM motifs in their cytoplasmic regions, have been recently considered as potential targets for specific immunotherapy and/or markers for diagnosis or prognosis of B cell malignancies. Burkitt's lymphoma (BL), with germinal center-B cell origin, is an aggressive tumor that occurs in endemic, sporadic, and immunodeficiency associated variants. In this study the expression pattern of FCRL1-5 molecules was studied for the first time in BL cell lines by semi-quantitative RT-PCR and Flow cytometry. **Methods:** 18 sporadic (n=7) and endemic (n=11) Burkitt`s lymphoma cell lines were studied. Amplification of FCRL1-5 and β-actin mRNA was performed using specific primers and the band densities of FCRL and β-actin PCR products were determined. Surface expression of FCRL1, 2, 4 and 5 but not FCRL3 proteins was also analyzed in 7 cell lines using specific antibodies by Flow cytometry. **Results:** At mRNA level, FCRL1 to 5 were detected in 10-15 cell lines, of which FCRL3 (15/18) and FCRL4 (10/18) displayed the highest and the least expression frequency, respectively. Of the FCRL genes, FCRL1 ($P< 0.05$) and FCRL5 ($P< 0.01$) were expressed at higher levels. Comparison between the sporadic and endemic BL samples revealed higher expression frequency of FCRL4 in endemic (8/11) compared to sporadic (2/7) cases. Other FCRL genes were expressed similarly in these two subtypes of BL. At protein level, FCRL1, 2 and 5 molecules were expressed on the surface membrane of 4, 4 and 3 out of 7 BL cell lines tested, respectively. FCRL4 protein was totally negative in all samples. **Conclusion:** Our results indicate the potential implication of FCRL molecules, particularly FCRL1 and 5 in targeted immunotherapy of Burkitt`s lymphoma.

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# 221Anti-Tumor and Anti-Angiogenic Effects of ACA1 Plant-Product on Gastric Adenocarcinoma Cell Line

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**Background & Objective:** ACA1 is an aqueous extract of a plant-product that has been used in traditional Iranian medicine. In this study, inhibitory effect of ACA1 on growth and metastasis of gastric cancer cells and the mechanism of its action was evaluated. Also, its effect on angiogenesis of HUVEC cells was studied. **Methods:** Gastric adenocarcinoma cell line (AGS) and fibroblast cells (HgF) were incubated with different concentrations of ACA1 and proliferation of treated cells was determined using MTT assay. Death pattern of AGS cells was assayed using Annexin V-FITC and Propidium Iodide staining method. Tube formation by HUVEC cells for determination of angiogenesis. **Results:** Obtained results from MTT assay were shown a significant and dose-dependent inhibition of AGS cells after treatment with ACA1 product. But, fibroblast cells were shown less sensitivity to ACA1. ACA1 induced early and late apoptosis and necrosis in AGS cells. Also, ACA1 decreased rube formation (angiogenesis) by treated HUVEC cells. **Conclusion:** It has been shown that ACA1 product has a significant toxic effect on gastric cancer cells. Its mechanism of action was mostly through the induction of apoptosis. In addition to killing of gastric cancer cells, ACA1 could also decreased angiogenesis. So, it could be a good candidate as an anti-cancer agent against gastric cancer cells.

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# 222Evaluation of the Cytotoxic Activity of Different *Artemisia Khorasanica* Samples on Cancer Cell Lines

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**Background & Objective:** Artemisia spp. are important medical plants in the world. Cancer cell toxicity of fractions and compounds from different Artemisia species had been shown. **Methods:** In present study, three samples of Iranian *Artemisia khorassanica* were collected. Cytotoxicity of their isolated fractions was studied on cancer cell lines. Ethanol, ethylacetate, dichloromethan and hexan fractions were isolated from three Artemisia samples of different places in Khorassan province. Gastric (AGS), colon (HT-29), breast (MCF-7) and cervix (Hela) cell lines were incubated with different concentrations of fractions for 72 h. Then, cytotoxicity was measured using MTT assay and reported as IC 50. **Results:** All fractions showed strong inhibitory effects on cancer cells in a dose-dependent manner. But, it was different for same fractions from three samples. The strongest fractions were ethylacetate of sample 1, dichloromethane of sample 2, dichloromethane of sample 3 and hexan fraction from sample 1 of *Artemisia khorassanica*. MCF-7 and Hela were the most sensitive cell lines. **Conclusion:** With regard to significant toxicity of isolated fractions, they could be evaluated in prevention and treatment of different cancers.

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# 223Cancer Immunotherapy Using Dendritic Cells Matured with *Listeria Monocytogenes* Components

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**Background & Objective:** The use of DCs as a cellular adjuvant is a promising approach in immunotherapy of cancer. We have been previously demonstrated that dendritic cells (DCs) matured with *Listeria monocytogenes* antigens induce a specific anti-tumor immunity. In the present study, we used tumor antigen pulsed DCs matured in the presence of *L.monocytogenes* components such as protein ,DNA, lysate and heated antigen for induce a potent anti-tumor response in a mouse model of fibrosarcoma. **Methods:** Bone-marrow derived DCs (BMDCs) were cultured in the presence of GM-CSF and IL-4.After 5 days, tumor lysates with/without *L.monocytogenes* or protein or DNA or heated antigens were added to the culture for another 2 days. For immunization, mice received mature and tumor antigen pulsed dendritic cells subcutaneously around the tumor site. Tumor growth was monitored and two weeks after DC immunotherapy cytotoxic activity and infiltration of $CD8^{+}$T cell monitored in different groups. **Results:** Immunotherapy with dendritic cells treated with *L.monocytogenes* proteins led to a significant delay in tumor growth as compared to the other groups. In the other hand, immunotherapy with proteins and lysate of *L. monocytogenes* matured dendritic cells led to a significant increase in activity of cytotoxic T cells.

**Conclusion:** The current study suggests that specific anti-tumor immune response can be induced by DCs matured with proteins of *Listeria monocytogenes* and provide the basis for the use of them in DC-targeted clinical therapies.

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# 224In vitro Analysis the Effects of Heat Shock Proteins, HSP70 and GP96 on Cross-Presentation of Tumor Cell Antigens, and IFN-γ Production by TCD8+ Cells

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**Background & Objective:** Dendritic cells (DCs) based immunotherapy has received increased interest in the treatment of specific malignancies including breast cancer. In this in vitro study, T cell responses which induced by heat treated breast tumor cell lysate (TCL) pulsed monocyte-derived DCs were analyzed in terms of IFN-γ production by $TCD8^+$. **Methods:** Breast cancer cell line (MCF-7) was heated up to 41ºC for 1 hour prior to preparation of tumor cell lysate (five round freeze/thaw) as antigens to load immature dendritic cells. Non adherent PBMCs as source of T cells were then stimulated by antigen-loaded DCs for a week and $TCD4^+$:$TCD8^+$ ratios as well as the intracellular IFN-γ produced by TCD8+ cells were analyzed by Flow cytometry. **Results:** We found that $TCD4^+$:$TCD8^+$ ratio was increased when T cells were stimulated by heat treated antigen pulsed DCs. It is revealed as well, that, production of intracellular IFN-γ by $TCD8^+$ cells were increased non significantly ($P< 0.05$). **Conclusion:** Our findings indicated that induction of HSPs in tumor cells will results in increase of immune response against breast tumor, so, it is recommended that tumor cells are heated up to 41ºC for 1 hour prior to preparation of antigen-pulsed monocyte- derived DCs for cancer immunotherapy.

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# 225Heat Stressed Apoptotic Tumor Cell Loaded Autologous Dendritic Cells Induce More Potent T Cell Mediated Immune Responses against Breast Cancer in vitro

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**Background & Objective:** Dendritic cells (DCs) as potent antigen presenting cells are currently used in various therapeutic settings in particular in cancer research. There are so many factors that enhance the antigen presenting capabilities of DCs, it is recently assumed that heat shock proteins [(HSPs) (e.g. HSP70 and gp96)] have critical role in MHC-I restricted $CD8^+$T responses. In the present study, we analyzed the production of IFN-γ by $CD8^+$T cells in response to heat treated apoptotic tumor antigens. **Methods:** Breast cancer cell line (T47D) was heated up to 41ºC for 1 hour prior to preparation of apoptotic tumor cells as antigens to pulse monocyte-derived dendritic cells. Nonadherent PBMCs as source of T cells were then stimulated by antigen-loaded DCs and $CD4^+$T: $CD8^+$T ratios as well as the production of intracellular IFN-γ by $CD8^+$T cells were analyzed by Flow cytometry. **Results:** Our results showed that $CD4^+$T: $CD8^+$T ratio as well as intracellular production of IFN-γ by $CD8^+$T cells were increased when using heat treated apoptotic tumor cells as source of antigen to pulse DCs non significantly ($P< 0.05$). **Conclusion:** Regarding to our findings and results of others, it is recommended that tumor cells are heated up to 41ºC for 1 hour prior to preparation of antigen-pulsed monocyte- derived DCs for cancer immunotherapy.

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## 226In vitro Analysis of LAK Cell Activity against Breast Cancer Cell Lines

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**Background & Objective:** Lymphokine-activated killer cells (LAK Cells) which can be elicited in the presence of IL-2 from peripheral blood mononuclear cells (PBMCs), contains mainly NK cells. These cells are able to kill numerous tumor cells, but this ability varies from one tumor to other. So in the present study we analyzed LAK cell cytotoxicity against breast cancer cell lines MCF-7 and T47D as well as K562 and LNcap as NK cell target and unrelated cell line respectively. **Methods:** LAK cells are generated in the presence of 1000 U/ml IL-2 for five days in 37ºC and 5% CO2. The Flow cytometry analysis of LAK cell cytotoxicity against target cells were carried out in the 1:5, 1:10 and 1:20 target: effecter ratios using Annexin V/PI fluorescent dyes. **Results:** Our results showed that LAK cells could kill all four cell lines with optimum lysis in 1:10 target to effecter ratio They killed NK cell sensitive K562 cell line more than others significantly ($P < 0.05$). **Conclusion:** In accordance to other studies, our findings implicated that administration of LAK cells could be used for adaptive immunotherapy of breast and prostate cancer.

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# 227Evaluating of TH1 Response Induction of Dendritic Cells Matured with *Listeria Monocytogenes* Components

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**Background & Objective:** Dendritic cells (DCs) are professional antigen presenting cells (APCs), which direct the immune response through antigen presentation in the presence of appropriate co stimulation and cytokine production. In this study, we evaluated effects of different *Listeria monocytogenes* components on TH1 response induction of DCs. **Methods:** Bone marrow cells flushed from mouse tibia and femur. Cells were cultured in RPMI-1640 complete medium in the presence of GM-CSF, IL-4 for 5 days. Tumor antigens and different *listeria monocytogenes* components such as proteins, DNA, lysate and heated antigen added to DCs culture for 36h.T cell proliferation assay cell carried out by co culturing of tumor antigen primed BALB/C mouse spleen cells as responder and various numbers of irradiated DCs as effector cells. After 5 days of co culture, the proliferation of responder cells was estimated by Cell Proliferation ELISA BrdU kit. Cytokine production in BMDC culture and co culture supernatant of DC and splenic cells evaluated by ELISA kit. **Results:** The highest stimulation index was seen in DCs treated with *L.monocytogenes* lysate. In cytokine production by DCs all the mature DCs groups product low amount of IL-10 in compare with immature DCs and protein group have the highest amount in IL-12 and IL-18. Lymphocytes that co-cultured with DCs matured by protein have highest IFN-γ and cells co-cultured with lysate-DCs have lowest IL-10 production. **Conclusion:** These findings indicate that DC maturation with protein components have significant impact on their antigen capacity. In the other hand maturation with protein components trigger type 1 of cytokine that essential for cancer immunotherapy.

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# 228Evaluation of siRNA in Silencing CCND1 Gene in Esophageal Cancer (KYSE – 30 Cell Line)

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**Background & Objective:** RNAi (RNA interference) is a new strategy in the treatment of different disease such as cancer and viral disease. In this process a target mRNA degrades in a sequence specific manner, so target gene silencing occurs. siRNAs, 21-23 nt dsRNAs, are the gene silencing mediators in this process. CCND1 which is a key gene in cell cycle is amplified and over expressed in esophageal cancer. In this study we survey effectiveness of our synthesized siRNA in treatment of cancer on KYSE 30 and HN5 cell lines. **Methods:** Three methods with different level were used for evaluation for our synthesized siRNA. Microscopic observation was done by imaging in 0 and 24 hours after siRNA transfection. In second method, MTT assay was done as triplicate in 96 wells plate after 48 hours by adding 100 micro litter of working solution (with PRMI-1670) to the wells , after designing Cyclin D1 primers for Sybr Green Method, Real – Time PCR was done as duplicate in 24 wells plate. RNA extraction was done by RNeasy Mini Kit. **Results:** In microscopic observation, after 24 hours, significant changes were observed in cell phenotype and division in both applied cell lines (KYSE-30 & HN5). Also result from MTT Assay indicated 61.5% reduction in KYSE-30 cell line and 43.2% in HN5 cell line. InReal – Time PCR, 98% reduction was observed in both cell in HN5 cell line, level of reduction was more significant compared to KYSE-30. **Conclusion:** Our result indicate more reduction in cell activity and division in KYSE-30 cell line that could be result from importance and causality of cyclin D1 in formation and progression of this type of the cancer. Also our result confirmed the probable role of cyclin D1 in ESCC cancers, and its usage as candidate gene in gene therapies.

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## 229Inhibitory Cytokine Profiles in Peripheral Blood of Ovarian Cancer Patients

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**Background & Objective:** Helper type 2 (Th2) cytokine profiles such as o interleukin-4 (IL-4), IL-10 and transforming growth factor-β1 (TGF-β1) prevent anti-tumor response that induced by Th1 lymphocytes. In many solid tumors, expression of these cytokines increased in favor of tumor progression. In this study, we evaluated expression of IL-4, IL-10 and TGF-β1 transcripts in peripheral blood of ovarian cancer patients. **Methods:** To detect mRNA of these genes, peripheral blood sample of 50 confirmed ovarian cancer patients compared with 50 age/sex matched healthy women. Then expression of IL-4, IL-10, TGF-β1 and β-actin as housekeeping gene were determined using Real-Time PCR method. **Results:** IL-4, IL-10 and TGF-b1 mRNA levels were more increased in peripheral blood of patients than healthy women were ($P< 0.05$). Positive correlation between IL-10 and TGF-β1 expression was observed ($P< 0.05$); while there is not a link between IL-4 and others ($P \approx 0.147$ and $P \approx 0.086$). **Conclusion:** Over-expression of these cytokines that was observed in this study confirmed pervious information about them. In ovary cancer, tumor metastasis to pelvic cavity is common predicament and constitutively expression of these cytokine perhaps is major cause of tumor escaping from immune system and metastasis into there.

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# 230Interleukin-12 and Interferon-Gamma Gene Expression in Peripheral Blood of Ovarian Cancer Patients

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**Background & Objective:** Interleukin (IL)-12 and Interferon-gamma (IFN-γ) are two major cytokines that secret through T helper 1 lymphocytes. These cytokine have important antitumor property such as increase tumor immunogenicity, disrupt proliferation of tumor cells and inhibit angiogenesis in tumor site. The antitumor activity of IFN-γ is mediated by IFN regulatory factor-1 (IRF-1) and may be blocked by IRF-2. In this study, we proposed to evaluate expression of these cytokines in peripheral blood cells of ovarian cancer patients. **Methods:** To reach this goal, gene transcript of IFN γ and IL-12 in peripheral blood sample of 50 confirmed ovarian cancer patient compared with 50 age/sex matched healthy women. Expression of IFN-γ and IL-12 p35 transcript was investigated with Real-Time RT-PCR and Sybergreen as reporter dye. **Results:** Our data shows that expression of IFN-γ and IL-12 p35 mRNA was increased 1.6 and 2.9 fold in ovarian cancer patients compared with healthy controls but this is not statistically significant. **Conclusion:** Despite these cytokines slightly increase in peripheral blood, but cannot help immune system to distinguish cancer cells. This data confirmed systemic apply of these cytokines could not efficient treatment, however, locally treatment maybe associate with better result.

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# 231Expression Profile of SDF-1/CXCR4/CXCR7 as Angiogenic Factors in Peripheral Blood Mononuclear Cells of Patients with Bladder Cancer

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**Background & Objective:** Tumor angiogenic ability is one of the most important predictors of cancer progression. SDF-1/CXCR4/CXCR7 axis closely correlates with this process and contributes to the bladder cancer growth, production of proangiogenic factors and metastasis. **Methods:** This study examined the expressions of SDF-1/CXCR4/CXCR7 mRNA levels in peripheral blood mononuclear cells (PBMCs) of 50 bladder cancer patients using real-time quantitative RT-PCR method. Results were compared to 50 healthy control samples which were matched in sex and age with patients. **Results:** As a result, SDF-1 mRNA had lower expression in patients than controls. However there were no significant different in the expression of CXCR4 and CXCR7 mRNAs between PBMCs of patients and normal individuals. **Conclusion:** The analysis of this data showed that there is no correlation between bladder cancer and SDF-1/CXCR4/CXCR7 axis.

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# 232CTLA- 4 and FOXP3 Genes Expression in Peripheral Blood of Bladder Cancer Patients, Using Real-Time PCR

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**Background & Objective:** Urinary bladder cancer is the second most common diagnosed malignancy in men and the tenth most common diagnosed malignancy in women worldwide. Generation of T- Regulatory cells (Tregs) is known to play a major role in progression and modulation of the immune escape mechanisms in cancer. These cells express Forkhead/winged helix transcription factor (FOXP3) also a marker of Treg activation and Cytotoxic T-lymphocyte antigen-4 (CTLA-4), a negative regulatory molecule which, is a potential target for immunotherapy. In this study, we aimed to examine gene expression of FOXP3 and CTLA-4 genes in bladder cancer patients using real-time PCR. **Methods:** Gene expressions of FOXP3 and CTLA-4 were evaluated in peripheral blood sample of 50 patients with bladder cancer and 50 healthy volunteer using quantitative RT-PCR. **Results:** This data has shown patients had significantly higher expression in FOXP3 and CTLA-4 genes in peripheral blood sample than healthy control. **Conclusion:** Consequently, up-regulation of FOXP3 and CTLA-4 displays their essential role in tumor progression and can be used as suitable prognostic biomarkers in bladder cancer and immunotherapy target.

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# 233Quantitative Real-Time RT-PCR Assay, a Suitable Method for Detection of Apoptotic and Anti-Apoptotic Markers in Peripheral Blood of Bladder Cancer Patients

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**Background & Objective:** Transitional cell cancer (TCC), which is the most frequent type of urothelial cancer, is the second most common genitourinary malignancy, accounts for more than 90% of all primary bladder cancers. The prognosis of patients with advanced bladder cancer is still extremely poor in spite of recent therapeutic advances in cancer research and many ongoing works have tried to find a prognostic biomarker in peripheral blood. In this study, the alteration in the mRNA expression of some apoptotic and anti-apoptotic gene in bladder cancer patients were compared with normal group. **Methods:** p53, Fas and Bcl-2 mRNA was assessed in peripheral blood of 50 patients with bladder cancer by real-time quantitative reverse transcription-PCR (QPCR) and compared with 50 sex/age matched healthy controls. **Results:** Data of this study demonstrates that Fas, Bcl-2 and p53 gene transcripts have significantly increased in bladder cancer patients' peripheral blood in comparison with healthy control. **Conclusion:** Data of this study showed that overexpression of p53 and Bcl-2 may be considered as a prognostic biomarker in management of bladder cancer.

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# 234Specific Single Chain Antibodies against Prostate Cancer Cells

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**Background & Objective:** Recently, several members of a vertebrate protein family containing a six transmembrane (6TM) domain involved in apoptosis and cancer (e.g. STEAP, STAMP1, TSAP6) have been identified in Golgi and cytoplasmic membranes. STEAP (six-transmembrane epithelial antigen of the prostate) was the first described member of this family and identified as a prostate-specific cell-surface antigen overexpressed in cancer, located at the cell-cell junction of the secretory epithelium of prostate, and found as well in both colon and bladder cancer cell lines. It has been shown that antibodies against STEAP are able to block cell junctions and lead in apoptosis of the cancer cells. Here we describe the selection of specific human recombinant antibodies against STEAP2 in order to be used for prostate cancer immunotherapy. **Methods:** Antibody engineering technology was applied to lymphocyte mRNA of a non-immune donor and a scFv (single chain antibody) library was constructed and selected against STEAP2 using the panning process. The selected clones reacted with the STEAP2 epitope in ELISA. The chains VH/linker/ VL of the selected scFv were amplified and sequenced using a dye termination method. **Results:** The selected scFvs produced positive ELISA with the corresponding peptide. None of the antibodies reacted with the control peptides. The amino acid alignment of heavy and light chains of the antibodies with VH and VL gene families revealed specific changes in some amino acids which represent the specificity of the antibody molecules. **Conclusion:** The highly specific anti-STEAP2 scFvs show potential for the development of a new reagent against prostate cancer cells. The structural studies that display the composition of complementary-determining regions (CDRs) of specific antibodies would be helpful in the creation of synthetic antibodies for immunodiagnosis or immunotherapy.

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# 235Immunophenotying of Neuron Stem Cells in Tumor Tissue Removed from Manangioma Patients

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**Background & Objective:** Meningiomas originate from cells of arachnoids granulations, they are known as benign tumors, and rarely may transform to malignant features. Meningiomas form one-fifth of all primary brain tumors and account for approximately 15% of all brain tumors with a slow pattern of grow and development. There is an association between clinical presentation of epilepsy and incidence of meningioma. The aim of this study was to investigate the presence of neuron stem cells in meningioma tissues after surgery by Flow cytometry analysis. **Methods:** Surgically removed tumors were received in sterile conditions. Single cell were prepared and cultured in DMEM, 10% FFP and 10%FCS. Tumor Cells were propagated for 3 weeks. Cells from passage 1 and 4 were assessed for expression of CD166, CD105, CD44, CD133, CD14, CD45 and CD34. **Results:** Isolated tumor cells were highly positive for CD166, CD105 CD44 and CD133 and were negative for CD34, CD45 and CD14. **Conclusion:** Results of our investigation clearly indicate that a portion of tumor cells derived from meningioma tissues express most of specific markers of mesenchymal and neuron stem cells. To understand the significant of theses finding, works are required to study the functional aspects of neuron stem cell in the process of tumor genesis of meningioma.

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# 236IGF-1, HGF, VEGF Expression Profiles in Breast Cancer Tissues and Adipose-Derived Stem Cells (ASCs) of Breast Cancer Patients

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**Background & Objective:** Insulin like growth factor (IGF-1), hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) are reported to play crucial roles in cancer development and metastasis. It has been shown that MSCs recruit to the tumor microenvironment and may contribute to the production of proangiogenic factors. Herein, we investigated the expressions of HGF, VEGF and IGF-1 mRNAs in breast cancer tissues and adipose-derived stem cells (ASCs) of twenty one women with breast cancer and fifteen normal women. Data of breast cancer tissues were compared between high stage and low stage patients. **Methods:** mRNAs were extracted and the expressions of described genes were determined by real-time quantitative RT-PCR using specific primers and SYBR Green Master Mix kit in compare to a housekeeping β-actin gene. **Results:** As a result, in breast cancer tissues, IGF-1 mRNA had 28.6 folds more expression in high stage compared to low stage patients. No apparent difference was observed in the expressions of VEGF and HGF mRNA levels between both groups. In ASCs relative quantification (RQ) of VEGF was about 2.4, HGF and IGF-1 were about 2 folds higher in patients than controls. **Conclusion:** Expression of growth factors in ASCs may show the important role of these cells in tumor microenvironment. Thus, these cells might be introduced as potential therapeutic targets for human breast cancer.

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## 237Assessment of Monoclonality in Large B Cell Non-Hodgkin's Lymphoma

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**Background & Objective:** To assess the value of polymerase chain reaction (PCR) technique in determination of the clonality of immunoglobulin heavy chain gene rearrangements for diagnosis of large B cell non-Hodgkin´s lymphoma in paraffin embedded tissue samples. **Methods:** DNA was extracted from paraffin embedded tissue of 44 diffuse large B cell lymphoma (DLBCL) cases and 20 samples of reactive lymphoid tissues of appendix and tonsil as control. Framework 3 to joining region (FR3/JH) of the variable segment of the immunoglobulin heavy chain gene was amplified using degenerate primers. Fifteen µl of PCR-products from each case were analyzed on 8% polyacrylamide gels following AgNo3 staining on the gel. **Results:** Monoclonal rearrangements were identified in 33 of 44 cases (75%) of diffuse large B cell lymphoma (DLBCL). Monoclonal IgH gene rearrangements were not detected in any of the reactive lymphoid hyperplasia samples and all of these cases showed polyclonal pattern. **Conclusion:** PCR analysis, using degenerate primers outlined in this study, monoclonality was demonstrated about 75% in Diffused large B cell lymphoma (DLBCL). This approach can be used as a sensitive, reliable and valuable diagnostic adjunct to conventional morphological and immunocytochemical evaluation of lymphoproliferative disorders particularly in cases with limitation in quantity and type of diagnostic material (needle aspirates and cellular fluids).

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# 238SDF-1 and CXCR-4 Gene Overexpression in a Large Cell Lung Cancer Cell Line (Mehr-80) Increased Bcl-2 Expression

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**Background & Objective:** The chemokine Stromal-Derived Factor-1 (SDF-1/CXCL-12) and its receptor, CXCR4, play a crucial role in tumor growth and metastasis. Our current understanding about the role of SDF-1/CXCR4 axis in the promotion of lung cancer is limited. Mehr-80 is a Large Cell Lung Cancer cell line that previously has been established in Shiraz University of Medical Sciences. In this study the hypothesis that overexpression of SDF-1 or CXCR4 may change the expression of some important genes related to angiogenesis, survival and metastasis of tumor was tested in Mehr-80 cell. **Methods:** For transfection, five different non-viral gene delivery systems including Calcium Phosphate, DEAE-Dextran, Superfect, Lipofectamine 2000 and Electroporation were optimized in Mehr-80 using plasmid encoding GFP. Electroporation was selected for SDF-1 and CXCR4 gene delivery. mRNAs were extracted from Mehr-80 before and after transfection, cDNA was synthesized using cDNA synthesis kit. The expression of desired genes including SDF-1, CXCR4, Bcl-2, IL-8 and VEGF were evaluated using quantitative Real-time PCR (q-PCR) with proper primers. Beta-actin was used as a reference gene. **Results:** As a result, mean transfection efficacies were 8.4 ± 2.69% with Calcium Phosphate, 8.20 ± 1% with DEAE-Dextran, 4.9 ± 2.69% with Superfect, 40.14 ± 2.39% with Lipofectamine 2000 and 34.13 ± 3.7% with Electroporation. Analysis of different genes expression using q-PCR showed that transient overexpression of SDF-1 could increase the CXCR4 mRNA. Also, after SDF-1 and CXCR4 genes transfection, overexpression of Bcl-2 but not IL-8 or VEGF was observed. **Conclusion:** In conclusion, our results provide possible evidence that SDF-1 and CXCR4 axis may promote lung cancer progression through enhanced Bcl-2 expression. Multiple studies have linked Bcl-2, an anti-apoptotic factor, to the aggressiveness of tumors such as lung cancer. For confirmation of our results about the role of SDF-1/CXCR4 axis in the lung cancer promotion and metastasis further investigations are essentially needed.

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# 239IL-8 and MMP2 Expression Profiles in Breast Cancer Tissues and Adipose-Derived Stem Cells (ASCs) of Breast Cancer Patients

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**Background & Objective:** There are a plenty of studies show the important role of CXCL8 (IL-8) and matrix metalloproteinases (MMPs) in cancer development and metastasis. It has been shown that MSCs recruit to the tumor microenvironment and may contribute to the production of proangiogenic factors. Herein, we investigated the expression of IL-8 mRNA in breast cancer tissues and also MMP2 mRNA level in adipose-derived stem cells (ASCs) of twenty one women with breast cancer and fifteen normal women. Data of breast cancer tissues were compared between high stage and low stage patients. **Methods:** mRNAs were extracted using TRizol reagent and the expressions of described genes were determined by real-time quantitative RT-PCR using specific primers and SYBR Green Master Mix kit in compare to a housekeeping β-actin gene. **Results:** In breast cancer tissues, IL-8 mRNA had 56 folds more expression in high stage compared to low stage patients. In ASCs relative quantification (RQ) of IL-8 was about 2 folds higher in patients than controls. No difference was found in the expression of MMP2 between ASCs of patients and controls. However, the expression of this molecule was significantly higher in estrogen receptor and progesterone receptor (ER and PR) positive patients compared to ER and PR negative ones ($P< 0.05$). **Conclusion:** These data suggest that the higher expression of IL-8 by breast cancer tissue and ASCs of breast cancer patients can probably change the prognosis and susceptibility of women to breast cancer. Thus, this molecule might be introduced as potential therapeutic targets for human breast cancer.

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## 240PD1.5 Renders Susceptibility to Head and Neck Squamous Cell Carcinoma

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**Background & Objective:** PD-1, which was originally isolated by the subtractive-hybridization technique, is an immunoglobulin gene super family member that is expressed on activated T, B as well as myeloid cells. PD-1 strongly inhibits both proliferation and cytokine production by CD4 and CD8 T lymphocytes after interaction with its two ligands PDL-1 (B7-H1) and PDL-2 (B7-DC). The negative immune-regulatory effect of co-inhibitors, such as PD-1, has been hypothesized to be a potential cause of immune dysfunction in tumor immunity. The aim of this study was to investigate if a single nucleotide polymorphism at position +872 in PD1 gene (PD1.5) is associated with susceptibility and/or progression of Head and Neck Squamous Cell Carcinoma (HNSCC). **Methods:** One hundred ninety seven pathologically confirmed HNSCC patients as well as 152 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 using salting out method and genotyping was performed using nested polymerase chain reaction following by restriction fragment length polymorphism (Nested PCR-RFLP) reactions. **Results:** No deviation was observed from Hardy Weinberg Equilibration as confirmed by Arlequin 3.1 software package. Statistical analysis revealed a significant increase in the frequency of +872 CC genotypes in PD1 gene in patients than control group ($P< 0.05$). When compared to controls, this genotype was also specifically and significantly higher in HNSCC patients whose larynx was involved by tumor. No association was found between this genotype and clinicopathological parameters in patients. **Conclusion:** Results of this investigation suggest that PD-1 gene polymorphism is probably associated with susceptibility to HNSCC in Iranian population.

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# 241Strong Association of CTLA-4 Variation (CT60A/G) and CTLA-4 Haplotypes with Predisposition of Iranian Population to Head and Neck Cancer

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**Background & Objective:** CTLA-4 gene variations may influence CTLA-4 expression and function and as a result, involve in genetic susceptibility of individuals to cancer and/or cancer progression. For instance CTLA-4 +49 G allele is believed to reduce CTLA-4 expression on the cell membrane (mCTLA4) but a G allele at locus CT60 decrease the soluble CTLA-4 (sCTLA4) isoform up to 50 percent. The aim of the study was to evaluate the distribution of +49 A/G, +1822 C/T and CT60 A/G genetic variations as well as the merged haplotypes in CTLA-4 gene in the patients with head and neck cancer. **Methods:** Eighty five patients with confirmed head and neck cancer (age 55.3 ±14.8 yrs.) and 85 healthy age and sex-matched controls with no personal and familial history of cancer or autoimmune diseases (age 56.3 ± 12.4) were enrolled in this case-control study. Genotypes were investigated by the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assays. Arlequin software package was used to check for Hardy-Weinberg equilibration and generate haplotypes. **Results:** At position CT 60, AA genotype as well as Aallele was significantly decreased in patients than controls (18.8% vs. 38.8% and 46.5% vs. 61.7% respectively, P< 0.007). The genotypes and alleles at other positions were not significantly differ between patients and controls, however ACG, GTA and GCA haplotypes emerged from three loci occurred with significantly more frequencies in patients (P< 0.001). Haplotype ACA and GTG, from the other hand, were more frequent in controls (P< 0.001). Significancies of haplotype resisted the Bonferroni correction. **Conclusion:** Our results suggest a strong genetic association of CTLA-4 genetic variants and emerged haplotypes with susceptibility to head and neck carcinoma. Also it revealed that higher incidence of CT60 A allele may have a protective role against cancer, probably by affecting sCTLA-4/mCTLA-4 isoforms ratio.

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# 242CTLA-4 Over-Expression in Intracellular Compartments as Well as on the Surface of Lymphocytes Derived from Patients with Lung Cancer

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**Background & Objective:** CTLA-4 is a homodimeric glycoprotein which behaves as a negative regulator of T as well as B lymphocytes. Intra-cellular storage of CTLA-4 supports the rapid surface expression following lymphocyte re-activation. Previous investigations indicated that lymphocytes with intracellular expression of CTLA-4 show an impaired response following Ag presentation. Background & Objective: Considering immune inhibitory state in patients with cancer we aimed, in the present study, to investigate, in patients with non small lung cancer (NSCLC), the percentages of $CD4^+$T, $CD8^+$T as well as B lymphocytes expressing CTLA-4 both on the surface and in intracellular compartments. **Methods:** Twenty one new cases with NSCLC who received no prior treatment as well as sixteen sex-age matched healthy donors were recruited. Mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation. Intracellular staining of CTLA-4 and surface staining of CD4 and CD8 as well as CD19 molecules followed by Flow cytometric analysis was used to evaluate the prevalence of the targeted cells. **Results:** In both groups CTLA-4 presence in intracellular compartments of lymphocytes was significantly higher than that expressed on the surface ($P< 0.05$). Compared to the controls the expression of intracellular as well as surface CTLA-4 was independently increased in lymphocytes of the patients: total T cells from the patients had significantly higher CTLA4 expression both in intracellular compartments and on the surface (Intracellular: 19.2 ±17.0 vs. 7.3 ± 12.2, Surface: 0.71 ± 0.56 vs. 0.46 ± 0.19¸ $P< 0.05$). Nearly the same results were observed for $CD4^+$, $CD8^+$ T cell subsets as well as B lymphocytes. **Conclusion:** Our data suggest that parts of lymphocyte response problems in patients with cancer may come from high storage as well as high surface expression of CTLA-4 molecule, negative regulator which suggest to be, and has recently been, targeted for immunotherapy of the cancers including NSCLC.

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# 243Cytotoxic Effect and Inducing of Apoptosis by *Ornithogalum Caspidatum* Herb Medicine extract on WEHI-164 (Fibrosarcoma Model) in Comparison with Taxol

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**Background & Objectives:** Fibrosarcoma is one of the soft tissue sarcomas types. They include 5% of neoplasias in adult patients and 10 % of children tumors. Using herbal medicines that has apoptotic induction impact is one of ways in cancer treatments. In this study, for the first time we examine cytotoxic effect and inducing of apoptosis *Ornithogalum Caspidatum* extract ( an Iranian plant) have been assayed on cancer cell line WEHI-164 , model of fibrosarcoma and comparing with Taxol. **Methods:** This is a basic study that the cell line WEHI-164 have been under gone different concentrations of *Ornithogalum Caspidatum* extract and Taxol ,in various times of 6 , 12 and 24 hours treatment and after that we examined cell viability and cytotoxic effects by MTT Assay. For studying apoptosis, we chose 12 hours and the concentrations before and after of IC50 extract, using ELISA (Cell Death Detection kit) and Flow cytometry (Annexin V) to assay intra and extracellular changes in WEHI-164, that happen during apoptosis. **Results:** *Ornithogalum Caspidatum* extract have cytotoxic effects in three mention times. Cytotoxic effects of extract increase with time and concentration, when cytotoxicity increasing the cell viability decreasing. In ELISA, the results showed that the extract causing apoptosis. In Flow cytometry, we found that there are four group of cell including: viable cells, necrotic cells, apoptotic cells and apo-necrosis cells. When the concentration increased the group of apo-necrosis cells and necrotic cells increasing and in low concentration of extract the viable cells and apoptotic cells increasing. Taxol has cytotoxic effect and inducing programmed cell death in WEHI-164 in lower concentration than *Ornithogalum Caspidatum* extract ($P< 0.001$). **Conclusion:** In general, the cytotoxic effect and induction of apoptosis by *Ornithogalum Caspidatum* extract depend on time and concentration. Toxol causing apoptosis and cytoxicity on WEHI-164 in low concentration because, Taxol is more pure than this herb extract. These data are first report on potential anticancer activity of *Ornithogalum Caspidatum* extract on fibrosarcoma.

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# 244Evidences of Treg Cells Increase before Metastasis in Patients with Non-Small Cell Lung Cancer

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**Background & Objective:** Recent studies have shown that the function of Regulatory T (Treg) cells plays a pivotal role in the suppression of effective immune responses against cancer. Background & Objective: The purpose of the study was to evaluate the prevalence of FoxP3 positive $CD4^{+}CD25^{+}$ Treg cells in both metastatic and non-metastatic stages of non small cell lung cancer (NSCLC) patients and healthy controls. **Methods:** Twenty one new cases with NSCLC who received no prior treatment as well as sixteen sex-age matched healthy donors were recruited. Mononuclear cells were isolated and intracellular staining of FoxP3 and surface staining of CD4 and CD25 molecules followed by Flow cytometric analysis was used to evaluate the prevalence of targeted cells. **Results:** Compared with healthy donors¸ NSCLC patients had an increased percentage of Regulatory T cells (7.91 ± 4.13 vs. 3.85 ± 1.76¸ P< 0.05). The proportion of Treg cells in the patients was directly proportional to stage increase (stage II= 5.16 ± 2.4¸ stage III= 7.94 ± 4.3¸ stage IV=11.96 ± 2.2¸ P< 0.05) and was also significantly higher in metastatic than non metastatic stages (11.96 ± 2.22 vs. 6.83 ± 3.8¸ P< 0.05). Additionally and interestingly, looking at the sub-stages indicated that the prevalence of Treg cells is the same among pre-metastatic (IIIB) and metastatic (IV) sub-stages, but at the same time, significantly different from non metastatic sub-stages (10.29 ± 3.and 11.96 ± 2.22 vs. 6.30 ± 3.76¸ P< 0.05). **Conclusion:** Our data not only verify the previous data of Treg cell increase in cancer but also provide new insights in the relation of metastasis and Treg cell increase. The results indicated that a significant increase in Regulatory T cells precede, and accordingly may accelerate, metastasis; a finding which suggest the use of Treg targeted immunotherapy even for patients with late stages before metastasis.

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# 245Selection And Assessment of Human Recombinant Antibodies against HER2/neu Tumor Antigen

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**Background & Objective:** Advances in recombinant DNA technology facilitated the production of smaller recombinant antibody fragments such as scFv.s (single-chain fragment variable). The rapid blood clearance and good tumor penetration of scFvs offer potential advantages over longer antibody molecules for cancer therapy. Moreover, these characteristics of scFv are applicable to cancer imaging (tumor detecting). In this study we selected specific scFvs against HER2/neu, a tumor antigen which its overexpression is associated with markedly aggressive forms of cancer, and the effects of the antibodies on the breast cancer cells were assessed in vitro. **Methods:** Four rounds of panning were performed to select specific and high affinity scFv antibodies against three immune-dominant epitopes of HER2/neu from a phage-display library of scFv. PCR and Fingerprinting were done on the clones after the panning process. The proliferation assay was performed using MTT assay on HER2/neu positive cell line, the SKBR3, and on HELA cells as the negative HER2 expressing cell line. **Results:** PCR products of 20 selected clones against each epitope showed the presence of scFv genes in all of them. One specific clone was isolated against each epitope using the fingerprinting patterns. The clones inhibited the proliferation of the HER2/neu positive cell line in the MTT proliferation assay significantly while they didn't show any inhibitory effect on the HeLa cell line. **Conclusion:** Herceptin (Trastuzumab) is an antibody against HER2/neu which is approved by the FDA for treatment of metastatic breast cancer. Researches have shown multiple life-threatening side effects of Herceptin on the patients. Moreover the HAMA (Human Antimouse Antibody) reaction has limited the uses of this antibody. Therefore, there is a need to develop a new generation of therapeutic anti-HER2/neu reagents. Our results suggest that the selected scFvs which are human antibodies and showed significant inhibitory effects on cancer cells can be used as a new alternative for inhibiting the proliferation of cancer cells which are associated with HER2/neu overexpression.

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# 246Evaluation of the Binding Specificity of Single Chain Antibodies against HER2/neu

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**Background & Objective:** Antibodies displayed on phages are powerful tools for the identification of ligand specificity to targets of interest and also are high affinity binders in order to be used for cancer immunotherapy. Binding ligands from a library are selected by panning rounds usually performed on pure antigens on a solid phase. The specificity of the selected antibodies has been evaluated by different methods. Flowcytomety has shown the most accurate and precise results. **Methods:** In this study eight single chain antibodies were selected against three linear sequence of HER2/neu antigen using panning process. The ability of the selected antibodies to bind the positive and negative HER2/neu expressing cell lines, SKBR3 and HELA cells, was tested by Flow cytometry. **Results:** The results derived from FACS analysis showed intensity range 73%-85% when the binding of single chain antibodies were tested against SKBR3 cells. The binding of the antibodies to HELA cells showed intensity range 10%-19%. **Conclusion:** All the selected clones against HER2/neu epitopes bound specifically to HER2/neu expressing cell line. These data demonstrated that the specific single chain antibodies have the potential to be used as useful tools for immunodiagnosis of cancers overexpressing HER2/neu. Further researches are needed to show the therapeutic effects of the specific antibodies.

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# 247Comparative Proteomic Analysis of SKBr3 and MCF7 Breast Cancer Cell Line

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**Background & Objective:** Overexpression of HER2, a poor prognostic marker in breast cancer, is correlated with down-regulation of estrogen receptor (ER), a good prognostic marker in breast cancer. Both HER2 and ER have been implicated in the regulation of distinct genes with important roles in tumor genesis. Breast cancer cell lines, SKBr3 and MCF7, are known to highly express HER2 and ER cancer biomarkers, respectively. **Methods:** In this study their global protein profiles were compared using two-dimensional electrophoresis. Fourteen out of 32 differentially expressed proteins ($\geq$ 2 fol and P value $<$ 0.05) were identified by mass spectrometry. **Results:** The most significant upregulated proteins in SKBr3 cells were Rho GDP dissociation inhibitor-α (RhoGDI-α), voltage-dependent anion channel 2, lactate dehydrogenase A, capping protein (actin filament) muscle z-line alpha 2 and apo-lipoprotein binding-protein, and in MCF7 cells were cellular retinoic acid binding-protein 2 (CRABP2), Hsp27 and profilin 2. **Conclusion:** The data of this study show that proteins involved in HER2 signaling and glycolysis are upregulated in SKBr3 cells, while proteins involved in ER signaling are upregulated in MCF7 cells. Most of the identified proteins have been a candidate marker for cancer aggressiveness or drug resistance, but their differential expressions between SKBr3 and MCF7 cells were not known. Apo-lipoprotein binding-protein has not been described in cancer so far. Further studies are required to clarify the importance of differential expressions of these proteins particularly RhoGDI-α in SKBr3 and MCF7 breast cancer cell line models.

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# 248Gene Expression Profile of IL-17, TGF-β and IL-6 in Peripheral Blood Samples of Bladder Cancer Patients

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**Background & Objective:** Despite the important role of TH-17 cells in the pathogenesis of many autoimmune diseases, their prevalence and the mechanisms by which they are generated and regulated in cancer remain unclear. They are the third subset of $CD4^+$ T-helper cells recently been described that express IL-17. IL-17 is also expressed by $CD8^+$ T cells, NK-T cells, γδ T cells and neutrophils. Recent studies show that IL-6 and TGF-β may increase IL-17 under certain conditions in cancers. We therefore examined IL-17 TGF-β and IL-6 gene expression in blood samples of bladder cancer patients. **Methods:** Peripheral blood was collected from 50 men with bladder cancer and 50 healthy men from Shiraz University of Medical Sciences hospitals. Then PBMCs were isolated and assessed for IL-6, TGF-β and IL-17 gene transcript using quantitative Real-time PCR. **Results:** Analysis of cytokine production profiles revealed that patients with bladder cancer showed a significant increase in TGF-β but no change in IL-6 and IL-17 in comparison with normal controls. **Conclusion:** Despite of increase of IL-17 and IL-6 in other cancers, these data did not show any change in their expression level. However, the expression level of TGF-β increased which suggests there is a role for TGF-β in bladder cancer development and metastasis. In addition IL-6 and TGF-β may not be involved in the IL-17 development in bladder cancer. Future work on these cytokine will further benefit our understanding of the immune responses and may help to develop new treatments of immune diseases and cancers.

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# 249Gene Expression Profiles as Prognostic Markers in Women with Ovarian Cancer

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**Background & Objective:** This study aims to identify reliable prognosis markers of blood sample of ovarian cancer patients by a real-time quantitative PCR. Most studies trying to identify molecular targets were single-marker studies. However, expression of a prognostic tumor profile may be of interest. New sensitive, specific, accurate, and reliable technologies should be applied that focused on readily available patient resources, such as blood. In this study we determined the gene expression profile of blood cells sample of patients with ovarian cancer. **Methods:** We performed Real time-PCR analysis for peripheral blood samples of 50 patients with ovarian cancer and compared to a control group of 30 healthy women. CTLA-4, Foxp3, IL-4, IL-10, IL-12, IFN-γ, IL-23, IL-27, IL-6, IL-17 and TGF-β were analyzed. **Results:** IL-12, IFN-γ, IL-23, IL-27, IL-6 overexpression was found in 5o% of ovary cancer cases, CTLA-4, Foxp3, IL-17 in more than 70% and IL-4, IL-10 in 40%. In this study, TGF-β did not show any significant correlation to ovarian cancer. Besides single gene analyses, gene profiles were additionally evaluated. Highly significant correlations to ovarian cancer were found in single gene analyses of CTLA-4, Foxp3 and IL-17 and in gene profile analyses of (CTLA-4, Foxp3) and (IL-17, IL-6). **Conclusion:** We show a blood-based assay using 11 analytes that may distinguish women with ovarian cancer from healthy women. We demonstrate that (CTLA-4, Foxp3) and (IL-17, IL-6) may serve as potential biomarkers and molecular targets for ovarian cancer and a variety of other solid tumors.

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# 250Gene Expression and Novel Bood Biomarkers for the Early Detection of Breast Cancer

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**Background & Objective:** Early detection of breast tumor is of key importance for patient survival. The diagnostic tools to predict the prognosis in patients suffering from breast cancer need further improvements. New technological achievements like the gene profiling could help identify new prognostic markers in the clinical setting. Novel diagnostic blood biomarkers either generated by the tumor and released into the blood, or generated by non-tumor cells as a response to the tumor presence, can now potentially help improve the accuracy of early-stage breast cancer detection. **Methods:** We performed Real-time PCR analyses for peripheral blood samples of 55 patients with early stage breast cancer and compared to 30 healthy women. p53, bcl-2, Fas, CTLA-4, Foxp3, IP-10, CXCR-3, CXCR-7, CXCR-4, SDF-1, IL-12, IL-2, IFN-γ, IL-23, IL-27, IL-6, IL-17 and TGF-β were analyzed applying Real-time-PCR. **Results:** P53, Bcl-2 , Fas, IL-12, IL-2, IFN-γ, IL-23, IL-27, IL-6 overexpression was found in about 6o% of breast cancer cases, CTLA-4 , Foxp3, IL-17 in 80%, IP-10, CXCR-3, CXCR-7, SDF-1 in only 10% and CXCR-4 in 40%. In this study, TGF-β did not show any significant correlation to breast cancer. Highly significant correlations to breast cancer were found in single gene analyses of CTLA-4, Foxp3 and IL-17 and in gene profile analyses of (P53, Bcl-2 and Fas), (CTLA-4, Foxp3) and (IL-6, IL-17). **Conclusion:** The multigene analyses found highly positive levels in breast cancer patients. Our study shows that may not single gene analyses but subtle patterns of multiple genes lead to rising accuracy and low loss of specificity in detection of breast cancer cases.

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# 251Monitoring of Minimal Residual Disease in Patients with Acute Lymphoblastic Leukemia Using Wilms' Tumor Gene 1 (WT1) Expression

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**Background & Objectives:** Screening of residual neoplastic cells (minimal residual disease-MRD) during treatment of solid and hematopoietic malignancies has remarkably reduced disease recurrence and relapse. In patients with acute lymphoblastic leukemia (ALL) different methodologies including analysis of Ig-TCR gene rearrangement and expression pattern of leukemia specific fusion genes or tumor-associated antigens (TAA) are routinely used for MRD monitoring. In this study, the expression of WT1, a tumor suppressor factor involved in the development of Wilms' tumor, was longitudinally determined by RT-PCR as a marker for prediction of clinical relapse in Iranian ALL patients. **Methods:** In this study 25 Iranian ALL patients positive for WT1 were followed for 9 months after chemotherapy. Sampling was performed at days 0, 14 and 28 bone marrow and peripheral blood (PB) and days 56, 90, 180 and 270 [PB only]. A semi-quantitative RT-PCR method was used for determination of WT1 mRNA levels in different samples. To minimize the experimental variations of pre-PCR steps, the housekeeping gene β-actin was also amplified in all samples and the results were presented as the ratio of the density of WT1 to β-actin PCR products. Immunophenotyping of leukemic cells was also performed by Flow cytometry. **Results:** Of the 25 ALL WT1+ patients, 4 patients eventually relapsed as evidenced by WT1 expression in their PB as a marker of molecular relapse at 1.5-5 months before clinical relapse. **Conclusion:** Our data suggests WT1 as a suitable and reliable marker for monitoring of MRD in ALL patients. Detection of WT1 expression during therapy could be a useful molecular tool for prediction of clinical relapse in this malignancy.

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# 252Immunomodulatory and Cytotoxic Effect of *Alhagi Pseudalhagi* on Different Tumor Cell Lines

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**Background & Objective:** Medicinal plants have been used as a remedy for various diseases in folk medicine. Objective: In the present study, the methanolic extract of *Alhagi pseudalhagi* medicinal plant was investigated for its possible immunomodulatory and cytotoxic effects. **Methods:** Peripheral blood lymphocytes separated from healthy individuals were stimulated with phytohemagglutinin (PHA) and cultured with different concentrations of the extract. *Alhagi pseudalhagi* was also tested for its possible cytotoxic effect on human tumor cell lines. This effect was assessed by MTT colorimetric assay. **Results:** The extract caused an increase in lymphocyte proliferation examined by BrdU incorporation assay at concentrations 10-50 μg/ml (maximum stimulation index, 1.45). *Alhagi pseudalhagi* showed no significant inhibitory effects on the proliferation of Hela, Fen and Raji cell lines. However, two leukemic cell lines including Jurkat and K562 were sensitive to the various concentrations of the extract. In the culture of Jurkat cells treated with 200 μg/ml of the extract, 62% decrease in cell proliferation was observed. Corresponding value for K562 cells was 41%. **Conclusion**: *Alhagi pseudalhagi* showed mild lymphocyte stimulatory effects and cytotoxic activity on some tumor cell lines particularly Jurkat leukemia cells.

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# 253Impacts of Anti-EGFR Monoclonal Antibody in Prostate Cancer PC3 Cell Line: Downstream Signaling Pathways

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**Background & Objective:** Prostate cancer (PCa) is the most commonly diagnosed form of malignancy and the second leading cause of cancer related deaths in men. Among many biomarkers, the epidermal growth factor receptor (EGFR) appears to be expressed in many cancers, at which it is considered as a promising novel molecular target for anticancer therapy. It has been well documented that the expression of epidermal growth factor receptor (EGFR) increases in prostate cancer. In this investigation, we studied the effects of anti-EGFR monoclonal antibody (mAb) on the expression of EGFR and some important apoptosis signaling molecules in androgen-independent human prostate carcinoma (PC3) cell line. **Methods:** Flow cytometry and fluorescence microscopy analysis were used for survey specificity of mAb binding to PC-3, A431 (positive EGFR control) and CHO cells (negative EGFR control). MTT assay was performed to determine cell proliferation. Semi-quantitative RT-PCR was used to look at the expressions of EGFR, MAPK-1, AKT-1 and STAT-3. **Results:** The Flow cytometry and fluorescence microscopy analysis showed that mAb can bind to the surface of PC3 and A431 cells (but not to CHO cells) with high specificity. MTT assay showed that 50 and 100 µg /ml of mAb for 48, 72 and 96 hrs can inhibit cell growth in PC3 cells. RT-PCR analysis revealed that the expression of some important apoptosis signaling molecules can be decreased (such as AKT-1) or without any change in other signaling molecules (such as MAPK-1). **Conclusion:** This study proved that anti-EGFR monoclonal antibody has anti-proliferative effect on PC3 cells. As well as with decreasing of some signaling molecules, mAb can inhibit cell growth, thus our results highlight impacts of such modality in inhibition of prostate cancer.

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# 254Construction of T Lymphocyte Chimeric Receptor Consisting OX40 Gene Targeting MUC1 Antigen

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**Background & Objective:** The specific activation of the immune system to control cancer growth has been a long-lasting goal in cancer immunotherapy. Employment of T cells for tumor therapy is an attractive approach. In order to redirect and enhance the ability of the patient's own immune cells to fight cancer, the chimeric receptor (CR) approach that endows lymphocytes with antibody specificity was developed. Nanobodies are the smallest fragments of antibodies that have great homology to human VH and low immunogenic potential. VH is variable heavy chain of camel antibody that is named VHH. We use MUC1 VHH for targeting in chimeric receptor. T cells require both primary and costimulatory signals for optimal activation. Tumor cells rarely provide costimulatory signals and hence CAR receptors that transmit just a CD3ζ signal can only initiate target cell killing and Interferon-gamma release and fail to induce full activation. Although incorporation of a CD28 component results in IL-2 (Interleukin 2) release and limited proliferation, T cell activation remains incomplete. To optimize CAR signaling, tripartite endodomains were constructed. OX40 transmits a potent and prolonged T cell activation signal and is crucial for maintaining an immunological response. **Methods:** We design to overlapping primer that contains 60% of OX40 sequence then we synthesized CD28-Ox40-CD3ζ segment by SOE PCR and replaced this segment with CD28-CD3ζ in VHH-Hing-CD28-CD3ζ construct. Then transfected this construct in Jurkat T cells by lipofectamin and detect CAR receptor function and CAR expression with IL-2 detection kit and RT PCR and Real Time PCR. **Results:** It was showed that VHH-Hing-CD28-OX40-CD3ζ construct have more function and more expression. **Conclusion:** We showed that the CD28-OX40-CD3ζ tripartite cytoplasmic domain provided a full complement of activation, proliferation and survival signals for enhanced anti-tumor activity.

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# 255Analysis of IL-4 and IL-10 Serum Levels in Patients with Head and Neck Squamous Cell Carcinoma (HNSCC)

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**Background & Objective:** Immunosuppression is commonly associated with head and neck squamous cell carcinoma (HNSCC). Interleukin (IL)-4 and IL-10 are considered as anti-inflammatory, immunosuppressive cytokines whose serum concentrations are elevated in some types of cancer. So the aim of this study was to investigate serum concentrations of these cytokines in Iranian HNSCC patients, and their correlations with clinicopathological findings at diagnosis. **Methods:** The cytokines were quantified in serum by ELISA commercial kits. Study groups consisted of 92 untreated patients and 51 healthy donors. **Results:** Mean serum level of IL-4 in the patients group was significantly higher than in the controls group ($P< 0.05$). Serum level of IL-10 was not statistically differed from controls. There was no significant association between serum levels of IL-4 and IL-10 and stage, grade, and age at diagnosis. **Conclusion:** An increase in IL-4 level in the patients group may represents a shift from Th1 to Th2 in HNSCC patients, although no significant increase in IL-10 was detected.

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# 256Gene Expression Profile of IL-17, TGF-β and IL-6 in Peripheral Blood Samples of Breast Cancer Patients

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**Background & Objective:** Despite the important role of TH-17 cells in the pathogenesis of many autoimmune diseases, their prevalence and the mechanisms by which they are generated and regulated in cancer remain unclear. They are the third subset of $CD4^+$ T-helper cells recently been described that express IL-17. IL-17 is also expressed by $CD8^+$ T cells, NK-T cells, γδ T cells and neutrophils. Recent studies show that IL-6 and TGF-β may increase IL-17 under certain conditions in cancers. We therefore examined IL-17 TGF-β and IL-6 gene expression in blood samples of breast cancer patients. **Methods:** Peripheral blood sample was collected from 50 women with breast cancer and 50 healthy women from Shiraz University of Medical Sciences hospitals. Then PBMCs were isolated and evaluated for IL-6, TGF-β and IL-17 gene transcript using quantitative Real-time PCR. **Results:** Analysis of cytokine production profiles revealed that patients with breast cancer showed a significant increase in IL-17 and IL-6 but no change in TGF-β in comparison with normal controls. **Conclusion:** Taken together, these data suggest that IL-6 may be involved in the IL-17 development in breast cancer. So future work on these cytokine will further benefit our understanding of the immune responses and may help to develop new treatments of immune diseases and cancers.

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# 257High Concentration of Soluble Vascular Endothelial Cadherin in Sera of Patients with Prostate Cancer

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**Background & Objectives:** For many years, prostate-specific antigen (PSA) was used to screen prostate cancer (PC) patients. However, recent controversial findings have cast doubt on the accuracy of this biomarker for diagnostic and prognostic purposes, and have stimulated the search for new candidates. This study was conducted to determine the capability of a soluble adhesion molecule known as soluble vascular endothelial Catherin (sVEcadherin) or CD144 to distinguish prostate cancer or benign prostate hyperplasia (BPH) patients from healthy individuals. **Methods:** Patients recently diagnosed as having PC (n=35) or BPH (n=35) and age-matched controls (n=30) were enrolled. The concentration of sVE-cadherin and PSA was measured by ELISA. Gleason score in patients with PC was determined by pathological examination of tumor biopsies. **Results:** The concentration of sVE-cadherin in the serum of patients with PC and BPH was significantly higher than that in the healthy men. No association was found between the concentration of this soluble adhesion molecule and PSA values. Moreover, concentrations of sVE-cadherin did not correlate with Gleason scores in patients with PC. **Conclusion:** The high concentration of sVE-cadherin in our patients suggests that this bio-marker is a potentially useful tool to identify high-risk patients. However, further research in patients with PC and other pathological conditions is needed to support the efficacy of this molecule in PC screening.

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# 258High Concentration of Serum Soluble FAS in Patients with Head and Neck Carcinoma: A Comparative Study before and after Surgical Removal of Tumor

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**Background & Objectives:** Alternative splicing of Fas transcript can produce a natural secreted isoform of this molecule. Some cancer cells also can make soluble Fas (sFas) which may have suppressive effects on anti-tumor response of the immune system. Elevated concentration of sFas has been detected in serum of patients with different malignancies. To correlate the sFas concentration in sera of head and neck cancer patients with disease indices. **Methods:** Concentration of sFas in sera of patients with head and neck carcinoma (n=98) and healthy individuals (n=30) were measured by Sandwich ELISA and compared to values obtained 6 months after surgical removal of tumor (n=48). Data were correlated with different clinical findings of the patients. **Results:** Serum sFas in sera of HNC patients were found to be significantly higher in patients with different tumor stages. Concentration of sFas was not correlated with age or tumor invasiveness however more sFas was found in sera of patients with higher grades of tumor. Surgical removal of tumor in patients resulted in substantial decrease in sFas concentration. **Conclusion:** Initial raise in sFas concentration in sera of HNC patients and its consequent decrease could be regarded as a sign of suppressive mechanisms by tumors. Additional studies are needed to fully elucidate this mechanism however these finding may show the prospective use of such biomarkers of the immune system for prognosis of the disease and even immunotherapeutic applications.

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# 259TGF-β1-509 Codon Polymorphism in Southern Iranian Patients with Malignant Bone Neoplasms

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**Background & Objective:** Primary bone tumors are a heterogenous group of malignant neoplasms presenting predominantly in children and adolescents. They include Ewing sarcoma, Chondrosarcoma and osteosarcoma. Transforming Growth Factor-Beta1 (TGF-β1) gene, located on chromosome 19 (19q13), has been involved in various malignancies. The aim of this study was to investigate the association of polymorphism of Transforming Growth Factor-β1 gene at position-509 among a group of southern Iranian patients with malignant bone tumors. **Methods:** Fifty seven patients with malignant bone tumors and 139 healthy individuals as control group were recruited. DNA specimen was extracted from peripheral blood mononuclear cells in controls and from tissue specimens respectively among patients. Genotyping was performed by an allele-specific polymerase chain reaction.

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# 260Investigation of CTLA-4 gene Polymorphisms in Patient with Ovarian Cancer

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**Background & Objective:** Ovarian cancer is a relatively common cancer among post menopausal women which is usually discovered in late stages because it shows very few and vague clinical signs. Nowadays there is a lot of Conclusion about immunotherapy of ovarian cancer and interleukins such as interferon are used, producing good results in prognosis of patients under chemotherapy. CTLA-4 is a gene which has an important role in homeostasis and regulation of immune response. CTLA-4 inhibitory role has been proved to be of significance in autoimmune diseases as well as in cancer and their progress. In this study we intended to find out the relationship between polymorphisms in CTLA-4 gene at positions +49 A/G as well as -318 C/T and ovarian cancer. **Methods:** A PCR-RFLP method and an AS-PCR procedure was used to investigate polymorphisms at positions +49 A/G in exon 1 and -318 C/T in promoter region, respectively. Deviation from Hardy Weinberg Equilibration was investigated by Arlequin software package and statistical analysis was performed by SPSS 11.5. **Results:** No statically significant differences were seen in genotypes and alleles prevalence at locus +49 A/G in exon-1 and position -317 C/T in promoter region of CTLA-4 gene between two investigated groups. **Conclusion:** Current study revealed no association between CTLA-4 +49 and -318 with susceptibility to ovarian cancer.

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# 261The Effect of STAT3 Knockdown on EGFR, SMAD7, SMAD2 & KI67

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**Background & Objective:** Transforming Growth Factor-Beta (TGF-β) and Epidermal Growth Factor (EGF) signaling pathways are both independently implicated as key regulators in tumor formation and progression. Stat3 as a signaling molecule activated specifically and persistently by overexpressed EGFR, but not by normal levels. Importantly, Stat3 is responsible for the reduced TGF- β sensitivity, since its knockdown by siRNA restored TGF- β signaling sensitivity. **Methods:** Tumors (Knockdowns) were lysed in a lysis buffer and clarified by centrifugation. Proteins were then separated by SDS-PAGE (Invitrogen), blotted onto nitrocellulose and probed with the primary antibodies (Anti EGFR, Stat3, Erk1, pErk1, Smad2, pSmad2, Actin). The signal was visualized using the ECL chemoluminescence detection kit (Amersham Biosciences) following incubation with appropriate secondary antibodies. Also, paraffin-embedded tumor sections were stained indirectly by primary antibodies (Smad7, Ki67, pSmad2) and Alexa Fluor 488-conjugated, Alexa Fluor 546-conjugated secondary antibodies. Then, they visualized with using fluorescence microscopy. **Results:** The knockdown of Stat3 caused deletion of Stat3 and reduction of Ki67, Smad7 in tumors. Also it made to increase of pSmad2 and no effect in EGFR amount in tumors. **Conclusion:** This study shows that Stat3 makes to increase of Ki67 and Smad7 and growth of tumors. Therefore, the knockdown of Stat3 can help to reduce of tumor growth.

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# 262Production of Immunotoxin against EGFR and Evaluation of Its Effect in Apoptosis Induction on Tumoral Cell Lines

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**Background & Objective:** Tumor growth and progression depends largely on the activity of cell membrane receptors like epidermal growth factor receptor (EGFR). Epidermal growth factor receptor (EGFR), play an important role in the growth and survival of many solid tumors and has been proven that it is an encouraging target for cancer treatment. Immunotherapy is the best strategy in cancer treatment and immunotoxins have predominant position herein. **Methods:** For production of monoclonal antibody against human EGFR, first BALB/c mice were immunized against A431 tumor cells. Then the most immune mouse was selected for fusion. For production of recombinant toxin PE38, toxin gene and pET-22b vector were digested by NotI and NdeI enzymes and then the gene was cloned in the vector by Ligase enzyme. The recombinant vector was transformed to *E.coli* bacterium by electrical shock. The production of toxin was induced by IPTG and then, produced toxin was purified by Nickel Column. The toxin was conjugated with antibody by chemical method. The effects of immunotoxin in induction of apoptosis on tumor cells were assayed by ELISA method. **Results:** In this study, 118 clones were obtained. MTT assay showed that produced monoclonal antibodies against EGFR prevented 35% of A431 cell growth in culture in comparison with control group. The immunotoxin induced of 62% apoptosis in tumor cells too. **Conclusion:** These results indicate that the produced Immunotoxin and monoclonal antibodies against EGFR can be used in treatment of tumors with membranous EGFR if produce in recombinant forms.

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# 263Identification of Autoantigens in Breast Cancer by Two Dimensional Immunoblot

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**Background & Objective:** Endeavors to identify breast cancer-associated antigens by high throughput techniques are being increased. These antigens may be useful in cancer diagnosis, prognosis, and immunotherapy. The aim of our study was to identify antigens eliciting a humoral immune response in breast cancer by a two-dimensional polyacrylamide gel electrophoresis, Western blotting, and mass spectrometry. **Methods:** Sera from breast cancer patients and healthy volunteers were individually investigated for antibodies against MCF7 lysate. Reactive protein spots in immunoblots were matched to Coomassie Brillant Blue-stained gels. Matched spots were excised from the gel and subjected to MALDI-TOF/TOF MS analysis. **Results:** Some of the identified antigenic proteins were aldehyde dehydrogenase, adenosine kinase, heat shock protein beta-1, kinesin family member 11, chromosome 1 open reading frame 38, isoform CRA-a, heat shock 90kDa protein 1, glucose-6-phosphate dehydrogenase. **Conclusion:** This study further strengths the usefulness of an immunoproteome analysis in the successful identification of immunoreactive proteins.

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# 264Toll-Like Receptor 4 Gene Polymorphism in Breast Cancer Patients

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**Background & Objective:** Recent studies suggested that toll-like receptors (TLRs) signaling is involved in the induction of anti-cancer immunity. TLR4 gene is located at chromosome 9q32-33; it has three exons spanning about 10kb. It seems that TLR4 expresses on dendritic cells (DC) are require for the cross presentation of tumor derived antigens and the promotion of tumor specific cytotoxic T-cell responses. Breast cancer patients harboring the loss-of-function Asp299Gly polymorphism of TLR4 relapsed earlier after receiving anthracycline based chemotherapy. This investigation is aiming to genotyping tlr4 Asp299Gly single nucleotide polymorphism (SNP) in breast cancer patients compared to healthy matched control subjects. **Methods:** 107 breast cancer patients and 179 controls were genotyped for the Asp299Gly using RFLP-PCR method. The mean age at the time of sampling was 48.9 ± 11.5 years for cases and 46.3 ± 12.6 years for the control group. **Results:** Statistical analysis revealed no significant differences in the frequencies of genotype and alleles ($P \approx 0.42$) between breast cancer patients and controls. No homozygous Asp299Gly mutation was detected in our studied populations. **Conclusion:** We could not find any significant association for tlr4 Asp299Gly in breast cancer patients compared to control subjects.

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## 265Investigation of PD-1.5 Genetic Marker in Colorectal Cancer in Iranians

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**Background & Objective:** A programmed death-1 (PD-1) molecule has been found to be a critical component in immune regulation. Genetic association of PD-1 has been observed in certain autoimmune diseases and cancers. Herein, we examined the polymorphism of C+872T (PD1.5) in colorectal cancer patients to investigate the role of this genetic marker in colorectal cancer. **Methods:** Two hundred patients with confirmed colorectal cancer and 200 healthy Iranian controls from the same geographic area were age and sex matched and recruited. The polymorphisms of PD-1 at position C+872T (PD1.5) were genotyped by using nested PCR- Restriction Fragment Length Polymorphism (RFLP) test. Differences between patients and controls were analyzed by using Fisher Exact Probability and Pearson Chi-Square tests. **Results:** The frequencies of CC, CT and TT genotypes for the patients and controls were respectively 29.5% vs. 37.5%, 54.4% vs. 44.5% and 16% vs. 18%. The frequency of C and T alleles for the patients and controls were respectively 56.7% vs. 59.7% and 43.2% vs. 40.3%. No deviation was observed from Hardy Weinberg Equilibration as confirmed by Arlequin 3.1 software package. Statistical analysis revealed no significant differences in the frequencies of genotype and alleles between patients with colorectal cancer patients, although a trend toward higher occurrence of CT genotype was observed in patients ($P \approx 0.057$). **Conclusion:** As the first study to investigate PD1.5 genetic marker in colorectal cancer, current study revealed no association between this genetic marker with susceptibility to colorectal cancer in Iranian population.

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## 266CTLA-4 Gene Variants in Cervical Cancer

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**Background & Objective:** CTLA-4 is a costimulatory molecule expressed on activated T lymphocytes and constantly expressed on the surface of regulatory T cells (CD $4^+$ $CD25^+$) and plays a central role in immune tolerance. CTLA-4 genotypes have been associated with autoimmune diseases and cancers. In this study, we evaluated the role of four single nucleotide polymorphisms (SNPs) of CTLA-4 gene in cervical cancer. **Methods:** 55 patients with cervical cancer and 110 age/sex matched control were genotyped in four polymorphic sites, three in promoter region -1722(T/C), -1661(A/G),-318(C/T) and one in exon 1, +49(A/G). The allele and genotype frequency were determined using a PCR –ARMS and RFLP methods. **Results:** The genotypes distributions and allele frequencies at positions -1661 and -318 were significantly different among cervical cancer patients and healthy controls. At position -1661 the frequency of A/A homozygote and A allele were higher in control than in patients. ($P< 0.01$, $P< 0.05$; respectively), while at position -318 the frequency of C/C homozygote and C allele were increased in patients. ($P< 0.05$, $P< 0.05$; respectively). The haplotype analysis demonstrated 9 haplotypes, of which 5 were common between two groups. The TGTA haplotype frequency was only observed in cervical cancer patients (9.54%, $P< 0.005$) and the TGCG haplotype was only occurred in control group (6.48%, $P< 0.0005$). **Conclusion:** The allelic variation at -1661 position may imply a protective role whereas the variations at -318 might render susceptibility to cervical cancer. Investigation of CTLA-4 gene variants in other ethnic population will consolidate the finding of this study.

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# 267KIR and HLA Combinations in Patients with Lung Cancer Disease

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**Background & Objective:** Natural killer (NK) cells are a subset of lymphocytes that can play a crucial role in the early immune response to infection and tumors by lysis of infected/transformed cells. A family of 14 killer cell immunoglobulin-like receptors (KIR) regulates human NK cell response. The goal of the present research is to investigate how the host genes that encode variable KIR receptors and HLA ligands influence the susceptibility to lung cancer. **Methods:** 85 subjects with lung cancers disease and 278 controls were compared in KIR gene frequency and HLA class I ligands using two recently developed methods, duplex-PCR and direct DNA sequence-based typing of KIR-binding HLA-I ligands typing system. **Results:** A trend toward more activating KIR genes was observed in patients comparison to controls, although they weren't statistically different. In addition, more than 80% of patients carry Bx genotypes that encode 2-6 activating KIR receptors. The 3DL1+Bw4 combination was less frequent in patients than controls (P< 0.02, CI=0.027-0.92, OR=0.5). KIR-HLA pairs implicated for weak inhibition with more activating KIR genes may be contribute to pathogenesis of lung cancer disease. **Conclusion:** The data of this study conclude no significant association between KIR/HLA pairs in patients with lung cancer.

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# 268KIR3DS1-2DS1-2DS5 Occurred Most Frequently in Patients with Advanced Stage of Breast Cancer

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**Background & Objective:** The polymorphic KIRs are the key receptors of NK cells, a subset of lymphocytes that trigger early immune response against infection and tumors. Here we investigated if certain KIR genes and HLA-I ligands are associated with the development of breast cancer. **Methods:** DNA from 167 women and 278 healthy controls from the same geographical area were characterized for KIR gene polymorphism and HLA-I ligands using two novel duplex-PCR and direct DNA sequence-based typing methods. **Results:** The frequency of Bx genotypes that possess 2-6 activating KIR genes were predominant in the patients compared to the controls (84.4% vs. 72.6%, P< 0.01; 95% confidence interval (CI), 1.24-3.34, Odds ratio (OR)=2.04). Particularly group-B haplotype-associated activating KIR genes, 3DS1 (46.7% vs. 34.1%, P< 0.01; 95% CI, 1.14-2.5, OR=1.68), 2DS1 (55.6% vs. 36.3%, P< 0.0001; 95% CI, 1.48-3.25, OR=2.2) and 2DS5 (42.5% vs. 25.5%, P< 0.0005; 95% CI, 1.43-3.24, OR=2.15) were significantly increased in breast cancer compared to the controls. Moreover 36.4% of the patients carried all these three KIR genes (we previously named them as T4 cluster group) compared to only 21.4% controls (P< 0.001; 95% CI, 1.37-3.2, OR=2.09). Furthermore, overrepresentation of Bx genotypes and T4 cluster group was more pronounced in patients with advanced stage. **Conclusion:** The data of this study suggests that KIR genotypes might be used as predictor of susceptibility to breast cancer. Moreover, these data suggest that NK cells expressing activating KIR receptors 3DS1, 2DS1 and 2DS5 may trigger an inappropriate localize hyperresponsiveness exacerbating the risk of caner growth.

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# 269A Study of T Helper 1 & 2 Associated Chemokine Receptors on Peripheral Blood Lymphocytes of Gastric Cancer Patients in Pre- and Post- Operation

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**Background & Objective:** Based on the differential expression of chemokine receptors on immune cells, we hypothesized that there would be an alteration in the relative expression of these receptors by Th1 and Th2 cells during a malignancy progression. **Methods:** The PBMC isolate from twenty-seven patients with gastric cancer (19 male and 8 female) and twenty-seven controls were studied. The percentage of $CD4^+$ T cells expressing chemokine receptors (before and after gastrectomy) were determined by three-color Flow cytometry using anti-CD45/CD14, anti-CD4 and one of the following chemokine receptor antibodies: anti-CCR5, anti-CXCR3, anti-CCR3 and anti-CCR4. **Results:** The mean expression of $CD4^+$ $CCR5^+$ was 0.83 ± 0.34 in pre-gastrectomy group, and 1.34 ± 0.74 in post-operation patients with a $P< 0.005$ when those were compared. The mean of $CD4^+$ $CXCR3^+$ expression was 16.95 ± 5.71 in pre- and 25.08 ± 9.31 in post-gastrectomic patients with a $P< 0.001$. Moreover, the mean of $CD4^+$, $CXCR3^+CCR5^+$ expression was 0.86 ± 0.49 in pre-operation and 1.57 ± 0.67 in post-operation with a $P< 0.001$. The mean expression of $CD4^+CCR3^+CCR4^+$ was 1.57 ± 0.83 in pre-gastrectomy and 1.27 ± 0.66 in post- gastrectomy group with a $P< 0.001$, once those were compared. Pearson correlation analysis shows that there is a correlation between CCR3 and CCR5 expression with $r=0.321$, $P< 0.05$, and between CCR4 and CCR5 on CD4 T cell in post-treatment status with $r=-0.401$, $P< 0.05$. However it was not reach to significant level for the other chemokine receptor expression. **Conclusion:** Our data shows that CXCR3 and CCR4, but not CCR5 or CCR3 expression serve as the useful markers for Th1 and Th2 effector cells in peripheral blood. In addition, the data indicate a reduction in cell immunity against the tumor, but if this reduction would restore, the immune cells may cope with the tumor cells more efficiently.

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# 270Comparison of Low-Dose Cyclophosphamide Treatment with Artemisinin Treatment in Reducing the Number of Regulatory T Cells in Murine Breast Cancer Model

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**Background & Objective**: Artemisinin (ART) is a sesquiterpene lactone. Posesing an endoperoxide bridge is unique among antimalarial drugs, and now much attention is focused on the anticancer properties of ART. In this study we aimed at immunomodulatory effects of artemisinin in treatment of breast cancer in comparison to the conventional anti cancer drug, cyclophasphamide (CTX). **Methods:** IL-4 and IFN-γ production, tumor volume, tumor infiltrated regulatory T cells (Treg) and spleen lymphocyte proliferation assay was examined. Briefly three groups of five 4-6 weeks old female BALB/c tumor bearing mice (mouse mammary tumor) were treated with 2.8mg/kg ART and 20mg/kg CTX intraperitonealy for six consecutive days. Tumor volume was measured using a digital vernier calliper (with accuracy of 0.01). Mice were sacrificed and percentage of tumor infiltrating Tregs was obtained using Flow cytometry (BD, USA). Proliferation of splenocytes was obtained using BrdU proliferation assay (Roche). **Results:** Our results show that ART can reduce the number of Tregs in tumor stroma ($P< 0.05$) as compared to CTX ($P> 0.05$) and control. Furthermore ART increases IFN-γ/IL-4 ratio produced in splenocyte culture ($P< 0.001$). Proliferation assay did not show any significant difference. **Conclusion:** Early accumulation of Treg cells in the tumor tissue correlates with tumor progression and is an indication of bad prognosis. In this regard an efficient drug that reduces the number of infiltrating Tregs would be of choice. According to the obtained results, ART can reduce the number of Tregs. Cancer is a multi-factorial disease which needs a multi approach treatment. We suggest using artemisinin; with its dual action mechanism, it can effectively kill cancer cells along with reducing the suppressive environment within the tumor.

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# 271Malignant Lymphomas: HIV/EBV Association

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**Background & Objective:** HIV infection is reported to be associated with some malignant lymphomas (ML) so called AIDS-related lymphomas (ARL), with an aggressive behavior and poor prognosis. Furthermore, ML biological characterization as well as EBV association is sketchy, thus restraining comparison, prognostication and application of established therapeutic protocols. **Methods:** Archival, diagnostic ML biopsies (n=336) collected at Muhimbili National Hospital (MNH) in Tanzania between 1996 and 2006, a fraction (n=150) were analyzed by histopathology and immunohistochemistry (IHC). Selected biopsies were characterized by *in-situ* hybridization (ISH) for EBV-encoded RNA (EBER, n=37). Available sera (n=35) were screened by ELISA for HIV antibodies. **Results:** Most (83.6%, 112/134) non-Hodgkin lymphoma (NHL) were B-cell lymphomas [(BCL) ($CD20^+$)]. Of these, 50.9%, (57/112) were diffuse large B-cell (DLBCL). Most (74.1%) DLBCL showed completely diffuse histology and 25.9% had follicular remnants. Furthermore, out of the 60 FC analyzed ML cases, 27 (M:F ration 2:1) were DLBCL, the majority (55.6%, n=15/27) with activated B-cell like (ABC) and 45% (12/27) with germinal center B-cell like (GCB) immunophenotype. EBV infection seemed more (41/%, 5/12) frequent in GCB cases than in ABC (33.3%, 5/15). Of the serologically tested MLs, 40.0% (14/35) were HIV seropositive, mostly with high (≥40.0%) Ki-67 reactivity. **Conclusion:** Extranodal presentation was common among MNH lymphoma patients who also showed tumor proliferation (KI-67) and EBV infection. DLBCL was frequent and phenotype heterogeneity appeared similar to observations in Western countries suggesting applicability of established intervention approaches. Malignant lymphomas apparently, were predominantly among the young, HIV infected and AIDS patients. The frequent aggressive clinical and histological presentation as well as the dominant B-immunophenotype and the HIV serology indicate a pathogenic association with AIDS.

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# 272Oral Kaposi's Sarcoma (OKS) in Endemic Areas: Presentation and Human Herpes Virus-8 (HHV-8)/HIV Association

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**Background & Objective:** Seventy eight (78) patients biopsied for oral Kaposi's sarcoma [OKS] at Muhimbili University College of Health Sciences (MUCHS) Tanzania corresponding to about 10% of registered KS biopsies between 1990-2005, were diagnosed by serology (ELISA) for HIV infection (OAKS) [74/78] and as endemic (OEKS) cases (4/78) and evaluated clinically, immunologically (Flow cytometry or FACS) and histologically. **Methods:** Immunohistology was performed on OAKS (29) and comparable cutaneous AKS (CAKS) [21] for Human herpesvirus-8 (HHV-8) latency-associated antigen [LANA], KS spindle-cell (SC) [CD34] and proliferation [Ki-67] markers. Fifty four (54/78) of these patients were females (69.2%) with a median age of 31 and 24/78 were males (38.8%) median age 38. Males had a greater proportion (50%) of systemically disseminated KS than females (37%) and were 4-times more likely to have multicentric OKS. **Results:** All immunofluorescence assay (IFA)-tested OKS (34) sera were HHV-8 positive. Most OKS (61.5%) were nodular histological stage. By immunohistology adult, male nodular OAKS had significantly higher frequency $LANA^+$ SC and significantly more Ki-$67^+$ [median=24.1%] cells compared to females (17.2%). Furthermore, juvenile nodular OAKS had also more LANA+ and Ki-$67^+$ cells than corresponding adult cases. Significantly more $LANA^+$ and Ki-$67^+$ expression was also found in nodular OAKS compared to nodular CAKS. A positive correlation (60%) was found between proliferation index (Ki-$67^+$ cell frequency) and $LANA^+$/$CD34^+$ SC. **Conclusion:** Oral Kaposi's sarcoma in Tanzania is since 1990, usually associated with HIV infection and advanced (nodular) histological stage. Males appear to have more tumor burden while females seem increasingly affected by oral AKS. More HHV-$8^+$ tumor cells are found in nodular male compared to female oral AKS, in juvenile compared to adult oral AKS and in oral compared to cutaneous AKS. Furthermore, higher lesional HHV-8 content appeared correlated to increased tumoral proliferation index (PI) in all examined KS biopsies.

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# 273KIR2DS3 is Associated with Protection against Acute Myeloid Leukemia

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**Background & Objective:** Interaction between killer cell immunoglobulin-like receptors (KIR) and human leukocyte antigen (HLA) class I molecules is important for regulation of natural killer (NK) cell function. The aim of this study was to investigate the impact of compound KIR-HLA genotype on susceptibility to acute leukemia. **Methods:** Cohorts of Iranian patients with acute myeloid leukemia (AML; n=40) and acute lymphoid leukemia (ALL; n=38) were genotyped for seventeen KIR genes and their three major HLA class I ligand groups (C1, C2, Bw4) by a combined polymerase chain reaction–sequence-specific primers (PCR-SSP) assay. The results were compared with those of 200 healthy control individuals. **Results:** We found a significant decreased frequency of KIR2DS3 in AML patients compared to control group (12.5% vs. 38%, odds ratio=0.23, P< 0.005). Also, the KIR3DS1 was less common in AML group than controls (27.5% vs. 44.5%, P< 0.05, not significant after correction). Other analyses including KIR genotypes, distribution and balance of inhibitory and activating KIR+HLA combinations, and co-inheritance of activating KIR genes with inhibitory KIR+HLA pairs were not significantly different between leukemia patients and control group. However, in AML patients a trend toward less activating and more inhibitory KIR-HLA state was observed. Interestingly, this situation was not found in ALL patients and inhibition enhancement through increase of HLA ligands and inhibitory combinations was the main feature in this group. **Conclusion:** Our findings may suggest a mechanism for escape of leukemic cells from NK cell immunity.

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# 274Effect of TLR3 Agonists on the Functionality and Metastatic Properties of Breast Cancer Cell Model

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**Background & Objective:** TLRs are expressed on a wide variety of tumors suggesting that TLRs may play important roles in tumor biology. Several studies shown that TLR3 agonists can directly affected human cancer cells but the role of poly A:U in human breast cell line is unclear. The aim of this study was investigated of anti-cancer effect of TLR3 agonist in human breast cell line. **Methods:** We assessed potential effects of poly A:U on human breast cell line (MDA-MB-231) on a dose-response and time-course basis. Human breast cell line MDA-MB-231 was treated with poly A:U and lipopolysaccharide (LPS) at 10, 50, 100 μg/ ml and 5, 10 μg/ ml concentrations, respectively. Following treatments, dose- response and time-course cytotoxicity using a colorimetric method, Metalloproteinase-2 (MMP-2) activity (using gelatin zymography), apoptosis (using Annexin V Flow cytometry method) assays and expression of TLR3 and MMP-2 genes (using PCR method) were performed. **Results:** Cytotoxicity and Flow cytometry analysis of poly A:U showed that poly A:U does not have any cytotoxic and apoptotic effect in different concentration ($P > 0.05$). MMP-2 activity analysis showed significant decrease in higher concentration (50 and 100 μg/ ml) between treated and untreated cells. Moreover, poly A:U treated cells demonstrated a decrease expression of MMP-2 gene in higher concentration. **Conclusion:** Collectively, our data indicated that human breast cancer cell line (MDA-MB-231) is highly responsive to poly A:U effect in vitro and inhibit metastatic aspect of this cell line. The direct poly A:U and TLR-3 interactions in MDA-MB-231 cells could provide new approaches in malignant tumor therapeutic strategy.

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# 275Establishment of Murine Model of Human Breast Cancer Expressing Human Her2/neu

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**Background & Objective:** Most studies in tumor immunology depend upon the appropriate animal models. Although some murine models of human breast cancer have been reported, they mostly need such immune compromised mouse strains as nude mice which are expensive and require specific housing conditions. Indeed, successful targeted immunotherapy requires cells expressing a well-established tumor marker. 4T1 mammary carcinoma is a transplantable tumor cell line derived from BALB/c mice, which is highly tumorigenic and invasive and induce tumor with the very similar kinetics of human breast cancer in terms of tumor growth and metastasis. But the lack of expression of a known tumor marker in this cell line restricts its application in tumor immunological studies. Here we introduce a new cell line derived from 4T1 which is genetically modified to stably express highly immunogenic tumor antigen Her2/neu (Her2) expressed in most human breast cancers. **Methods:** 4T1 cell line was stably transfected with pCMV6-Neo plasmid vector containing full-length of human Her2 gene. Expression of this tumor marker was evaluated by RT-PCR, Flow cytometery and immunocytochemical methods. In order to assess their capacity to induce tumor, transfected cells were then subcutaneously injected into BALB/c mice and the tumorigenic propensity was investigated. **Results:** Transfected 4T1 cell line expressed Her2 both at gene and protein level. These cells expressed well Her2 over 50 rounds of cell passage. Interestingly, transfected cells retained their tumorigenic capacity in transplantation experiments. **Conclusion:** Here we introduce a new cell line which provides an invaluable tool for in vivo studies of human breast cancer. This new cell line will greatly facilitate the study of immunological aspects of human breast cancer and preclinical assessment of cancer drugs and other immunotherapeutics particularly those targeting Her/neu tumor-associated antigen.

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# 276Potential Application of Quantum Dot Nanoparticles for Early Detection of Ovarian Cancer

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**Background & Objective:** Ovarian cancer is the most common cause of mortality among gynecological cancers which in most cases is not diagnosed until later stages. As a matter of fact, detection of ovarian cancer in later stages dramatically reduces the chances of survival and early detection is a prerequisite for successful treatment. Quantum dots (QDs) are fluorescent nanoparticles which offer several advantages over conventional organic dyes including higher quantum yield and thereby higher sensitivity. We report here potential application of QDs for early detection of ovarian cancer. **Methods:** For immunocytochemical applications, anti-CA 125 monoclonal antibodies (mAbs) were produced and characterized. FITC (Fluorescein Isothiocyanate) and QD 525 were conjugated with Streptavidin and used as fluorescent probes for detection of CA 125 expression on human ovarian carcinoma cell line, OVCAR-3, using labeled-Streptavidin biotin (LSAB) immunofluorescence technique. Optical properties of these conjugates including fluorescent intensity, staining index and photostability were then quantified by “image J” image processing program and compared. **Results:** Our results showed that QD 525 are significantly brighter ($P< 0.0001$) and have a very high photostability as compared to FITC. Indeed, staining index of QD 525 was at least 3 times higher than that of FITC. **Conclusion:** We demonstrated that QDs have great potential in the imaging and labeling of CA 125 in immunofluorescent applications as a new type of fluorescent probes that exhibit superior characteristics. Based on their higher quantum yield, excellent photostability and very high staining index reflected by well discrimination between signal and background, these types of fluorescent probes would be a suitable candidate for early detection of ovarian cancer.

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# 277The Role of Platelet in Cancer Growth and Metastasis

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**Background & Objective:** Potential biological significance of tumor-cell-platelet aggregates was first recognized in the 1960s. Further studies revealed that most tumor cells can activate platelets via direct contact through surface molecules on platelets (e.g. GPIIb/IIIa), tumor cells (e.g. α3 integrins), and production of soluble factors (e.g. thrombin and ADP). **Methods:** A combination of these agents forms platelet-tumor-cell aggregates. Regarding to metastasis, platelets on the surface of tumor cells create a barrier that protects them from attacking by immune cells and facilitates their adherence to endothelial cells which is result of retarding movement of tumor cells in the blood by platelets and releasing contractive mediators of endothelial cells. On the other hand, platelets produce substances such as lysophosphatidic acid (LPA) and platelet derived- endothelial cell growth factor (PD-ECGF) that promote tumor growth by inducing angiogenesis and inhibiting apoptosis. In spite of the apparent promotion of tumor growth and metastasis, some of recent in vitro observations indicate that platelets may deliver certain inhibitory signals to tumor cells at the same time. **Results:** These signals induce apoptosis or block transition of G0/G1 phase into S phase and decrease DNA synthesis activity. **Conclusion:** In addition to platelet beneficial effects on tumor growth and metastasis as reported earlier, they might also exhibit certain non-beneficial effects on tumor growth that be considered for further therapeutic and etiologic studies of cancer.

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# 278Role of Cancer Microenvironment in Tumor Growth and Metastasis: Focus on Colorectal Cancer

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**Background & Objective:** Colorectal cancer (CRC) is the third most current form of cancer in the many countries of the world and similar to other cancers, two major factors involved in tumor advent and development are intrinsic genetic alterations, and effects of stromal and immune cells forming microenvironment in relation to cancer cells. **Methods:** One of the most important stromal cells surrounding tumor mass are cancer associated fibroblasts (CAFs) producing cytokines such as transforming growth factor (TGF-β) inducing cancer cells to neoplasm. The function of immune cells which exist around tumor cells undulates between immune surveillance, a protection of the host against tumor extension, and immunoediting, a process in favor of the growth of tumor cells with reduced immunogenicity. **Results:** The presence of $CD45RO^+$ T cells in CRC mass is a good factor for protection against cancer relapse. In contrast, tumor associated macrophages (TAMs), chiefly promote tumor incidence and development by secreting cytokines and growth factors such as TGF-β and vascular endothelial growth factor (VEGF). On the other hand, a morphogenetic process nominated epithelial-to-mesenchymal transition (EMT) occurs in cancer such as CRC. This phenomenon accompanies with loss of E-cadherin-mediated cell-cell adhesion and Wnt-catenin signaling pathway activation which induce cell proliferation and less adhesive traits in tumor cells. **Conclusion:** In summaries, we don't neglect to examine individually each microenvironmental agents engaged in CRC progress because of their two-edged sworn function requiring to pay attention in etiology and immunotherapy of CRC.

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# 279Potential of Nucleostemin Gene Silencing for Cancer Stem Cells Therapy

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**Background & Objective:** Recent studies provided evidence of the existence of a subpopulation of cells within a variety of tumor types with a tumourigenic potential that is lacking in the rest of the cells within these tumors, such cells called cancer stem cells (CSCs). CSCs have stem-like properties, capability of self-renewal and multi-potency of differentiation. These cells are responsible for cancer recurrence and resistance to current anticancer therapies. There is mounting evidence that such cells exist in almost all tumor types. In the other hand, it has been shown that the nucleolar protein Nucleostemin (NS) is preferentially and exclusively expressed in the stem cells, some types of cancer cells and other stem cell-enriched populations, but not in differentiated adult tissues and cells. NS is likely to take part in controlling the proliferation and differentiation switch in stem cells and progenitor cells. Its deregulation in cancer also contributes to the elevated proliferation and undifferentiation of cancer cells. **Results:** We speculate that only small number of cancer cells, cancer stem cells, in at least some tumors having stem-cell like properties can express NS since it has been shown that tumors are heterogeneous populations. **Conclusion:** Gene silencing of NS in tumor-derived cancer stem cells may be a new approach for fighting against cancers. Such approach may changes CSCs fate or trait and perhaps their sensitivity to current anticancer therapies and/or even eradicates CSCs, the origin of cancer resistance and relapse, and hence CSCs therapy.

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# 280Tumor-Derived Cancer Stem Cells Therapy: Niche Modulation versus Manipulation of Cancer Stem Cells Themselves

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**Background & Objective:** A great deal of studies have been shown that a growing tumor is a heterogeneous mix of mostly differentiated cancer cells and cancer stem cells (CSCs), a rare population that have stem-like properties and having capability of self-renewal and multi-potency of differentiation . By contrast to differentiated cancer cells, CSCs exhibit more resistance to conventional therapy. To date, current concepts for treatment and/or cure of tumor-cancer stem cells are based on differentiation therapy and/or eradication of neoplastic stem cells as the root of cancers. It seems that the role of CSCs niche in tumor-derived cancer stem cells therapy is underestimated since all of the supposed therapies are only based CSCs themselves. **Methods:** Modulation of CSCs niche is as pivotal as or more pivotal than manipulation of CSCs themselves since current therapies are emphasizing on eradication or differentiation of CSCs, while recurrence of cancers is inevitable because although they may manipulate CSCs but the cancer niche is in the original state (cancerous state), in turn, normal tissue-resident stem cells or progenitors will be exposed to already existing transforming cues and maybe leading to new CSCs formation and finally cancer relapse. **Results:** It seems that new therapies should be directed against both CSCs niche and neoplastic SCs or their niche at least. The concept of the role of CSCs niche and its modulation appear to be a pivotal approach for treatment and/or cure of cancers. **Conclusion:** An understanding of the CSCs niche in addition to the CSCs themselves lead to a better understanding of the therapeutic approaches for tumor-derived cancer stem cells therapy.

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# 281Efficacy of Hepatitis B Vaccine in Paramedical Students of Bojnoord University

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**Background & Objective:** Hepatitis B virus (HBV) is a common viral pathogen that causes a substantial health burden worldwide. HBV infection is hyperendemic in the South-east Asia and Middle East. Vaccination against hepatitis B is the most effective measure to control and prevent hepatitis. Vaccination of adolescents especially who their job is related to medical profession is necessary. So we investigated the efficacy of recombinant HBV vaccine in medical students in Iran. **Methods:** In 2009, 83 paramedical students that had been vaccinated against HBV infection enrolled in this study. According to the Iranian guidelines, vaccination schedule consist of three doses of recombinant hepatitis B vaccine (heberbiovac-HB) given at 0, 1 and 6 months. A blood sample was taken at least one month and a maximum of two years after the third dose of vaccine. Blood samples were sent to North Khorasan Blood Transfusion Organization to assay for HBsAg and anti-HBs antibody by ELISA kit. A questionnaire was used to record sociodemographic information. **Results:** Based on our results mean age of students was 20 ± 2.6 and the number of men and women was 7(8.4%) and 76 (91.6%), respectively. All students were negative for HBsAg. The mean serum level of anti-HBsAg antibody was 573 IU/ml. Among total students, 96.5% showed anti-HBs responses at level considered protective (≥10 mIU/ml). At least 19% of total cases had anti-HBsAg concentrations less than100 mIU/ml. Also10% had anti-HBsAg concentrations more than 1000 mIU/ml. **Conclusion:** We concluded that the majority of students have protective level of anti-HBsAg antibody.

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# 282Murine Antibody Response to Intranasally Administered Chitosan Microparticles Loaded with Catalytic Domain of Pseudomonas Exotoxin A (PEIII)

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**Background & Objective:** *Pseudomonas aeruginos*a is one of the most important opportunistic bacterial pathogens in humans and animals. This organism is ubiquitous and has high intrinsic resistance to antibiotics due to the low permeability of the outer membrane and the presence of numerous multiple drug efflux pumps. Therefore, vaccine development against this bacterium is required. One of the best target for this purpose is exotosin A. Exotoxin A include 3 domain (catalytic, translocation and bindind domain). This study was designed to compare the immunogenicity between encapsulated catalytic, or third domain of Pseudomonas exotoxin A (PEIII) ( PEIII-chitosan) and uncapsulated chitosan. **Methods:** Recombinant PEIII was purified and encapsulated in biodegradable chitosan microsphere. PEIII-chitosan and PEIII were administered intranasally (IN) to BALB/c response in serum and mucosal secretion following each of the three inoculations. **Results:** Mice vaccine with two or three doses of PEIII –chitosan demonstrated a significantly greater rise in serum anti-PEIII IgG and mucosal IgA titer values 7-fold and 4.4-fold greater , respectively, than three dose of PEIII ($P< 0.02$). **Conclusion:** Intranasally administered PEIII to mice is safe and highly immunogenic either alone or when encapsulated in microspheres. Chitosan microsphere encapsulation of PEIII significantly augments the antibody response to that antigen when administered to a mucosal surface.

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# 283Cloning, Expression and Purification of Enterotoxigenic *Escherichia Coli* Colonization Factor B as a Component of Vaccine Candidate

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**Background & Objective:** Among the bacterial causes, Enterotoxigenic *Escherichia coli* is the most important etiologic agent of childhood diarrhea and represents a major public health problem in developing countries. Although ETEC possesses numerous antigens, the relatively conserved colonization factor (CF) antigens and the heat labile enterotoxin (LT) have been associated with protection and most vaccine candidates have exploited these antigens. A safe and effective vaccine against ETEC is a feasible goal as supported by the acquisition of protective immunity. Bacterium adhesion is mediated by colonization factor antigens, with Colonization Factor Antigen I (CFA/I) a commonly isolated virulence factor. ETEC fimbriae (CFA/I), consist of the stalk forming major subunit cfaB and a tip localized minor adhesive subunit cfaE, both of which are necessary for fimbrial assembly. Cfa/B subunit is a glycoesphangolipid binding protein and therefore it seems that this subunit has a critical role in attachment of bacterium to epithelial cells of small intestinal and supposed that this protein is an appropriate candidate for vaccine development. In this work, we attempted to produce recombinant colonization factor B in *Escherichia coli* with the goal of studying immunogenicity of this protein as a component of candidate vaccine. **Methods:** A synthetic sequence encoding the CFAB gene was designed using *E.coli* codon bias. The optimized gene was cloned into appropriate expression vector (PET28a).The protein was overexpressed in E. coli BL21 (DE3) cells under the control of the T7 promoter. Expression condition was 1mM IPTG, 3 hours, and 37◦ C. The identity and the antigenicity of CFAB were confirmed on western blots using anti-His and anti-cfaB antibodies. Immobilized Ni-ion affinity chromatography was used to purify the expressed protein. **Results:** Correspondent 20kDa band in SDS PAGE and Immunoblot Techniques was confirmed the cfaB expression. Purification result implicated that a yield of 1mg of CFAB per liter of induced *E. coli* culture. **Conclusion:** Codon optimization, Gene cloning and expression in heterologous hosts is one useful approach for obtaining large quantities of individual proteins for such studies.

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# 284Fusion of the *Leishmania Major* Amastin Antigen to HSV-1 VP22 and EGFP Enhances DNA Vaccine Potency in a BALB/c Mice Model

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**Background & Objectives:** Leishmaniasis is one of the most important diseases in tropical and subtropical regions of the world. Yet, there is no available vaccine against this disease. Amastins are members of a large surface protein gene family in Leishmania. Our aim was to employ an intercellular spreading strategy using herpes simplex virus type 1 (HSV-1) VP22 protein for enhancing DNA vaccine potency of *Leishmania major* amastin antigen in BALB/c mice model. **Methods:** C-terminal truncated VP22 (UL49) gene of HSV-1 was amplified from purified genomic DNA of HSV-1. Then, the open reading frame of the *L. major* amastin gene (LmjF08.0810, 600 bps) was amplified from genomic DNA of *L. major* Friedlin strain. In addition, the fusion construct of VP22-amastin was obtained. After sequence confirmation, all genes cloned in pEGFP-N1 and the expression was confirmed using COS-7 transfection by fluorescent microscopy. The recombinant VP22 were expressed and purified using NI-NTA affinity chromatography performed by FPLC. Two groups were immunized with pEGFP-amastin (group 1) and pEGFP-VP22-amastin (group 2) plasmids twice at a three-week interval. In addition, three control groups were also included. All mice were challenged with 3.3 □ $10^6$-stationary-phase parasite 3-weeks after last immunization. Footpad swelling was measured weekly and parasite burden in spleen was determined at 12-weeks post-challenge. The humoral and cellular immune responses were evaluated before and after challenge. **Results:** Our study indicated that DNA immunization with VP22-amastin-EGFP induced higher response in terms of IFN-gamma production and protection against infectious challenge in mice. The level of parasite load in this group was also significantly lower than the other groups. In addition, DNA immunization with VP22-amastin-EGFP can slightly delay swelling of cutaneous lesions almost two weeks as compared to other groups. **Conclusion:** These results suggest that the development of DNA vaccines encoding VP22 fused to a target Leishmania antigen would be a promising strategy to improve immunogenicity and DNA vaccine potency.

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# 285Formulation of Selected Leishmania DNA Vaccine Candidate in Solid Lipid Nanoparticles: Characterization and in vitro Evaluations

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**Background & Objective:** Leishmaniasis is a major health problem in many tropical and sub-tropical countries and safe easily-available vaccine development is highly demanded against this disease. Amongst antigens potentially capable of inducing protective immunity, Cathepsin L-like cysteine proteinase type I (CPB) has been used in a heterologous prime-boost vaccination regime in BALB/c mice and dogs exhibiting both type 1 and 2 immune responses. Highly immunogenic C-terminal extension (CTE) of this protein directed the immune response toward Th2. Therefore, there was a need for further investigating and examining the protective potential of the mentioned vaccine candidate without CTE fragment (cpb-CTE). Here we aimed to construct recombinant pEGFP, pcDNA and PET vectors carrying cpb-CTE gene. Cationic solid lipid nanoparticles (cSLNs) were used for this gene formulation and in vitro delivery. **Methods:** cSLNs were formulated of cetyl palmitate, cholesterol, DOTAP and Tween 80 via melt emulsification followed by high shear homogenization. Different formulations were prepared by anchoring pDNAs on the surface of cSLNs via charge interaction. The formulations were characterized according to their size, zeta potential, pDNA integrity and stability against DNase I challenge. Lipoplexes' cytotoxicity was investigated on COS-7 cells by MTT test. In vitro transfection efficiency was qualified by fluorescent microscopy and quantified using Flow cytometry technique. **Results:** cSLN-pEGFP-cpb-CTE complex was formulated with suitable size and zeta potential. Efficiency/cytotoxicity ratio of this formulation was comparable to linear PEI-25KD-pEGFP-cpb-CTE polyplexes while exhibiting significantly lower cytotoxicity. **Conclusion:** Tested formulation was able to deliver cpb-CTE gene efficiently. Further in vivo studies is underway to discover stimulation of the relevant immune responses induction necessary for anti-leishmanial protection using this novel recombinant pDNA. This data also proves the ability of cSLN as a promising carrier for the leishmaniasis to cover the main drawback of naked pDNA delivery that is rapid elimination from the circulation.

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# 286Antibody Response to a Booster Dose of Two Cellular DTP Vaccines in Iranian Children Low Responders to Primary Pertussis Vaccination

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**Background & Objective:** The present study was undertaken to compare the immunogenicity of two diphtheria-tetanus-whole cell pertussis (DTwP) vaccines administered to Iranian 18 months old children who displayed low antibody response to primary vaccination with the same vaccines. **Methods:** In this prospective case-control study, 558 infants were administered with either a local DTwP vaccine [(DTwP-Local) (n=283)] or a commercial vaccine manufactured by Sanofi-Pasteur [(DTwP-Pasteur) (n=275)]. All subjects received DTwP vaccine at 2, 4 and 6 months of age, following the national vaccination schedule of Iran. The infants who had low responses (< 16 EU/ml) to pertussis, were revaccinated with DTwP-Local (n=55) or DTwP-Pasteur (n=57) at 18 months of age. Blood samples were collected 2-4 weeks after booster dose (18th month) of vaccination. Immunogenicity of the vaccines was assessed by ELISA using commercial kits. **Results:** There was no significant difference between the immunogenicity of the two vaccines against diphtheria and tetanus. The geometric mean titers (GMT) of antibodies produced against pertussis were 7.36 and 21.35 EU/ml for DTwP-Local and 10.09 and 41.43 EU/ml for DTwP-Pasteur vaccines, after primary and booster vaccinations, respectively (P< 0.01). **Conclusion:** Immunogenicity against diphtheria and tetanus was similar for the two vaccines, but the immunogenicity of the local vaccine against pertussis was significantly less efficient than that of DTwP-Pasteur. This difference could be due to the bacterial strain or the preparation or formulation protocol of the local pertussis vaccine.

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# 287Fused Recombinant Protein Production of N-Terminal GP96 Plus E7 Antigen and Its Evaluation in COS-7 Cell Line

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**Background & Objective:** Cervical cancer is often the most common cancer in women worldwide. HPVs are associated with most cervical cancers. E7 is a transforming protein which represents a perfect target antigen for vaccines or immunotherapies. Heat shock proteins facilitate cellular immune responses to antigenic peptides or proteins bound to them. The purified HSP/antigen complexes efficiently elicited antigen-specific CD8 T cell responses in mice when delivered as vaccines. The N-terminal of glycoprotein 96 (NTgp96) has adjuvant effect and induce CTL response. The aim of this study is to clone, express and produce recombinant antigen-HSP fusion protein (E7/NTgp96) for different applications in vaccination and immunotherapy against HPV infections. **Methods:** The NTgp96 and HPV16 E7 genes were cloned in pUC18 and pDrive, respectively. These genes were linked in pUC18 vector and confirmed by PCR, digestion and sequencing. The fused genes were subcloned into the pQE-30 vector and expressed in M15 strain. The purification procedure was performed by Ni-NTA affinity chromatography using FPLC. The protein was analyzed by SDS-PAGE and western blot analysis using Anti-His antibody. To verify the in vitro expression of the fused genes, the pEGFP fusion gene (pEGFP-E7/NTgp96) was constructed and a transfection assay using PEI was conducted on COS-7 cells. **Results:** The expression level of the 6xHis-tage fusion protein was noticeable 2hr after IPTG induction. The soluble fusion protein was purified by affinity chromatography on Ni-NTA column under native condition. The protein migrated about 60KDa on SDS-PAGE and was confirmed by western blot. The expression of pEGFP-fusion construct in COS-7 was detectable at 24 h post-transfection. **Conclusion:** Highly immunogenic fusion protein was constructed by linking the N-terminal of gp96 to E7. The recombinant E7/NTgp96 fusion protein is a promising tool for vaccination against HPV infections. The immune responses induced by E7/NTgp96 protein in C75BL/6 mice are under study.

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# 288Investigation of the Immunogenicity of Microencapsulated *E.coli* Antigen for their Use in Vaccine against Collibacilosis

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**Background & Objective:** A commonly used system for microencapsulation is a polyion complex system based on alginate and poly-L-lysine. Targeted delivery of microencapsulated bacterial cells has strong potential for application in treating various diseases such as colibacillosis. The disease is a zoonosis which caused by *E.coli*. Microencapsulation technique has been developed to formulate vaccine therapeutic with improved stability and immunogenicity. The aim of this study was to preparation of alginate microcapsules entrapping *E.coli* antigen and investigation of the antigen immunogenicity. **Methods:** Alginate microcapsules were prepared by emulsification method. The concentration of antigen before and after loading in microcapsule was determined by Kejehldal method. The shape and size alginate microcapsules before and after loading antigen were characterized by electron microscopy. Then sustain release of antigen from alginate microcapsule evaluated at 37oC at different time. The immunogenicity of microencapsulated *E.coli* antigen was evaluated in chickens. The chickens were challenged with the homologous and heterologous strains. The antibody titers were tested using the microagglutination procedure. **Results:** The microcapsules produced with Alginate gave optimal results and their morphological features ere good. The microcapsule showed good loading capacity to *E.coli* antigen as well as sustained release to a certain degree. The microencapsulated *E.coli* antigen showed the high level immunogenicity in chickens. **Conclusion:** The results showed that the antigen was loaded in microcapsules completely. The microcapsules had so obviously sustained antigen release and this showed that the alginate microcapsules may be used as a promising vaccine delivery system for prevention and control of the disease in poultry.

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# 289Molecular Identification, Cloning and Expression of Invasion Plasmid Antigen (IpaC) *Shigella Dysentria*

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**Background & Objective:** Shigellosis is an acute intestinal infection and a major public health problem. Estimations by The World Health Organization (WHO) indicate that the World population suffered from 4.5 billion incidences of diarrhea causing 1.8 million deaths in the year 2002. Approximately, 99% of the cases occurred in developing countries where poor hygiene and limited access to clean drinking water promote the spread of enteric diseases. Shigella species cause bacillary dysentery in humans. Epithelial cell invasion is an essential step in the pathogenesis of Shigella. IpaC of Shigella is essential for initial entry into epithelial cells. According to high frequency report of mortality and shigellosis, antibiotics resistance and lack of appropriate vaccine against this disease, ipaC was cloned for prepare vaccine. **Methods:** In this study, ipaC gene from gene bank was obtained and primers were designed. After genome extraction from *S. dysentria*, it was used as template for PCR amplification. The amplified ipaC gene by PCR was cloned into pTZ57R. Recombinant plasmid was digested by EcoRI and HindIII restriction enzymes; the released band was purified and subcloned into expression vector [pET-28a(+)]. For expression of the recombinant protein, pET-28a(+)/ipaC was transformed into *E. coli* BL21(DE3)(pLysS). ipaC was expressed by inducing early-log-phase cultures of BL21(DE3)(pLysS) containing pET-28a(+)/ipaC with 1 mM isopropyl-b-D-thiogalactopyranoside (IPTG). The protein was tested for expression by employing Ni–NTA agarose column, SDS-PAGE and Western-Blotting analysis. **Results:** Molecular techniques such as PCR, RFLP (restriction digestion), sequencing, electrophoresed on SDS-PAGE and western blotting confirmed that ipaC was cloned and expressed. **Conclusion:** In present study, a recombinant plasmid is produced harboring ipaC that can be used as a recombinant vaccine for perspective studies.

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# 290Superoxide Dismutase B1, a Novel Exacerbatory Antigen Elicits Interleukin-10 Production in Murine *Leishmania Major* Infection

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**Background & Objectives:** The induction of a Th1 immune response to Leishmania antigens using defined vaccination protocols is a potential strategy to induce protection against Leishmania infection. *Leishmania major* superoxide dismutase B1 (SODB1) has been described as prominent enzyme in parasite survival. Recently, we have shown that rSODB1 is highly immunogenic during human leishmaniasis and therefore, considered it as an attractive vaccine target. **Methods:** The protective potential of the rSODB1 against the infection with Leishmania was assessed in BALB/c mice. The animals were vaccinated with rSODB1 in Freund's adjuvant, challenged with live *L. major* promastigotes and the degree of protection was examined by measuring footpad lesion sizes. Furthermore, the levels of cytokine production (IFN-γ, IL-5 and IL-10) by splenocytes were determined by ELISA. **Results:** The results of the present study showed that rSODB1 immunization induce a Th1 response and increase ability in IFN-γ production along with little, if any, IL-5 production (respectively, 878.297 pg/ml and 39.862 pg/ml). However, no evidence of protection was observed after challenge with *L. major*. In fact, the lesions size was bigger in vaccinated mice compared to control groups. At 8-week post-infection, in vitro evaluation of immune responses against the rSODB1 suggested a mixed TH1/TH2 response. Cytokine production analysis showed higher pre-challenge IL-10 production in vaccinated group ($P< 0.005$). As well, infection with parasite caused further production of IL-10 in test group. **Conclusion:** Though a significant production of IFN-γ by splenocytes from vaccinated mice in response to rSODB1 antigen was detected, we did not observe a reduction in lesion size of vaccinated animals. The lack of protection observed may be explained by a significant production of IL-10 induced by this protein.

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# 291In Silico Prediction and in vitro Evaluation of *Leishmania Major* Specific Peptides Eliciting HLA Class I Restricted CD8+ T Cell Responses

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**Background & Objective:** As a potent CTL response activator, peptide vaccine has found its way in vaccine development against intracellular infections and Cancer, but not in Leishmaniasis yet. The first step toward a peptide vaccine is epitope mapping of different proteins of an infectious agent according to the most frequent HLA types in a population. Using in Silico prediction and in vitro evaluation, we mapped potential epitopes of *L.major* proteins presented in HLA-A2 context. **Methods:** We screened L. major proteins that potentially activate $CD8^+$ T cells (CPB, CPC, LmsTI1, LeIF, TSA, LPG3), for 9-mer epitopes presented by HLA-A*0201 (most frequent HLA allele) through online soft-wares (SEFPEITHI, BIMAS, EpiJen, NetMHC, NetCTL, nHLApred). Peptides were selected only if predicted by all programs, according to their predictive scores (18 peptides). Pan HLA-A2 presentation of selected peptides was confirmed by NetMHCPan1.1. 67 *L. major* recovered individuals from endemic area and 66 normal volunteers from non-endemic area were subject to blood sampling. 20ml blood was collected for PBMC isolation. 1ml was used for DNA extraction and PCR-SSP HLA-A2 typing. We evaluated the immunogenicity of immunoinformatically predicted peptides by in-vitro stimulation of PBMCs from recovered $HLA\text{-}A2^+$ individuals. Recovered HLA-A2- and normal individuals were used as controls. PBMCs were stimulated with 4 peptide pools (CPB-CPC in pool-1, LmsTI1 in pool-2, LeIF-TSA in pool-3, LPG3 in pool-4) and rh.IL-2 for 10 days and restimulated with the same pool and anti-CD28/anti-CD49d antibodies plus brefeldin-A for 16hr. $CD8^+$/IFN-$gamma^+$ T cells were detected by BD FACScallibur using ICCS. **Results:** We detected specific response to pool-2 (4 peptides from LmsTI1) compared to background response of $HLA\text{-}A2^-$ recovered individuals in addition to negative response of Normal volunteers. Stimulation due to individual 9-mer peptides of LmsTI1 and its confirmation by T2-stabilization assay and ELISPOT is still under study. **Conclusion:** Among all four pools, pool 2 containing LmsTI1 is a potent $CD8^+$ T-cell activator. So epitopes from this protein will be good candidates for peptide vaccine in Iranian population.

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# 292Optimization of HSP70 and Gp96 Expression in Prostate and Erythroleukemia Cell Lines. Implication for Tumor Vaccine Preparation

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**Background & Objective:** Tumor cells harbor a repertoire of unique, mutated antigens but generally are incapable of provoking an effective immune response, likely because of inadequate antigen presentation by professional antigen-presenting cells. Heat shock proteins (HSPs) play important roles in eliciting innate and adaptive immunity by chaperoning peptides for antigen presentation and providing endogenous danger signaling. In the present study, we optimized HSP70 and gp96 in tumor cells in order to preparation of effective tumor antigens to use in immunotherapeutic settings. **Methods:** The human tumor cell lines LNcap (prostate cancer) and K562 (erythroleukemia) were treated with 41ºC, 42 ºC and 43 ºC for one hour and incubated for 3, 6 and 12 hours post treatment for maximum induction of HSPs. The expression of HSP70 and gp96 were then measured using commercially available ELISA kit and monoclonal anti gp96 antibody and Flow cytometry respectively. **Results:** Our results showed that heat stress of 41ºC for one hour and 12 hours post treatment incubation was induced maximum expression of gp96 in both LNcap and K562 cell lines. However, in the case of HSP70, heat treatment of 43 ºC for one hour and 3 hour post treatment incubation for K562 and 12 hour post treatment incubation for LNcap were resulted in maximum expression of this protein. **Conclusion:** We found that maximum expression of HSPs varies from one cell line to other in terms of temperature and incubation time, so it is recommended to optimize the expression of these proteins for each cancer before preparation of tumor antigens to use in immunotherapy.

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# 293Production and Characterization of Monoclonal Antibodies against an Opticin Peptides: Application for Detection of Recombinant Protein Expressed in Mammalian Cells

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**Background & Objective:** The leucine-rich repeat (LRR) is a molecular recognition motif found in proteins with roles in cell adhesion, signal transduction, DNA repair and RNA processing. Opticin belongs to class III of the small leucine-rich repeat that is mainly expressed in human eye. It is also expressed in human brain, ligament, liver and skin, but at lower levels than the eye. The precise function of opticin is unknown, but it may be involved in fibrillogenesis of collagen molecules to form the vitreous gel and maintaining the spacing between the collagen fibrils of the tissue. In the present study two monoclonal antibodies against two specific opticin peptides were produced and characterized. In addition, the expression of the recombinant opticin in SP2/0 cells was examined by these antibodies. **Methods:** Two peptides, one from the signal sequence and the other from the C-Terminal part of opticin were designed and conjugated with Keyhole limpet hemocyanin (KLH). Then, two BALB/c mice were injected by peptide-KLH conjugates, and monoclonal antibodies against them were produced by hybridoma technology. Reactivity of the antibodies was then evaluated in different immunological assays including ELISA, Western blotting. The opticin gene was cloned in expression vector pcDNA3.1(+) and expressed in eukaryotic mouse myeloma cell line SP2/0, and positive hybridoma clones were further characterized using the produced recombinant opticin proteins by Western blotting. **Results:** Totally 2 hybridoma clones were selected from two separate fusions by ELISA. Antibody isotyping revealed that the antibody against the signal peptide was of IgM class and that against the C-Terminal peptide was of IgG class. These monoclonal antibodies were able to react with recombinant opticin in Western blotting. **Conclusion:** These peptide-based mAbs that specifically bind to the opticin, are useful tools for detection and purification of opticin.

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## 294Isolation and Characterization of Targeting Nanobodies against ERBB2

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**Background & Objective:** The orphan receptor tyrosine kinase ErbB2 (also known as HER2) transforms cells when overexpressed, and it is an important therapeutic target in human cancer. Conventional antibodies and their several drawbacks such large size and difficulty in penetration to dense tissues such as solid tumors, has forced Investigators to search and evaluate alternatives that not only have reduced size and complexity, but also have superior production and stability characteristics. Nanobodies constitute alternatives to conventional monoclonal antibodies that can be produced from functional heavy-chain antibodies (HCAbs) devoid of light chains. **Methods:** Using a carefully designed selection strategy, biopanning on purified antigens and target cells, the nanobodies specific to ErbB2 were isolated from a large phagemid library of immunized camelid. Human tumor cell-based ELISA for ErbB2 were performed on a panel of cell lines with different ErbB2 expression level. Also, identification of a number of their intrinsic molecular properties, Including antigen binding activity (specificity and sensitivity) and measurement of affinity constant were determined using the purified nanobodies ELISA format. **Results:** Biopanning with this library resulted in the selection of various nanobodies which specifically recognized ErbB2 overexpressing cell lines such as SKBR3, T47D, BT474 and transformed NIH3T3, but not A431 and untransformed NIH3T3 as ErbB2 negative cell lines. The results of specificity, sensitivity and affinity demonstrated that the selected nanobodies are functional. **Conclusion:** These nanobodies are potentially effective immunoreagents for diagnostics and therapeutics of certain cancers after further improvement of the sensitivity and future assay validation.

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# 295Recombinant *Leishmania Tarentolae* Expressing the A2 Virulence Gene as a Novel Candidate Vaccine against Visceral Leishmaniasis

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**Background & Objective:** Visceral leishmaniasis is the most severe form of leishmaniasis. To date, there is no effective vaccine against this disease. The use of live attenuated vaccines has recently emerged as a promising vaccination strategy. *Leishmania tarentolae* is a non-pathogenic member of the genus Leishmania, which lacks the A2 virulence gene. Our aim was to evaluate the protective efficacy of *L.tarentolae* transfected with A2 as a live vaccine against visceral leishmaniasis. **Methods:** *Leishmania donovani* A2 gene was episomally transfected along with GFP as reporter gene into *L.tarentolae*. Expression of A2 gene was confirmed by RT-PCR, Northern Blot, and Western Blot analysis. Groups of mice were injected via i.p. or i.v. routes with either *L.tarentolae* GFP-A2 or *L.tarentolae* GFP. Six weeks later, mice were challenged with virulent *L.infantum* parasites. Both humoral and cellular immune responses were evaluated before and also four weeks after challenge by antibody (total IgG, IgG1, IgG2a) and cytokine (IFN-γ and IL-5) assays, respectively. Protection levels in test and control groups were measured by parasite burden quantification four weeks after challenge using Real time PCR method. **Results:** We showed that a single intraperitoneal administration of A2 recombinant *L.tarentolae* strain protects BALB/c mice against *L.infantum* challenge and that protective immunity is associated with high levels of IFN-γ production prior and after challenge. This is accompanied by reduced levels of IL-5 production after challenge, leading to a potent Th1 immune response. In contrast, intravenous injection elicited a Th2 type response, characterized by higher levels of IL-5 and high humoral immune response, resulting in a less efficient protection. **Conclusion:** Our findings for the first time explore the promise of A2-expressing *L.tarentolae* as a safe live vaccine against visceral leishmaniasis. This strategy benefits from both non-pathogenicity of *L.tarentolae* and the ability of A2 antigen to elicit potent Th1 immune response. The route of administration is also proved to have dramatic effects in vaccination outcome.

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# 296Identification and Characterization of Novel Nanobodies Reactive with Tumor-Associated Glycoprotein-72-Expressing Tumor Cells

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**Background & Objective:** Monoclonal antibodies have become a rapidly expanding class of pharmaceuticals for treating cancer. The antigen-binding fragments of camelid heavy chain-only antibodies which are comprised in a single-domain (called nanobodies) have superior properties compared with classical antibodies such as high solubility, high specificity and good affinity for the target antigen. The tumor-associated glycoprotein (TAG)-72 is expressed in the majority of human adenocarcinomas but is rarely expressed in most normal tissues, which makes it a potential target for the diagnosis and therapy of a variety of human cancers. In this report, we describe the successful selection and the characterization of anti-TAG-72 nanobodies by using a phage display strategy. **Methods:** Single-domain antibodies were directly selected by panning a *Camelus dromedaries*. (single-humped camel) library on purified TAG-72 antigen and LS-174T cells (TAG-72 positive cells). After screening of monoclonal phage antibodies by monoclonal phage-ELISA, the construct from selected positive clones were transformed into the non-suppressor strain of E. coli (Rosetta gami2) and reactivity of single-domain monoclonal antibodies against TAG-72 was tested by ELISA. The selected antibody fragments were tested for their properties including sensitivity, affinity, specificity and reactivity with colon carcinoma cells. **Results:** Affinity of selected nanobodies was in the nanomolar range. In addition, they showed no significant cross-reactivity with some of related proteins. Immunocytochemistry test showed strong binding of selected nanobodies to human TAG-72 expressing cells (LS-174T), while no binding was observed with the TAG-72-negative cell line (HT-29). **Conclusion:** These in vitro data indicate that these single domain antibodies have the appropriate specificity and affinity for further evaluation as potential immunotherapy for TAG-72-positive malignancies.

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# 297Topical Imiquimod is a Potent Adjuvant to a Leishmania SLA Antigens

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**Background & Objective:** The leishmaniasis are a group of diseases caused by protozoa of the genus Leishmania and affecting many millions of people worldwide. In humans, it manifests either as a cutaneous disease caused mainly by *Leishmania major*, *Leishmania tropica*, and *Leishmania mexicana*, as a mucocutaneous disease caused mainly by *Leishmania brasiliensis*, or as a visceral disease caused mainly by *Leishmania donovani* and *Leishmania chagasi*. Despite many efforts yet there is no vaccine available against any form of human leishmaniasis. Protection against leishmaniasis is depending upon generation of a Th1 type of immune response. Field trials of first generation Leishmania vaccine showed a limited efficacy even with multiple doses mainly due to lack of an appropriate adjuvant. Imidazoquinolines are attractive as novel adjuvants for weakly-immunogenic protein vaccines. Imidazoquinolines are a family of synthetic organic compounds, of which imiquimod is the best known, that regulate immune responses through interactions with to toll-like receptors (TLRs) on cells especially denderitic APC cells , and induce Il-12 production and promote Th1 response against leishmania antigen. Imiquimod is very suitable immunomodulators, the imidazoquinolines imiquimod and resiquimod bind to TLR7 in mice and TLR7 and 8 in humans. Activation of Toll-like receptors (TLRs) on antigen-presenting cells of the innate immune system initiates, amplifies, and directs the antigen-specific acquired immune response. Ligands that stimulate TLRs therefore represent potential vaccine adjuvant. In the present study, we determined whether imiquimod which when delivered topically. **Methods:** BALB/c mice were vaccinated by three doses of frees-thawed *Leishmania major* accompanied imiquimod as adjuvant. **Results:** IFN-γ, IL-5 and cellular proliferation in response to leishmania antigen in vitro were significantly higher in vaccinated mice as compared to the controls. Vaccinated mice showed better protection against leishmania major challenge. These results have shown that vaccination with these adjuvants mediated a Th1 response against *L. major* antigen, which appeared to suppress the Th2 response following a challenge infection. **Conclusion:** These observations suggest that topically administered imiquimod represent potential vaccine adjuvants to enhance the TH1 response, which can be used with existing or new vaccine formulations.

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# 298Evaluation of Immune Responses Induced by Multigene HIV-1 Recombinant Adenovectors in Mice

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**Background & Objective:** Infection with HIV is an epidemic very difficult to control, so development of a new, safe and efficient preventive vaccine offers the best hope of controlling the HIV pandemic. Adenovectors are valuable tools for developing vaccines against various pathogens. In this study we evaluated the efficacy of patterns of combinations of Ad5 [E1-, E3-] vector expressing HIV-1 clade A env, and rev proteins when combined with other HIV genes to induce immune responses in mice. **Methods:** We used recombinant Adenovector-5 separately. The HIV-1 genes, sequence were amplified from HIV-1RNA (clade A) using nested polymerase chain strategy. The cDNA of each gene was cloned into a transfer vector. Transfer vector then co-transformed into *E.coli* strain BJ5183 together with pAdenovector ΔE1/E3. The recombinant adenoviral construct was transfected into QBI-293A cells to produce viral particles. Recombinant viruses were purified using CsCl gradient centrifugation and titrated on 293 cell plates. Expressions of transgenes were evaluated using western blotting and then injected at 1012 viral particles into 6 groups comprised of 5 mice as a single injection. After 2 weeks, the Humoral responses were evaluated using ELISA and neutralization assay and cellular immune responses checked by cell proliferation and ELISpot assay (IL-2, IL-4 and IFN-γ). **Results:** It was demonstrated that each of the genes was expressed. The responses were mostly toward Th1. Cell proliferation index and ELISpot results showed strong cellular responses. When env+rev were combined with tat and rt the responses was better comparing to other groups. **Conclusion:** Choice of antigen and the pattern of combination are very important in vaccine design. It was showed that the simultaneous injection of tat, rt, env and rev could enhance the humoral and cellular responses and also was able to increase the immune response comparing to other groups harboring other combination of HIV genes.

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# 299Induction of Immune Responses to HIV-1 Clade A Genes by Simultaneous Recombinant Adenovector Injection in Mice

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**Background & Objective:** The correlates of protection against HIV remain undefined, but an effective AIDS vaccine will need to generate both humoral and sustained cellular immunity to more than one HIV antigens. The HIV-1 regulatory proteins rev, tat, are expressed at early time post-infection and showed attractive targets to be included in a vaccine candidate and studies show tat expression is required for efficient HIV-1 reverse transcription. **Methods:** We used recombinant Adenovector-5 separately. The HIV-1 genes, sequence of rev, rt and exon 1 of tat were amplified from HIV-1RNA (clade A) by nested polymerase chain plan. Reveres transcription activity of RT prohibited by site-directed mutagenesis. The cDNA of each gene was first cloned into a transfer vector. Transfer vector then co-transformed into *E.coli* strain BJ5183 together with pAdenovector ΔE1/E3. The recombinant adenoviral construct was transfected into QBI-293A cells to produce viral particles. Recombinant viruses were purified using CsCl gradient centrifugation and titrated on 293 cell plates. Expressions of transgenes were evaluated using western blotting and then injected at 1012 viral particles into 6 groups of 5 mice as a single injection. After 2 weeks the Humoral immune responses evaluated using ELISA (antibody titers and subtype) and cellular immune responses checked using cell proliferation and ELISpot assays (IL-2, IL-4 and IFN-γ). To enhance the immunogenicity rGM-CSF was used. **Results:** It was shown that each of the genes was expressed both independently as well as together with other genes. The response targets were mostly toward Th1. rAd5-tat, rAd-rev and rAd5-rt have induced strong cellular as well as antibody responses. **Conclusion:** It might be claimed that the combination of specific HIV genes such as tat, rev and rt could be more useful comparing to other combinations.

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# 300Characterization of Immune Responses Induced by Combined HIV-1(clade A) Recombinant Adenovectors in Mice

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**Background & Objective:** Numerous evidences exist at present indicating that in some HIV-1 patients the humoral and cellular immune responses are induced against some HIV-1 genes and this is inversely correlated to infection progress. **Methods:** We have used recombinant Adenovector-5 harboring single HIV genes. The HIV-1 genes including gag (p24), rev, nef and exon 1 of tat were amplified from HIV-1RNA (clade A) using nested polymerase chain strategy. The cDNA of each gene was cloned into a transfer vector. Transfer vector then co-transformed into *E.coli* strain BJ5183 together with pAdenovector ΔE1/E3. The recombinant adenoviral construct was transfected into QBI-293A cells to produce viral particles. Recombinant viruses were purified by CsCl gradient centrifugation and titrated on 293 cell plates. Expression of transgenes was evaluated using western blotting and then injected at 1012 viral particles into 15 groups of 5 mice and all pattern of combination of these 4 HIV-1 genes were evaluated. After 2 weeks Humoral and cellular immune responses were evaluated using ELISA, cell proliferation and ELISpot (IL-2, IL-4 and IFN-γ) assays consecutively. **Results:** It was that demonstrated that each of the genes was expressed independently as well as together with other genes, and the expression of each gene did not interfere with the expression of other genes. The response targets were mostly toward Th1, though several Th2 responses were also observed. nef has induced the least IgG2a response comparing to other groups and exerts its effect in other groups much greatly. Single injection in our study induced a good cellular response but the humoral responses were not as strong as the cellular ones. **Conclusion:** Considering and comparing all results and evaluating the various possible interactions revealed that simultaneous injection of tat and gag has enhanced the humoral and cellular responses.

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# 301Comparative Evaluation of Adenovector - Protein Prime - Boost Strategy in HIV-1 Clade A Vaccine in Mice

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**Background & Objective:** The development of a safe and effective AIDS vaccine is urgently needed. Recombinant Adenovirus serotype 5 (Ad5) vectors have been used as vaccine in numerous animal and human clinical studies. But immunological efficiency of the vaccine decreases in body of most patients against this vector due to pre-existing Ad5 immunity Numerous strategies are followed to undertake the problem, one of which is to use prime-boost strategy. **Methods:** The HIV-1 nine genes sequence were amplified from HIV-1RNA (clade A) using nested polymerase chain strategy .We used recombinant Adenovector-5 separately. The cDNA of each gene was cloned into a transfer vector. Transfer vector then co-transformed into *E.coli* strain BJ5183 together with pAdenovector ΔE1/E3. The recombinant adenoviral construct was transfected into QBI-293A cells to produce viral particles. Recombinant viruses were purified and titrated. Expressions of transgenes were evaluated using western blotting and then injected at 1012 viral particles into 18 groups comprised of 5 mice as a single injection. After 2 weeks, 9 groups of mice scarified and another 9 groups received recombinant protein based of specific groups and then after 2 weeks the mice scarified. The Humoral responses were evaluated using ELISA and cellular immune responses checked by cell proliferation and ELISpot assay (IL-2, IL-4 and IFN-γ). **Results:** It was established that each of the genes was expressed. Cell proliferation index and ELISpot results showed very strong cellular responses as well as strong humoral responses in prime-boost groups but in adenovector alone groups only cellular response strong and humoral response are weak. The enhancement of response with prime-boost strategy not same in all groups and in some groups was not as much as predicted. **Conclusion:** It was showed that the prim-boost strategy in compare with adenovector couldn't enhance the humoral and cellular responses in all of the HIV genes.

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# 302Reduced Efficacy of Repeated Doses of CPG-Matured Dendritic Cell Tumor Vaccine in an Experimental Model

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**Background & Objective:** Dendritic cells (DCs) are the professional antigen-presenting cells of the immune system, with the potential to either stimulate or inhibit immune responses. Danger signals sensed by TLRs induce their maturation process. Unmethylated CpG motifs bind to TLR9 and induce DCs maturation. Base on our pervious study, a single dose of CpG-matured DCs could induce an anti-tumor immune response but was not sufficient for complete tumor eradication, so in the current study we evaluated the effect of repeated doses of CpG-matured DCs administration on antitumor immune response. **Methods:** For tumor challenge, WEHI-164 cell line was injected subcutaneously in the right flank of BALB/c mice. Bone marrow-derived DCs (BMDCs) were generated. During DCs culture, tumor lysate was added to immature DC and after 4-6 hours CpG-ODN was added to immature DC cultures for maturation of DCs. One, two or three doses of CpG-matured DCs were injected around tumors at 7, 10 and 13 days after tumor challenge, respectively. Tumor growth rate and survival of mice in different groups were determined. FoxP3 gene expression in tumor was evaluated by Real-time PCR. **Results:** Administration of three doses of CpG-matured DCs led to significant increase in tumor growth rate in compare to one dose. Treatment with two or three doses of CpG-matured DCs declined the survival percent compared with one dose. Repeated doses of CpG matured DCs significantly increased FoxP3 gene expression in tumor tissue samples obtained from immunized mice. **Conclusion:** Administration of repeated doses of CpG-matured DCs vaccine results in decreased anti-tumor immunity, and generation of regulatory responses could be responsible for tumor escape from immune responses.

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# 303Eradication of Tumor by Administration of Repeated Doses of *Listeria Monocytogenes* Activated Dendritic Cells Vaccine in an Experimental Model

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**Background & Objective:** According to our previous studies, *Listeria Monocytogenes (LM)* activated Dendritic cells (DCs) are potent inducers of anti-tumor immune responses. Using repeated doses of this vaccine caused augmentation of anti-tumor reactivity without complete eradication of tumor. In the present study we evaluated efficacy of repeated doses of *LM* activated DCs in complete rejection of tumor by modification of immunization schedule. In compare to previous report, reducing space of first immunization and tumor challenge and shorter intervals between three doses of vaccine were considered. **Methods:** WEHI-164 cell line was injected subcutaneously for induction of tumor in BALB/c mice. Bone marrow derived DCs (BM-DCs) were generated in the presence of GM-CSF and IL-4 for 5 days. Tumor lysates with/without *LM* lysate were added to the culture media for another 2 days. Mice received *LM* matured DCs around the tumor area, 7, 10 and 13 days after tumor challenge. Cytotoxic activity of $CD8^+$ T cells was evaluated by LDH release assay in different groups. Tumor growth rate and survival was monitored. **Results:** According to the findings, repeated doses of *LM* activated DCs vaccine led to a significant increase in the cytotoxic T cell activity and survival in immunized mices. Tumor growth rate was significantly decreased and complete tumor eradication in animals receiving three doses of vaccine was significantly higher than two and one doses. **Conclusion:** The results of the present study showed that tumor immunotherapy in earlier stages and shortening the time intervals between immunizations can cause strong immune response against tumor and its complete eradication.

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## 304Production of ESAT-6: Fcγ1 Fusion Protein as a New TB Vaccine Strategy

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**Background & Objective:** Tuberculosis is one the most important infectious diseases with high mortality rate in the world especially among developing countries. On the other hand, protection of BCG vaccine is proven to be inadequate especially in adult individuals and the urgency of designing new vaccines is clear. In this study, we wanted to produce a recombinant fusion protein including mycobacterial ESAT-6; a potent T cell antigen and FC portion of human IgG1 which is expected to be engulfed by Macrophages and Dendritic cells and induce a good cellular immune response. **Methods:** After preparation of mycobacterial DNA and lymphocyte mRNA, ESAT-6 and Fcγ1 genes were amplified by PCR and RT-PCR respectively. Then we recombined sequences by SOEing PCR and inserted the final product into PDR2EF1 expression vector. Then this vector was inserted into competent bacteria and after plasmid purification and confirmation of cloning by sequencing the expression vector was transfected to eukaryotic CHO cell line. Finally the transient expression of ESAT-6: Fcγ1 fusion protein was measured by Western blotting. **Results:** Western blotting results showed that ESAT-6: Fcγ1 fusion protein was expressed in CHO cell line successfully. **Conclusion:** We could produce this recombinant protein which is the basis of future studies to evaluate the efficacy of this protein as a novel new TB vaccine.

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# 305Plant-Base Production of Chimeric EspA, Intimin and Tir of *Escherichia Coli* O157:H7; An Insight into Its Immunological Evaluation in Animal Model

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**Background & Objective:** *Escherichia co*li O157:H7 is an important food-borne pathogen and cause hemorrhagic colitis and hemolytic uremic syndrome in humans. Cattle are an important reservoir of EHEC. Intimin, Tir, and EspA proteins are virulence factors expressed by LEE locus of enterohemorrhagic *E. coli*. EspA proteins are part of the type three secretion system needle complexes that delivers Tir to host cell, while surface arrayed intimin docks the bacterium to the translocated Tir. This intimate attachment leads to attaching and effacing lesions. We hypothesized that the chimeric forms of antigens would reduce colonization EHEC in cattle. **Methods:** To test this concept we constructed a trivalent recombinant protein (EIT) which is composed of C-terminal of EspA (E), C-terminal of Intimin (I) and Tir (T) which attached together by linkers (EAAAK). Two other constructs were assembled to complete the antigen combination. These construct composing EspA/Intimin and Intimin/Tir. Designing, synthesis and evaluation of these two constructs is under progress. Before synthesize of trivalent antigen, the chimeric gene (eit) adapted to coding sequence of tobacco plants and fused Kozak sequence before start codon and KDEL signal at the end. The synthetic gene was cloned into plant expression vector adjacent to CaMV35S promoter and was introduced into tobacco plant by Agrobacterium-mediated transformation. Three mice group were either immunized subcutaneously with recombinant EIT expressed in *E.coli* in our previous work, as a control group, fed by whole leaves of transgenic tobacco plant, as a test group or immunized with combination of these two procedures. **Results:** The amount of chimeric protein detected in transgenic tobacco leaves was near 2% of the total soluble (TSP) protein. Immunized mice generated an EIT-specific immune response when primed parent rally and then boosted orally with recombinant EIT protein. **Conclusion:** Here we show that oral based immunization with chimeric antigens in laboratory animal could apply successfully. The immunized mice exhibited a reduced duration of *E. coli* O157:H7 fecal shedding after challenge.

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# 306Expression of Fusion Protein, Colonization Factor B-Heat Labile Toxin B Subunit (CfaB-LTB) in Transgenic Colza Seeds and Evaluation of Its Immunological Properties

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**Background & Objective:** *Enterotoxigenic Escherichia coli (ETEC)* are the cause of diarrhea in fewer than 5 year old children living in developing countries. The prevalence of the disease is about 400 millions annually among which 800,000 lead to deaths. Vaccination against the disease is one of the significant objectives of World Health Organization (WHO). After intestinal colonization of *ETEC*, heat labile toxin is released which results to diarrhea. Colonization factor/I antigen and heat labile enterotoxin (LT) are the most important virulent factors of *ETEC*. Conclusively, the critical role of these two proteins in *ETEC* pathogenesis, candidate them for vaccine development. Mucosal immunization is necessary for efficient vaccination. Transgenic plants are able to administrate vaccines at mucosal surfaces and can induce local as well as systemic immunity. **Methods:** A plant optimized synthetic gene encoding for CfaB-LTB fusion protein was designed. After insertion into a plant expression vector with seed specific promoter, this gene has been introduced into colza plant. Ten independent transgenic lines were regenerated. After molecular analysis, we fed BALB/c mice with oral transgenic colza seeds on days 0, 7, 14 and 21 and collected serum and fecal samples weekly. Serum was analyzed for CfaB-LTB specific IgG and IgA, and feces was analyzed for CfaB-LTB specific IgA. **Results:** The sCFaB-LTB gene was detected in the genomic DNA of transgenic cells by PCR DNA amplification. RT-PCR and southern blot analysis confirmed stable integration of gene into the genome. Based on results of ELISA, CfaB-LTB protein comprised approximately 0.3-0.5 % of total soluble protein in transgenic colza seeds. We observed anti CfaB-LTB antibody response with high specific antibody concentrations in groups fed of transgenic seeds. **Conclusion:** Our results demonstrate that (CfaB-LTB) derived from transgenic colza is immunogenic when orally administered to mice. After this, mice will be orally challenged with *ETEC*. Also, the protection against enterotoxienic *E. coli* binding to Caco-2 cells generated by antisera from mice immunized with plant-synthesized antigen will be investigated.

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# 307Induction of Tumor-Specific Immunity by HER2/Neu-Derived Peptides of Various Lengths

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**Background & Objective:** In this study, we identified T cell-binding epitopes deduced from the Her2/neu protein. Peptides of various lengths were synthesized and applied in BALB/c mice following by immunologic evaluation. These data indicate that these peptides are able to generate antitumor responses. **Methods:** Synthetic peptide was designed according to on-line algorithms and used for vaccination of BALB/c mice. Cytokine Assays (IFN-γ and IL-4) were performed using mouse ELIspot kits as described by the supplier. An in vitro CTL assay was carried out using ex vivo expanded splenocytes and TUBO tumor cells labeled by calcein AM as targets. Splenocytes from vaccinated and control mice were Also cultured with synthetic peptides and then supplemented with WST-1 tetrazolium salt. Subsequently, the absorbance at 450 nm was measured. Vaccinated mice (five animals per group) were challenged (s.c) in a distant site of the vaccination with TUBO live cells. Mice were inspected weekly to monitor the tumor growth. **Results:** Peptide sequences with an MHC-I binding ability, designed using on-line algorithms. When individually BALB/c mice were vaccinated with these peptides, two of peptides were found to induce antigen-specific T-cell responses, as measured by IFN-γ and IL-4 ELISPOT assays. The results indicated that these peptides were the most effective peptides in generating $CD8^+$ T cells that reacted with TUBO cell line expressing Her2. Determination of specific $CD4^+$ T cell proliferation showed significantly improved proliferation. Mice receiving the complete vaccine showed decreased tumor growth against the tumor challenge. **Conclusion:** In conclusion, we describe two of the HER2/neu-derived peptides generating Tcell specific responses and immune-mediated rejection of transplantable tumors in vaccinated mice. Our results show that these peptides can be used as vaccine candidates in Her2 over-expressing tumors.

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# 308Construction of Recombinant BCG Harboring Hepatitis C Virus (HCV) Truncated Core and Enhanced Thymidine Kinase Genes as HCV Candidate Vaccines

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**Background & Objective:** Infection with Hepatitis C virus (HCV) is the leading cause of chronic liver disease, cirrhosis and carcinoma. Although no vaccine against HCV is available so far but cell mediated immunity (CMI) seems to play a determining role in natural HCV clearance. The tuberculosis vaccine, Bacillus Calmette-Guerine (BCG), carrying HCV antigens may be a promising candidate to induce HCV-specific CMI. In this study recombinant BCG (rBCG) was engineered to express the HCV truncated core (HCV-C) protein, as a potent and conserved HCV antigen to make a vaccine candidate in fusion with the enhanced thymidine kinase (en-TK) protein which enables Gancyclovir-mediated destroying of rBCG for increased presentation of its antigens to immune system. **Methods:** Sequences corresponding to the promoters of heat-shock proteins (HSP) 70 and 60, α-antigen signal sequence (αS), en-TK and HCV-C were PCR-amplified and by the application of SOEing-PCR were fused and finally cloned into the kanamycin-encoding pVN2 vector to construct the plasmids of pHSPTK, pHSPαS-C and pHSPTKHSPαS-C. BCG cells were transformed by the recombinant plasmids via electroporation. **Results:** Analyses by restriction enzymes and sequencing reactions confirmed the authenticity of the constructed recombinant BCG-plasmid encoding the proper sequence of pHSPTK, pHSPαS-C and pHSPTKHSPαS-C proteins. Optimization of electroporation parameters (such as: BCG washing prior to electroporation, plasmid and antibiotic concentrations, time constant and temperature electroporation) resulted to high transformation efficiencies Kanamycin-resistance BCG cells electrotransformed with corresponding plasmids. **Conclusion:** Recombinant BCG-expression strains for HCV-C protein were obtained and following confirmation of their expression capacities will be exploited for the immunogenicity analysis in vivo.

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# 309Induction of Interferon-Gamma Production in T Cells by Different Costimulations

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**Background & Objective:** Costimulatory interaction between antigen presenting cells and T cells is needed for full activation after antigen exposure. Quality of this interaction determines the differentiation pathway of the activated cell toward effector phenotype. The aim of this study was to compare the ex vivo ability of two pathways of costimulations, CD28 and 4-1BB, to activate T cells and stimulate IFN-g production. **Methods:** Human T cells were stimulated with anti-CD3 antibody while cells were costimulated with artificial antigen presenting cell of adenoviral infected A549 cells expressing CD80/86 and/or 4-1BBL. CFSE T cell proliferation assay and presence of intracellular IFN-γ were analyzed by Flow cytometry and compared to secreted cytokine in supernatant measured by ELISA. **Results:** Costimulation by the 4-1BB ligand enhanced IFN-γ production in lymphocytes during culture for 1 week with anti-CD3 stimulation. This effect was greater than effect of CD80 costimulation, but both ligands together showed some cooperative effects. **Conclusion:** The enhanced IFN-γ production with 4-1BB ligand costimulation and its cooperative interaction with CD28 support the potential usefulness of artificial antigen-presenting cells that express different costimulatory ligands for ex vivo T cell-based therapies.

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# 310Evaluation of the Adjuvant Activity of Naloxone, an Opioid Receptor Antagonist, in Combination with Heat-Killed *Listeria Monocytogenes* Vaccine

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**Background & Objective:** We have previously demonstrated the adjuvant activity of naloxone (NLX), a general opioid antagonist, using a DNA vaccine for herpes simplex virus type 1. Here, the adjuvant activity of NLX has been evaluated using a heat-killed *Listeria monocytogenes* (HKLM) vaccine as a model for general immunization against intracellular bacteria. **Methods:** BALB/c mice were divided into three groups: the Vac group received the HKLM vaccine alone; the NLX-Vac group received the HKLM vaccine in combination with the adjuvant NLX; and the control group received PBS. All the mice were immunized two times subcutaneously on days 0 and 14. Two weeks after the last immunization, lymphocyte proliferation, delayed-type hypersensitivity, skewing of the immune response toward a Th1/Th2 pattern and induction of protective immunity against *Listeria monocytogenes* were evaluated. **Results:** Our results indicate that the administration of NLX as an adjuvant enhances the ability of the HKLM vaccine to increase lymphocyte proliferation, delayed-type hypersensitivity, and skewing of the immune response toward a Th1 pattern. Additionally, combination of NLX with the HKLM vaccine improves protective immunity against *Listeria monocytogenes*. **Conclusion:** To our knowledge, this study is the first to show administration of an opioid receptor antagonist as an adjuvant for a vaccine against an intracellular bacterium can enhance cell-mediated immunity and shift the immune response to Th1.

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# 311The Effect of Alum-Naloxone, as a New Adjuvant, on the Efficacy of *Salmonella Typhimurium* Vaccine

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**Background & Objective:** We previously showed administration of naloxone, an opioid receptor antagonist, as a new adjuvant increased the efficacy of vaccines in stimulation of cellular immune responses. Vaccine induced resistance against many microbes needs both humoral and cellular immune responses and on the other hand, alum, which is approved by FDA as a vaccine adjuvant, enhances only humoral immune response efficiently. Therefore in the current research we tried to evaluate the effect of administration of mixture of alum and naloxone (alum-naloxone), as a new adjuvant on the efficacy of killed *Salmonella typhimurium* (KSM), as a vaccine model, in induction of protective immunity and stimulation of humoral and cellular immune responses. **Methods:** BALB/c mice were divided to four groups. The mice of the first group were received alum-naloxone in combination with KSM. Alum in combination with KSM was administered to mice of the second group. The mice of the third group were received only KSM. PBS was injected to the mice of the forth group, as negative control. All the mice were immunized two times subcutaneously on days 0 and 14. Two weeks after the last immunization, survival of the mice against challenging with *Salmonella typhimurium*, lymphocyte proliferation in response to antigens of *Salmonella typhimurium* and *Salmonella typhimurium* specific serum IgG1 and IgG2a were evaluated. **Results:** Our results indicated that the mice received alum-naloxone in combination with KSM had significantly more lymphocyte proliferation, IgG2a/IgG1 ratio and survival rate than those of mice received alum in combination with KSM. **Conclusion:** To our knowledge, this study is the first to evaluate alum-naloxone mixture use as a new adjuvant for vaccines.

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# 312Effects of Dendritic Cell Vaccine Activated with Protein Components of *Toxoplasma Gondi* on Tumor Specific CD8+ T Cells

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**Background & Objective:** Dendritic Cell (DC) is an important antigen-presenting cell that present tumor antigen to $CD8^+$ and $CD4^+$ T lymphocytes and induce specific anti-tumor immunity. In order to induce effective anti-tumor response, an option is increasing the efficiency of antigen presentation of dendritic cells and T cell activation capacity. The aim of the present study was to investigate the effect of dendritic cell maturation with protein components of *Toxoplasma gondii* on cytotoxic T lymphocyte activity and their infiltration in to the tumor. **Methods:** For DC generation, bone marrow cells were cultured in the presence of GMCSF and IL-4 for five days. After that, LPS, protein components and whole extract of *Toxoplasma gondii* were added to the culture media and incubated for another two days for DC maturation. To generate tumor, mice were injected subcutaneously with WEHI-164 cell line. For immunotherapy 106 DCs matured with different compounds were injected around the tumor site. Infiltration of $CD8^+$ T cells were determined by Flow cytometry and cytotoxic activity was measured by LDH detection kit. **Results:** Immunotherapy with DCs treated with protein components of *Toxoplasma gondii* led to a significant increase in the activity of cytotoxic T cells and infiltration of $CD8^+$ T cells in to the tumor. Immunotherapy using protein components of *Toxoplasma gondii* significantly improved the survival of the mice compared with other groups ($P< 0.0001$). **Conclusion:** Protein components of toxoplasma are able to increase DC capability in induction of CTL-mediated anti-tumor response and increase infiltration of these cells in to the tumor.

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# 313Evaluation of IDO Gene Expression after Vaccination with *Listeria Monocytogenes* Activated Dendritic Cell Vaccine in an Experimental Tumor Model

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**Background & Objective:** There is ample evidence in favor of multiple immunosuppressive mechanisms that considerably dampen tumor responses and weaken the activity of current immunotherapeutic regimens. Therefore, it will be necessary to exactly recognized tumor-mediated immunosuppression before immunotherapies can successfully be applied. Increasing evidence has been shown most tumors and tumor infiltrating cells express indoleamine 2, 3-dioxygenase (IDO) as an immunoinhibirory enzyme by which tumor cells escape from effective immune responses. However, whether regulatory responses that expand during tumor progression can modulate dendritic cell based vaccines is unclear. **Methods:** To address this issue, we have evaluated gene expression of IDO in tumor microenvironment fallowing dendritic cell based immunotherapy and its correlation to anti-tumor response. *Listeria monocytogenes* activated, tumor lysate pulsed dendritic cell vaccine was administered to tumor challenged mice with different schedules. Tumor growth rate, T cell cytotoxicity and mRNA level of IDO was evaluated in different groups of vaccinated mice. **Results:** Tumor growth decreased in prophylactic vaccination group. In addition, gene expression of IDO reduced in this group and there was a positive correlation between tumor growth rate and transcriptional level of IDO in tumor tissue in all vaccination groups. **Conclusion:** Our results suggest that regulatory agents such as IDO dampen T-cell immunity against tumor and is the main obstacle tempering successful immunotherapy and active vaccination. Therefore this agent must be taken into account for the future designation and application of more effective cancer vaccines.

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# 314Comparison of Antibody and Cell-Mediated Immune Responses after Intramuscular Hepatitis C Immunizations of BALB/c Mice

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**Background & Objective:** Current treatments for hepatitis C infection have limited efficacy, and there is no vaccine available. The goal of this study was to compare the immune response to several immunization combinations against Hepatitis C virus (HCV). **Methods:** Six groups of mice were immunized at weeks 0, 4, and 8 with different combinations of a candidate HCV vaccine consisting of 100 microgram recombinant HCV core/E1/E2 (rHCV) DNA plasmid and/or 25µg rHCV polyprotein and 50ml Montanide ISA-51. Four weeks after the last injection, all groups of mice were sacrificed and blood samples and spleens were collected for measuring the levels of specific HCV antibodies (total IgG, IgG1 and IgG2a). Cell proliferation and intracellular Interferon-gamma were also measured. **Results:** Among the groups of immunized mice, only the mice immunized with rHCV DNA plasmid, rHCV polyprotein, and montanide (group D) and mice immunized with rHCV polyprotein and montanide (group F) demonstrated a significant increase in the total IgG titer after immunization. IgG1 was the predominant antibody detected in both groups D and F. No IgG2a was detected in any of the groups. Proliferation assays demonstrated that splenocytes from group D and group C (rHCV DNA primed/rHCV polyprotein boost) developed significant anti-HCV proliferative responses. **Conclusion:** The combination of an rHCV DNA plasmid, rHCV polyprotein, and montanide induced a high antibody titer with a predominance of IgG1 antibodies and recognized the major neutralization epitopes in HVR1. In contrast, group C did not show an increase in anti-HCV antibodies, but did show a proliferative response.

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# 315In vitro Selective Depletion of $CD4^+CD25^+$ Regulatory T Cells from PBMCs Using Anti-tac-SAP

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**Background & Objective:** It has been shown that naturally occurring regulatory T cells ($CD4^+CD25^+$ $Foxp3^+$ T cells), have critical roles in tumor invasion and down regulation of immune response against established tumors. Highly expression of CD25 (α chain of IL-2 receptor) by regulatory T cells may cause an inefficient IL2 based cancer vaccines. It seems that selective elimination of regulatory T cells before treatment of tumor bearing T cells can strongly increase the efficacy of vaccine. The aim of this study was to set up an efficient and cost effective protocol to eliminate $CD4^+CD25^+$ T cells using an immunotoxin called anti-tac-SAP. **Methods:** PBMCs taken from colon cancer patients were treated with different concentration of immunotoxin. Flow cytometric analysis was preformed for CD4, CD25, CD3, CD8, and CD45 surface markers, and semi-quantitative fluorescent-PCR used for detection of Foxp3 expression before and after treatment. **Results:** Anti-tac-SAP effectively eliminates $CD4^+CD25^+$ regulatory T cells. This study revealed that 25µg/dl is the optimal concentration of anti-tac-SAP for selective depletion of $CD4^+CD25^+$ Treg cells omong different concentrations, from 5 to 100 µg/dl. These data was verified by detection of Foxp3 expression. **Conclusion:** The results indicated that immunotoxin had no non-specific effects on other T cells such as $CD4^+CD25^-$ T cells and $CD8^+CD45^+$ T cells. Future studies relating to the anti-tac-SAP should be focused on the survival and functional assessment of remaining T cells (CD8, CD4) using proliferation and peptide sensitization assays.