

POSTER PRESENTATIONS

P1)

THE ROLE OF MACROPHAGE MIGRATION INHIBITORY FACTOR IN MODULATION OF INTERLEUKIN-17 EXPRESSION

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Interleukin-17 (IL-17) is a pro-inflammatory cytokine, produced by recently described Th17 cells which have critical role in immunity to extracellular bacteria and pathogenesis of several autoimmune disorders. IL-6 and TGF- β are crucial for the generation of Th17 cells, while the production of IL-17 is supported by IL-1b, IL-15, IL-23 or TNF- α . In this study the influence of a multifunctional cytokine, macrophage migration inhibitory factor (MIF) on IL-17 production in mice was investigated. Treatment of lymph node cells (LNC) with recombinant MIF up-regulated, while deletion of *mif* gene (MIF^{-/-}) severely impaired concanavalin A (ConA)-stimulated IL-17 secretion and expression measured by ELISA or Real-time PCR, respectively. In addition to lower IL-17 production, MIF^{-/-} LNC displayed impaired production of IL-1 β , IL-6, IL-23, and TGF- β and elevated TNF- α production. When stimulated with recombinant IL-1 β , IL-23 or TNF- α , ConA-triggered MIF^{-/-} LNC were fully able to reach IL-17

production seen in MIF^{+/+} while the addition of IL-6 and TGF- β had no apparent effect. Furthermore, impaired IL-17 production in MIF^{-/-} LNC concurred with defective activation of p38, ERK, JNK and JAK2/STAT3 signaling pathways, as determined by cell-based ELISA. Conversely, NF- κ B and NFAT transcription factors remained entirely operative and possibly involved in IL-17 production in MIF^{-/-}LNC. Finally, after injection of mice with complete Freund's adjuvant, secretion of IL-17 as well as the number of IL-17-positive cells, were significantly lower in draining LN of MIF^{-/-} mice in comparison to MIF^{+/+} mice. The obtained results suggest that MIF could stimulate IL-17 production both directly and indirectly, and that targeting MIF biological activity could be a valid therapeutic approach for the treatment of autoimmune disorders mediated by Th17 cells.

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P2)

LOXORIBINE, A TLR-7 AGONIST MODULATES FUNCTIONAL PROPERTIES OF HUMAN MONOCYTE-DERIVED DENDRITIC CELLS

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Introduction: Toll-like receptors (TLRs) sense microbial products and trigger dendritic cell (DC) maturation and cytokine production. Recently small guanosine analogs such as 7-allyl-8-oxoguanosine (loxoribine) have been identified as a selective TLR-7 agonist. **Methods:** Human monocyte derived DC have been generated in the presence of GM-CSF and IL-4 for 6 days. Maturation was induced by different TLR agonist. Phenotype and different functional properties of such cells were evaluated. **Results:** We showed that loxoribine did not significantly modulate maturation of human monocyte derived DC but upregulated maturation of these cells in combination with TLR-3 agonist (poli I: poli C) or TLR-4 (LPS from *E. coli*). This conclusion is based on the upregulation of CD83, CD54 and CD86 and higher allostimulatory properties of these cells compared to the effect of a single agonist. Poli I: poli C significantly stimulated the production of both IL-12 p70 and IL-23, which was followed by strong production of IFN- γ and IL-17, respectively, by allogeneic CD4⁺ T cells in coculture with DC. However, this TLR-3 agonist also stimulated the secretion of IL-10 by DC. Simultaneous stimulation of DC by poli I: poli C and loxoribine resulted in additional production of IL-12p70, IFN- γ and down-regulation of IL-23, IL-17 and IL-10. LPS-triggered DC produced lower levels of IL-12p70 and moderate levels of IL-23 and IL-10. The treatment of LPS-stimulated DC with loxoribine augmented the production of IL-12 p70, but CD4⁺ T cells from the coculture produced higher quantity of both IFN- γ and IL-10. In **conclusion**, our results suggest that combination of TLR-7 and TLR-3 agonist favors the Th1 immune response in vitro and suppresses IL-17 and IL-10 production, which could be beneficial for anti-tumor immune response.

P3)

THE EFFECT OF FATTY ACIDS ISOLATED FROM ROYAL JELLY ON FUNCTIONAL PROPERTIES OF DENDRITIC CELLS IN VITRO

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Introduction: Royal jelly (RJ) was shown to exhibit different biological activities, but its effect on the immune system is still unclear. Aim: In this work we studied the effect of 10-hydroxy-2-decenoic acid (10-HDA) and 3,10-dihydroxy-decanoic acid (3,10-DDA), isolated from RJ, on functional properties of dendritic cells (DC).

Methods: Rat splenic DC were cultivated with different doses of 10-HDA and 3,10-DDA. Allostimulatory capacity of DC was measured by ³H-thymidine incorporation assays. Phenotypic analysis was performed by flow cytometry. Cytokine production was determined by ELISA.

Results: Splenic DC, cultivated with 10 μ g/ml of fatty acids down-regulated the expression of CD86 and the production of IL-12, but up-regulated the production of IL-10. In contrast, DC pretreated with 100 μ g/ml of 3,10-DDA, up-regulated the expression of CD86 and augmented the proliferation of allogeneic T cells. The highest dose (200 μ g/ml) of both fatty acids reduced the number of DC in cultures, down-regulated the expression of MHC class II and CD86, decreased the production of IL-12 and made these DC less allostimulatory. DC, pretreated with all three doses of RJ fatty acids (10, 100 and 200 μ g/ml), significantly augmented the production of interferon- γ (IFN- γ) by autologous T cells in the presence of an agonistic anti-T cell receptor antibody and pretreatment of DC with the highest dose of fatty acids resulted in a decrease of IL-4 production. **Conclusion:** Our results showed that RJ fatty acids differently modulated the DC-mediated proliferation of T cells and Th1/Th2 cytokine production in vitro and suggest that DC could be a significant target of RJ immunomodulatory activity.

P4)

THYMIC NURSE CELL LINE-DRIVEN DEVELOPMENT OF CD4⁺FOXP3⁺ REGULATORY THYMOCYTES IN VITRO

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Introduction: Regulatory T cells (Treg) play a key role in the control of a wide range of immune-mediated reactions. Accumulated findings indicate that the thymic epithelial cells (TEC) have been implicated in the induction and/or selection of Treg cells in the thymus. However, little is known about the cellular and molecular mechanisms that mediate this pathway of Treg generation. In our previous studies we have characterized a rat TEC cell line with nursing activity (R-TNC.1). We have demonstrated that cocultivation of rat thymocytes with R-TNC.1 line induces the generation of thymocytes (E-Treg) with regulatory activity in vitro. This study describes further phenotypic and functional characterization of E-Treg cells. **Methods:** Experiments were performed on inbred AO rats. Detection of forkhead box protein p3 (Foxp3) positive thymocytes was performed by flow cytometry using a rat Foxp3-specific monoclonal antibody. The suppressive capacity of E-Treg cells was assessed in vitro on the proliferation of ConA stimulated responder cells (freshly isolated syngeneic thymocytes). **Results:** We found that E-Treg cells, isolated after 48h preincubation of thymocytes with R-TNC.1 cells, showed some changes associated with Treg cells. Namely, E-Treg cells expression of Foxp3 transcription factor was increased compared to control (thymocytes cultivated alone). E-Treg cells gained the ability to significantly inhibit ConA stimulated responder T cell proliferation in vitro. In the presence of ConA, E-Treg cells did not proliferate, their expression of CD25 molecule was increased but IL-2 production was decreased, and they were arrested in Go/G1. Using a transwell system we demonstrated that direct cell-to cell contact is necessary for the E-Treg cell induction. Generation and regulatory

activity of E-Treg cells were independent of contaminating dendritic cells. The suppressive capacity of E-Treg cells could be dependent on IL-10, since we detected increased production of IL-10 in the secondary culture of E-Treg cells and responder thymocytes. In **conclusion**, our results support a potential role of thymic nurse cells in Treg cells development.

P5)

THE EFFECT OF BLOCKADE OF NITRIC OXIDE FORMATION ON RAT IgA

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Whereas secretory IgA exerts its function as first line of defence in mucosal secretions by limiting invasion of pathogens, serum IgA may function as a second line of defence by eliminating pathogens that have breached mucosal surface. Although a growing body of evidence indicates that nitric oxide (NO) is a regulator of inflammation and immunity (Coleman, 2001, Korhonen et al., 2005), the effect of NO on IgA is largely unknown. The aim of the present study was to investigate whether endogenous NO modulates serum IgA using N ω -nitro-L-arginine-methyl ester (L-NAME), which inhibits the activity of all isoforms of NO synthase. To this end, adult female Wistar rats showing diestrus day 1 were treated with L-NAME (30 or 50 mg/kg, s.c.). Saline-injected rats were used as controls. The animals were sacrificed by decapitation 3 h after L-NAME or saline injection. Serum concentration of IgA was measured by a sandwich ELISA. The results revealed that treatment with L-NAME significantly decreased the level of serum IgA (p<0.05). Similar influence of different doses of L-NAME (30 or 50 mg/kg) on IgA indicated that observed action was not dose-dependent. Decreased concentration of IgA in serum following the blockade of NO formation suggests immunomodulatory role of this signal molecule on IgA in female rats showing diestrus day 1.

P6)

INHIBITION OF WOUND GRANULOCYTE APOPTOSIS DURING BURN INJURY IN RATS

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Introduction: Granulocytes (Gr) are cells with a short lifespan, dying spontaneously via apoptosis, the process that has been recognized as a critical for the resolution of inflammation and prevention of tissue injury. Our previous studies demonstrated that severe burn injury impaired wound healing. The aim of this study was to investigate the influx of Gr at the site of burn areas and their apoptosis *ex vivo* and in cultures.

Methods: Inbred AO rats were subjected to nonlethal, full-thickness burn injury (80°C, 30s, 20% of the body surface area). Immediately after wounding, sterile polyvinyl sponges were implanted subcutaneously on the borderline of the burned skin in order to obtain wound inflammatory Gr (experimental group). Control group consisted of rats with subcutaneously implanted sterile sponges. Apoptosis was determined by morphology after staining with Türkck reagent and by annexin V-FITC/propidium iodide (PI) labeling and flow cytometry. **Results:** Burn injury was followed by a reduction in the number of Gr at the wound site, but in contrary the number of Gr in peripheral blood increased significantly. Apoptosis rate of freshly isolated Gr from burned animals was significantly lower ($2.75 \pm 1.1\%$) compared to control wound Gr ($6.0 \pm 2.03\%$), $p < 0.05$. Cultivation of inflammatory and peripheral blood Gr from both groups resulted in the same phenomenon- decreased apoptosis rate of inflammatory ($19.8 \pm 0.3\%$ vs. $33.1 \pm 5.5\%$, $p < 0.01$) as well as peripheral blood Gr (62.7 ± 3.8 vs. $75.6 \pm 4.95\%$, $p < 0.02$) from burn-injured animals compared to control. In order to determine whether factors present in wound fluids (WF) were responsible for inhibition of apoptosis, we cultivated control and burn-inflammatory Gr with corresponding WF (6%) or *vice versa*. Contrary

to our expectation, control WF exerted a significantly higher anti-apoptotic effect on both control and burn-inflammatory Gr compared to the WF from burn-injured animals. **Conclusion:** Burn injury decreased Gr accumulation at the wound site and inhibited apoptosis of these cells both systemically and locally, although it seems that the systemic effect was predominant.

P7)

IMMUNOMODULATORY EFFECTS OF ORAL WARFARIN APPLICATION IN RATS

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Warfarin (4-OH coumarin) and its congeners are anticoagulants which biological activity is based on vitamin K (VK) antagonism. Warfarin (WF) inhibits vitamin K epoxide reductase (VKOR) preventing thus production of complete proteins involved in normal hemostasis. By inhibiting VKOR, warfarin affects generation of biologically active VKD proteins required for processes other than hemostasis including those involved in bone growth and calcification, cell signaling and several with yet unknown function. Our previous studies demonstrated proinflammatory activity of intraperitoneal and epicutaneous application of warfarin in rats. The aim of this study is to get initial informations on immune modulating effects of oral warfarin administration in rats. Changes in peripheral blood leukocytes and spleen activity were examined following subchronic (30 days) and chronic (90 days) of warfarin administration in drinking water (initial doses of 0.05 and 0.5 mg/kg body mass). Increase in prothrombin time was noted following application of higher WF dose, documenting access of WF to general circulation. No numerical changes in total and differential peripheral blood leukocyte counts were noted, with no direct cell toxicity, evaluated by MTT reduction assay. Both doses of warfarin exerted priming effect (evaluated by cytochemical nitroblue tetrazolium, NBT, reduction assay of respiratory

burst) on peripheral blood granulocytes at subchronic and chronic treatment, while chronic exposure to higher WF dose resulted in granulocyte activation as well. A tendency of decrease in spleen cellularity was noted following subchronic treatment with higher WF dose, which was accompanied by lower cell viability, survival and Con-A-stimulated proliferation. No such changes were noted following 90-days treatment. Presented data demonstrated differential effects of oral warfarin application on granulocytes and lymphocytes. Obtained data are relevant for recognition of biological effects of anticoagulants, other than those affecting hemostasis.

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P8)

STEREOLOGICAL STUDY OF THYMIC VASCULAR NETWORK AFTER DEXAMETHASONE AND MEDROXYPROGESTERONE ACETATE APPLICATION

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The aim of our investigation was to determine the effect of dexamethasone and medroxyprogesterone acetate (MPA) on the morphological and stereological characteristics of thymic parenchyma and thymic vascular network. 120 female Wistar rats were divided into 5 groups. The control group of rats was daily administered physiological solution, whereas the others, experimental groups were given dexamethasone at doses of 0,6 and 3 mg/kg bw, and MPA at doses of 30 and 150 mg/kg bw. One half of animals of each group was treated in a period of 7 days and the other half, 15 days. Thymus paraffin sections were stained according to the methods of hematoxylin-eosin, elastica Van Gieson, alcian blue PAS and Masson Goldner.

The histological analysis have shown distinct reduction of thymic parenchyma and enlarged vascular network. Stereological analysis revealed that volume density of thymic parenchyma significantly decreased in all groups of experimental rats. The volume density of thymic vascular network significantly increased after 7 days and insignificant increased after 15 days dexamethasone and MPA application. Our results suggested that marked reduction of thymic parenchyma, provokes masking perform to the angiostatic effect of the thymic vascular network after dexamethasone and medroxyprogesterone acetate application.

P9)

COMPARATIVE MORPHOMETRIC ANALYSIS OF THE EFFECTS OF DIFFERENT DOSES OF DEXAMETHASONE ON MESENTERIC LYMPH NODES

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The aim of our paper was to register the changes of the structural elements in mesenteric lymph nodes after application of different doses of dexamethasone. The experiment included 36 female Wistar rats divided into 3 groups. The control group of rats was administered physiological solution intramuscularly each day whereas the experimental group was treated with dexamethasone at therapeutic doses of 0,6 mg/kg/b.w. and maximal therapeutic dose of 3 mg/kg/b.w. The experiment lasted for 7 days. Paraffin slides of mesenteric lymph nodes were stained according to the methods of hematoxylin-eosin, elastica Van-Gieson and Masson-Goldner. Histological analyses were done with a light microscope. Microscopic characteristics of structural components of mesenteric lymph nodes in rats treated with different doses of dexamethasone have revealed a significant re-

duction of the cortex and paracortex and a significant decrease of cellular density, especially of the lymphocytic component of lymphoreticular tissue. Morphometric and statistical analyses have shown the following: diameters of lymph follicles from $316,71 \pm 22,61$ (mean value \pm standard deviation) in the control group of rats significantly reduced to $157,25 \pm 10,41$ in the group treated with 0,6 mg/kg/b.w. and $128,94 \pm 10,41$ in the group treated with 3 mg/kg/b.w. dexamethasone ($p < 0,01$). Diameters of germinal centers from $171,18 \pm 33,29$ in the control group of rats significantly reduced to $34,17 \pm 28,22$ in the group treated with 0,6 mg/kg/b.w. as well as disappearance of germinal centers $0,00 \pm 0,00$ in the group treated with 3 mg/kg/b.w. dexamethasone. The results obtained have shown that dexamethasone causes prominent atrophy of mesenteric lymph nodes.

P10)

HELMINTH TRICHINELLA SPIRALIS INFECTION - POTENT IMMUNOMODULATOR OF AUTOIMMUNE RESPONSE

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There are indications that, like other helminth infections, chronic *Trichinella spiralis* (TS) infection is associated with Th2 and regulatory responses that can enable long time survival of the parasite, restrict pathology and temper responses to third-part (non-helminth) antigens. Underlying mechanisms of immune response modulation are not clarified yet. We established model, in which chronic TS infection ameliorates, in a dose-dependent manner, the course of experimental autoimmune encephalomyelitis (EAE) in Dark Agouti (DA) rats. Rats were infected orally

with different doses of infective muscle larvae (TSL1, 100, 500, 1000, 2000, 5000), and were allowed to recover until day 28 post infection, before the induction of EAE. Among all applied doses of infective muscle larvae the dose of 500 TSL1 provided optimal effects on EAE: significantly reduced maximal severity score (2.7 ± 0.5 in infected rats vs. 3.1 ± 0.2 in control rats, $P < 0.05$), cumulative index (0.46 in infected rats vs. 0.89 in control rats, $P < 0.005$), duration of illness (6.4 ± 2.5 in infected rats vs. 12.2 ± 1.8 in control rats, $P < 0.005$). This model was used to investigate helminth-induced regulatory mechanisms that contribute to beneficial effects to the host. Cytokine profiles of TS infected and non-infected DA rats before and 8 days after EAE induction were analyzed by measuring lymph node cell cytokine production. The Th2 cytokine bias (increased levels of IL-4 and IL-10), observed in TS infected animals, was maintained even when EAE was induced. Adoptive transfer of spleen mononuclear cells from TS infected animals to animals 3 days after EAE induction significantly reduced the incidence and severity of the disease. This ability of infection-sensitized cells to transfer protective effects against EAE, together with TS induced production of IL-10, could indicate that helminth orchestrated regulatory cells are involved. (Grants No: 143047, 143029 and 145066)

P11)

FLOW CYTOMETRIC DETERMINATION OF PD-1+ AND TNF α + LYMPHOCYTES IN HIV-INFECTION

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Objectives: PD-1 receptor is inducibly expressed on CD4+T, CD8+T, NKT, B cells and monocytes.

The interaction of PD-1 with its ligands, transduces a signal that leads in an impaired ability of those cells to produce cytokines (termed "exhaustion") and also to survive and proliferate. The aim of this study was the development of a suitable flow cytometric TNF α staining method (surface or intracellular) and its correlation with lymphocytes PD-1 expression in HIV+ patients.

Methods: 17 HIV+ patients were studied (12 under HAART and 5 naïve for HAART). The percentage of PD-1+CD3+CD8- (PD-1+CD3+CD4+) lymphocytes, prior and after activation with PMA plus ionomycin, was assessed by direct surface staining method. The percentage of TNF α +CD4+ lymphocytes, prior and after activation with PMA plus ionomycin, was assessed by intracellular and surface staining methods. Data acquisition and analysis were performed on Epics XL MCL (Beckman Coulter) and FACSCalibur (Becton Dickinson) flow cytometers.

Results: A statistically significant negative correlation between the percentages of PD-1+CD3+CD8- lymphocytes (unstimulated) and TNF α +CD4+ lymphocytes was observed, with both staining methods ($R=-0.734$, $p=0.002$ for sTNF α and $R=-0.542$, $p=0.037$ for iTNF α). The TNF α staining methods showed high, statistically significant, correlation ($R=0.834$, $p=0.0001$) but surface staining showed significantly higher TNF α +CD4+ percentages than the intracellular one ($44,9\pm 15,4\%$ vs $27,8\pm 12,9\%$, $p=0.001$). Finally, no statistically significant difference was found between patients with and without HAART.

Conclusions: The expression of PD-1 receptor is negatively correlated with the expression of TNF α . The enhanced percentage of PD-1+ T-helper lymphocytes is correlated with the exhaustion of those cells to produce TNF α and the reduced percentage of TNF α +CD4+ lymphocytes. This is observed with both staining methods, although surface staining versus intracellular staining has some advantages for the determination of TNF α + cells such as higher percentage of TNF α + cells, less sample manipulation, time and cost of preparation.

P12)

EXPERIMENTAL DISSEMINATED ASPERGILLOSIS IN MICE

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Aspergillus fumigatus is the causative agent of aspergillosis defined as damage to the host tissue caused by invasion of tissues resulting in the diseases of variable severity. Our previous investigation demonstrated dose-dependent mortality of mice following intravenous application of $10(7)$ - $2 \times 10(7)$ conidia of *Aspergillus fumigatus*. In this study, dynamics of systemic response to i.v. administration of *A. fumigatus* conidia in female C57Bl/6 mice was evaluated by histopathological evaluation of lung, liver, kidneys, spleen, splenocyte proliferative characteristics and IL-17 production on the days one, three and seven following conidia inoculation. Dose of $10(6)$ conidia resulted in mortality in 20% individuals, with 100% survival following application of $10(5)$ conidia. Histological examination revealed presence of microabscesses in liver and peribronchial leukocyte infiltrates as early as one day following inoculation, while no changes were noted in kidneys. Increase in relative spleen mass was noted at dose of $10(5)$ at day seven and at dose $10(6)$ at days three and seven, with increased ex vivo spontaneous proliferation of splenocytes. Higher levels of proliferation in response to restimulation with heat-inactivated conidia by splenocytes from animals which received higher conidia dose was noted (compared to control animals), demonstrating responsiveness to antigenic restimulation. Levels of proliferation attained were lower in comparison to respective non-stimulated proliferation, suggesting presence of an inhibitory activity in these cultures. Absence of changes in metabolic cell viability in restimulated cells supports such an assumption. No IL-17 production was noted in the presence of conidia, but following

ConA stimulation at day seven by splenocytes from individuals which received 10(6) conidia. Presented data might aid understanding of mechanisms of survival vs fatal outcome in invasive aspergillosis and use of therapeutic strategies that improve survival or lessen the burden of pathology as well.

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P13)

SKIN ERUPTIONS CAUSED BY CMV INFECTIONS

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CMV is a group member of herpes virus and its features that after the first infection, to persist in tissues in a latent state during the whole life with the possibility of react. The skin eruptions are frequent rallies of viral infections. Symptomatic infections usually present at the newborns and to the people with compromised immunity. About 80% of the teenagers are infected by cytomegalovirus (CMV). It is supposed that the human being is the onliest reservoir of the human cytomegalovirus. It delivers during the intimate bodily liquids (saliva, the cervical secret, the seminal liquid, milk, feces, blood). There exist two vital periods during whom exists an appended risk for the infection: perinatal period and the reproductive period. The characteristic clinical rallies are: vesicles, urticaria, morbilliform exanthema, papules, petechies, ulcerations, dermatitis, interstitial pneumonia, myocarditis, pleuritis, arthritis, encephalitis, cervical lymphadenopathy, hepatosplenomegaly.

The scope of this presentation is to show the clinical rallies in the urticaria's form with the cytomegalovirus infections. There are introduced three cases at the children with intermittent chronic urticaria and exanthema morbiliforme, to whom is established by the TORCH test the presence of the CMV infection.

In 50% of the intermittent chronic urticaria cases,

it can not be attested the real aetiology and pathogenesis of the disease, therefore it might be in consideration the same aetiological factors, that provoke even acute urticaries, in particular the infective causatives (viral, bacterial, mycotic).

In cases of macular and urticarial changes, especially in the perinatal period, it might be investigated about the presence of the viral infections in order to define the causal therapy.

If the above note changes are escorted by the mononucleosis syndrome and cured by the penicillin's preparations (ampicillin) then, they are characteristics of the CMV infections.

P14)

DOES THE IMMUNOTHERAPY MODIFY NON-SPECIFIC INFLAMMATION TOO

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Introduction: Erythema multiforme is an allergic reaction to different triggers. Typically it starts with a red rash on palms, soles, and back of the hands, trunk, face and mouth. As main provocative causes viral, bacterial or fungal infections (including Mycoplasma pneumoniae, Herpes Simplex, Streptococcus), hypersensitivity to foods or drugs and immunisation have been implicated. Immunohistological analysis reveals granular deposits of IgM and Complement (C3 component) around superficial dermal blood vessels. Deposition of immune complexes (containing IgM) is seen in the superficial microvasculature of affected skin and oral mucous membrane.

Case report: Patient I.M., female 58 years old, admitted to our Clinic for ambulatory treatment with Dg Dermatitis cruris, Erythema regio faciei. She was hospitalized with suspicion of Erythema exudativum multiforme. Some of the laboratory findings were: ESR 30/66, RBC 5.21, PLT 246, Le 6.4, urea, creatinin, bilirubin, ALT and AST within normal limits. Protein C reactiv

= 2.40 (N=0.6), Rheumatoid factor= 12.0 (N=<12), ASTO=400 (N=200). She was previously tested with skin prick test and found to be positiv D. pteronyssinus and D. farinae, for which she was currently under treatment of Immuntherapy (Novo Helisen Depot D.pter.+D.farinae, Allergopharma, Germany).

Hallmarks of human allergic reaction are the activation of mast cells and basophils (IgE depend), and tissue eosinophilia in which cytokines play a major role. Following activation, Th1 cells produce interferon-gamma whereas Th2 cells produce mainly IL4, IL13 and IL5. IL4 directs B cell switching to IgE and IgG4. IL13 promotes B cell activation and IgE switch; promotes T cell growth and synergizes with IL3 for mast cell growth. IL5 is a major selective growth factor for the terminal differentiation, activation, and persistence of eosinophils in tissues. We hypothesize that immunotherapy modifies the nonspecific inflammation. The viral infections can precipitate an asthma attack through production of IL11 which is associated with bronchial hyper-reactivity. The IL11 further enhances IL 3 production and acts as mitogenic factor for plasma cells. Finally, IL3 promotes growth of early myeloid progenitor cells, eosinophils, mast cells and basophils and thus directs the immunologic response towards allergy.

P15)

PREVENTING ADVERSE OBSTETRIC OUTCOMES IN WOMEN WITH ACQUIRED AND INHERITED THROMBOPHILIA

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Inherited and acquired thrombophilia are associated with recurrent pregnancy loss. We have evaluated the efficacy and safety of the low molecular weight heparin (LMWH) and intravenous immunoglobulin (IVIg) during pregnancy for preventing recurrent pregnancy loss in women with adverse obstetrics outcomes.

Twenty one women with two or more pregnancy losses during the first and second trimester were treated during pregnancy. Fifteen of them (71.4%) are carriers of Factor V Leiden mutation, four (19.5%) – G202100A prothrombin mutation and two (9.5%) were homozygous for A2 allele of polymorphism A1/A2 in platelet glycoprotein IIb/IIIa. Thirteen (61.9%) of patients had positive antiphospholipid antibodies (anticardiolipin and/or anti-beta2-GPI).

Pregnant women with inherited thrombophilia received anticoagulant prophylaxis of LMWH - Enoxaparin 40mg/day during whole pregnancy. Women with combined acquired and inherited thrombophilic factors received additional therapy of intravenous immunoglobulin and aspirin 100 mg/day. Neither fetal losses nor fetal growth retardations were registered. All newborns were in the tenth centile or above. All babies were discharged in good clinical status. In the treated pregnancies, there were no pharmacological side effect or obstetric complications.

Both LMWH and IVIg are safe in preventing adverse outcomes in women carrying inherited or acquired thrombophilia with previous pregnancy loss.

P16)

PREVENTION OF PLATELET-LEUKOCYTE INTERACTION – SIGNIFICANCE OF LOW MOLECULAR WEIGHT HEPARIN FOR THE THERAPY OF PATIENTS WITH BOTH INFERTILITY AND THROMBOPHILIA

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Introduction: Platelet-leukocyte aggregates (PLA) are heterotypic cell complexes. In their limits the interaction between platelets and leukocytes influences on their function in the processes of hemostasis and inflammation. Increased PLA is detectable in patients affected by cardiovascular disease, cerebrovascular ischemia, diabetes, myeloproliferative disease, end-stage renal failure patients in hemodialysis, in patients with both infertility and thrombophilia. PLA may be important in thrombotic and inflammatory disease states like diagnostic marker and therapeutic target. Recent studies point towards the role of low molecular weight heparin (LMWH) on platelet-leukocyte interactions mediated by P-selectin.

The aim of this study is to investigate the role of PLA as a marker of thrombophilia in women carrying thrombotic risk factors such as inherited thrombophilia (MTHFR C677T, PTHR A20210G, Factor V Leiden polymorphisms) or acquired thrombophilia (antiphospholipid antibody) and affected by infertility as well as to trace the change of the levels of the PLA in the course of therapy with LMWH.

Patients and Methods: We selected 15 patients with infertility and with detected factors of thrombophilia. PLA were identified in whole blood by flowcytometry using CD45, CD14, CD61, CD41a, CD11b.

Results: PLA were increased in 13/15. In the course of therapy with LMWH was observed significant reduction of PLA (in 10/13).

Conclusion: Our results are in agreement with previous reports showing new mechanisms of antithrombotic effect of LMWH. LMWH has been shown to modulate the platelet-leukocyte interactions through several mechanisms: interference with P- and L-selectin – dependent cell adhesion, or prevention of platelet activation induced by proteases released from leukocyte. LMWH may inhibit the release of lysosomal enzymes and production of superoxide by activated leukocyte. This confirms that LMWH is

appropriate to be used in complex therapy of patients with both infertility and thrombophilia. Further studies are now required to assess the efficacy of LMWH on platelet-leukocyte interaction and the possible clinical benefit of these heparins on the inflammatory component of thrombotic disease, particularly inherited or acquired thrombophilia.

P17)

THE IMPORTANCE OF THE PRESENCE OF ANTISPERM ANTIBODIES IN SERUM AND EJACULATE OF MEN WITH INFERTILITY

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Introduction: There are multiple insults to the male genital tract that have been associated with an increased risk of antisperm antibodies (ASA) formation. The presence of ASA has been regarded as typical and specific for the immunological infertility. It has been suggested that the presence of agglutinated spermatozoa is suggestive of the existence of an immunological cause of infertility such as the existence of ASA.

Aim: The objective of this study was to determine the clinical significance of serum and seminal plasma ASA as well as whether varying degrees of sperm agglutination can be a predictive indicator of positive serum and/or seminal plasma ASA.

Patients and methods: 100 infertile and 30 fertile men were tested for ASA in seminal plasma and serum. The evaluation of patients included complete history, physical examination, scrotal ultrasound and semen analysis. ASA in serum and seminal plasma were tested by ELISA (Biosource, Belgium) and results were compared with the data of the semen analysis.

Results: 44 (44%) of the patients tested positive for ASA in seminal plasma, 9 of them had positive serum ASA. In the control group, seminal plasma ASA were not detected and two men (6.7%) tested positive for serum ASA. Correlation was established between seminal plasma ASA and two of the semen analysis markers: agglutination and increased viscosity.

Conclusion: ASA in seminal plasma are much more predictive than ASA in serum and have major role in the pathogenesis and diagnostics of male infertility.

P18)

ELASTIN TURNOVER MARKERS IN PATIENTS WITH RECURRENT PREGNANCY LOSS

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Problem: Elastin is one of the main structural proteins of the body and essential mechanical component of many tissues (arterial wall, skin, lung and uterus). During pregnancy the elastin content of the human uterus increases fourfold to fivefold. Antibodies to α -elastin (elastin breakdown product) and tropoelstin (elastin precursor) are found in the serum of healthy human subjects as a part of a homeostatic mechanism which clears altered elastin structures. A marked increase in anti- α -elastin IgG antibodies was found in patients with autoimmune diseases and their role in the pathogenesis of autoimmune alterations has been suggested.

Aim: The aim of the present study was to investigate the levels of anti-elastin antibodies (IgG and IgM) and elastin peptides (EP) in the sera of women with history of recurrent pregnancy loss (RPL, defined as two or more consecutive pregnancy losses), as an attempt to further explain the association between the activity of elastin turnover and RPL.

Method of Study: EA (IgG and IgM) and EP levels were measured by ELISA in serum samples of 15 female patients with RPL and 20 normal non pregnant women with a history of one or two successful pregnancies. One way analysis of variance (ANOVA) was used for statistical analysis.

Results: Serum anti-elastin IgG antibodies and EP were significantly higher in the study group compared to the controls. The levels of anti-elastin IgM antibodies did not show significant difference between the two study groups.

Conclusion: Serum anti-elastin IgG antibodies and elastin-derived peptides were significantly increased in patients with RPL. This data suggest an association between activity of elastin turnover and RPL. Further investigations are necessary to reveal the pathogenic role and the clinical relevance of elastin turnover (antibodies and peptides) for the pregnancy loss.

P19)

SERUM LEVELS OF ANTI-ELASTIN ANTIBODIES IN PATIENTS WITH RECURRENT PREGNANCY LOSS

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Introduction: Elastin is one of the main structural proteins of the body and essential mechanical component of many tissues (arterial wall, skin, lung and uterus). During pregnancy the elastin content of the human uterus increases four- to fivefold. Antibodies to α -elastin (elastin breakdown product) and tropoelstin (elastin precursor) are found in the serum of healthy human subjects as a part of a homeostatic mechanism which clears altered elastin structures. A marked increase in anti- α -elastin IgG antibodies was found in patients with autoimmune diseases and their role in the pathogenesis of autoimmune alterations has been suggested.

Aim: The aim of the present study was to investigate the levels of anti-elastin IgG and IgM antibodies (AEAbs) in the sera of women with history of recurrent pregnancy loss (RPL), defined as two or more consecutive pregnancy losses, as an attempt to further explanation the role of anti-elastin antibodies in RPL.

Method of Study: AEAbs (IgG and IgM) levels were measured by direct home-made ELISA in serum samples of 15 female patients with RPL and 20 normal non pregnant women with a history of one or two successful pregnancies. Results are expressed as means \pm SD and comparison between patients and controls were done using Post Hoc test - Least Significant Difference (LSD) with level of significance $p < 0.05$.

Results: Serum anti-elastin IgG antibodies were significantly higher in the study group compared to the controls. Anti-elastin Abs class IgM in the sera of the patients with RPL were non-significantly decreased compared to the levels, measured in the controls ($p = 0.073$), fact which was confirmed later in larger study groups.

Conclusion: Serum anti-elastin IgG antibodies were significantly increased in patients with RPL. Further investigations are necessary to reveal the pathogenic role and the clinical relevance of anti-elastin IgG antibodies for the pregnancy loss.

P20)

SIGNIFICANCE OF IMMUNOHEMATOLOGICAL MANAGEMENT IN PREGNANCY

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Fetomaternal hemorrhagias in pregnancy can activate the immune system in pregnant women to produce an Rbc alloantibodies, witch usually

leads to HDFN of various degrees.

Aim: To point the importance of immunohematological tests in first trimester of pregnancy.

Material and methods: 19 932 samples sierra of pregnant women (RhD positive and negative) were tested with screening tests of Rbc antibodies, in 10 years. The positive samples we tested on identification and quantification of the antibodies. The offspring's of sensibilisated women were tested on DAT, elution, identification and quantification of the alloantibodies and compare with clinical condition and therapy. 152 (72%) were anti-D alloantibodies with mild or siver HDFN . From non anti-D potential significant alloantibodies, 26 (40%) were from Rh system (C,c,E; 4 (3,1%)were from Kell system also with mild HDN. 35 (57%) were from other systems (Lea, Lab, I, M, Jkb), most of the them with non or moderate HDN. Management of sensibilisated pregnancy were provided in all women. There were 2 IUT, 1 plasmaferesis, 11 preterm delivers, 71 phototherapy and exanquine transfusion, and instead of that 14 foetuses and offspring died.

Conclusion: anti-D alloantibody is the most often antibody, but not the only one. Other antibodies can cause sever or moderate HDN and needs a special kind of management to prevent the complication of HDFN. The most important immunohematology tests in first 12 g.w. is blood group tipping and screening of Rbc alloantibodies in all pregnant women.

P21)

CHANGES OF THYMUS STRUCTURE OF MATURE WHITE RATS AFTER CYCLOPHOSPHANUM APPLICATION

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Introduction. All new facts about

immunopathogenesis of diseases, introduction of accessible methods of immunodiagnosis and immunocorrecting therapy come into the notice of doctors of different specialities. Last years immunotropic drugs are begun to use widely in general and pediatric practice, oncology, rheumatology, transplantology. The different displays of influences of damaging factors on the state of the immune system depend not only on force and on time of influence of factor but also from the state of organism protective forces. The last position is in a great deal related to the decline of immune defence of population because of worsening of the ecological state of our planet and considerable predominating among the microorganisms of their pathogenic or opportunistic forms. In accessible literature we did not find data about thymic morphoreactivity of animals after an experimental immunosuppression, because of what this problem causes considerable interest for a study. **Methods.** Research was carried out on 12 mature white rats-males in accordance to existing ethics norms during work with experimental animals. A single dose of cyclophosphanum (200 mgs/kg intramuscular) was administered. Thymus was extracted, weighed, exposed to the standard histological method after the finish of the experiment on 15 days of supervision. **Results.** Absolute mass of experimental rats' thymus was on 11,50% less than control value, relative mass of thymus in the group of intact animals exceeded the analogical index of rats of experimental group on 13,56% ($p < 0.05$). For the study of functional activity of organ a width and area of thymus cortex section was studied. These indexes on 46,21% and 18,93% accordingly were less than data of control group ($p < 0.05$). **Conclusions.** Thus, on the early terms of supervision thymus showed the high degree of reactivity after application of cytostatic, that showed up the development of involutive processes in an organ.

P22)

ANTIMITOCHONDRIAL ANTIBODIES OF IG G, IG M AND IG A ISOTYPES IN PRIMARY BILIARY CIRRHOSIS AND NON-HEPATIC AUTOIMMUNE DISEASES

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Introduction: The antimitochondrial antibodies (AMA) are one of the main immunological diagnostic parameter in Primary biliary cirrhosis (PBC). In spite of that the correlation between various isotypes of AMA and development of PBC or other autoimmune diseases is not well clarified. The aim of this study was to determine AMA titers of IgG, IgM and IgA class in two groups of patients - with PBC and with non-hepatic autoimmune disorders (NHAID), selected on the basis of previously proven positive AMA with non-defined Ig isotype in their sera.

Method: AMA titers of IgG, IgM and IgA isotypes in patients sera (10 with PBC and 10 with NHAID) were determined by a sensitive immunofluorescent assay using the serum-free McCoy-Plovdiv cell line and anti-Ig FITC conjugates.

Results: AMA of all three isotypes – IgG, IgM and IgA, were detected in high titers ($\geq 1/320$) in all sera from patients with PBC. In NHAID AMA-IgG, M and A at high titers were found in 60% of the patients and at lower titers ($\leq 1/40$) – in 40%. The fluorescent pattern was presented as granular or filamentous bright-green staining in the cytoplasm of serum-free cells. However, there was no significant difference between diagnostic sensitivity of the IFA-AMA test in terms of specific Ig class for both diseases ($p > 0.05$).

Conclusion: Our study implies that in patients with PBC and NHAID all three Ig isotypes participate in AMA production and the Ig class of AMA cannot be diagnostic criterion for these disorders.

P23)

IL-10 AND IL-12 RELATED CYTOKINE GENE EXPRESSION IN COLORECTAL CARCINOMA

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Colorectal carcinoma (CRC) is a common malignancy with high mortality rates worldwide. It is generally believed that cancer development requires the suppression of host-immune response and that cytokines play a central role in regulation of the anti-tumor immune response. IL-12p70 (IL-12p35/p40) exerts antitumor activity through activation of innate and Th1 adaptive immunity, while the effect of IL-23p19/40 on tumor development and progression is still in debates. On the other hand, IL-10 is the most potent inhibitor of IL-12 and IL-23 synthesis and suppresses the Th1 immune response.

Therefore, the aim of our study was to characterize the IL-10, IL-12p40, IL-12p35 and IL-23p19 cytokine expression in human normal and tumor tissues and their expression in patients' blood.

Paired human tumor and normal adjacent tissues were obtained from six patients undergoing routine therapeutic surgery. A venous blood sample from the same six patients was taken before surgery.

Blood samples from nine healthy donors were used as controls for cytokine expression. After total RNA extraction from tissue and blood, the samples were reverse transcribed and the level of gene expression was detected by quantitative real-time polymerase chain reactions (qRT-PCR) using TaqMan assay. qRT-PCR data were analyzed using the Relative Expression Software Tool (REST).

We didn't observe any significant differences in the relative expression of detected cytokine genes

in peripheral blood between CRC patients and control group. These results indicate that expression of IL-10, IL-12 and IL-23 mRNA on systemic levels are not indicative for colon cancer development in humans. However, we found substantial upregulation of the mRNAs encoding IL-10 and both subunits of IL-23 (p40/p19), but not of mRNA encoded IL-12p35, in tumor tissue. The expression of IL-23p19 mRNA was increased in the highest level (20.706 fold; p=0.002), followed by IL-10 mRNA (6.634 fold; p=0.002) and IL-12p40 mRNA (3.933; p=0.038) in tumor tissue compared to normal adjacent tissues. No significant difference of IL-12p35 mRNA expression in tumor compared to normal tissue was detected.

Our results indicated that local IL-10 and IL-23 expression probably regulates the tumour immune surveillance and had significant impact in promoting development of colorectal carcinoma.

P24)

AUTOANTIBODIES TO CYCLIC CITRULLINATED PEPTIDE AND RHEUMATOID FACTORS IN THE DIAGNOSIS OF RHEUMATOID ARTHRITIS

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Introduction: Rheumatoid arthritis (RA) is a common rheumatic disease of uncertain etiology. The rheumatoid factor (RF) that is one of the American College of Rheumatology classification criteria for RA, has a low sensitivity and specificity for this disease. Many studies have recently shown that the antibodies to cyclic citrullinated peptides (CCP) are highly specific serological markers for RA and are thought to be directly involved in the disease pathogenesis. Material and Methods: In order to evaluate the diagnostic value of anti-CCP and RF in RA we examined 49 patients sera coming from university rheumatological clinics with clinical diagnosis of RA, 3 with other rheumatic diseases and 11 controls. The RA patients were regrouped in

categories of adults (n = 33, mean age 47.8, female 24 /male 11) and of pediatrics (n =16, mean age 8.5, female 11 / male 5). The determination of antibodies to CCP was made by quantitative ELISA kit and the RF by laser nephelometry. Results: The control sera and the sera from other rheumatic diseases resulted negative for antibodies to CCP (specificity 100 %). There were no positive cases for CCP in the group of children with RA while the positivity for the RF was 18.7.2% (3/16). In the RA adults group the positivity for RF was found in 36.3% (12/33) and for antibodies to CCP in 24.2% (8/33) cases. 18.1 % of adult RA patients had double positivity tests (RF and anti-CCP). The rate of positivity were very high for anti-CCP (means value 859.3 UI/ml). Conclusion: In our study we confirm that there is a difference for sensitivity of the anti-CCP test in the childhood and adults form of RA, but this did not reach the statistical significance (p=0.082).. The sensitivity for anti- CCP was lower (24.2%) than in other studies, but this fact is to be appreciated with more large imunological and genetic studies and more accurate clinical criteria for RA.

P25)

CHARACTERISTICS OF LOCAL PULMONARY RESPONSE FOLLOWING INTRANASAL APPLICATION OF ASPERGILLUS FUMIGATUS CONIDIA IN RATS

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Majority of Aspergillus infections/diseases (aspergilloses) are caused by opportunistic fungi *A. fumigatus* which conidia enter the respiratory tract by inhalation and cause the infection. Pulmonary aspergillosis is most common and involves allergic bronchopulmonary aspergillosis,

aspergilloma and invasive pulmonary aspergillosis. With increasing numbers of immunocompromised and susceptible hosts, interest in aspergillosis arose. In almost all investigations of *Aspergillus fumigatus* infections immunocompromised animals were used. The aim of this study is to get initial data about local host lung immune response in a model of experimental pulmonary infection in immunocompetent rats. Development of response was monitored by measuring the presence of *A. fumigatus* conidia in homogenates of lung tissue by growth on selective Sabouraud medium on days one, seven and fourteen following intranasal conidia inoculation and by histological evaluation of pulmonary tissue. Lung response to conidia inoculation was evaluated by measuring nitric oxide (NO), IL-6 and IL-17 production by pulmonary cells obtained by collagenase/DNase digestion and by IL-17 production by draining pulmonary lymph nodes at day one. Mycological examination revealed no presence of conidia in lung tissue homogenates of inoculated animals. Mixed leukocyte infiltration was noted in lung tissue, with prevalence of neutrophils at day one and of lymphocytes at later time points following conidia inoculation. Increased spontaneous and LPS-stimulated NO and IL-6 production was noted by cells from digested lung tissue, while no difference was noted in IL-17 production by pulmonary cells. Increase in production of this cytokine was detected by draining lymph node cells following stimulation with ConA. Pulmonary response to intranasal *A. fumigatus* conidia administration is probably responsible for lack of pulmonary infection. Characterization of this response pave the way to investigation of factors/states which compromise it.

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P26)

BIOLOGICAL MARKERS OF INFLAMMATION IN PERIPHERAL BLOOD DURING TREATMENT WITH INHALED CORTICOSTEROIDS IN PATIENTS WITH BRONCHIAL ASTHMA

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Asthma is a chronic inflammatory disorder of the airways in which many cells play a role, in particular mast cells, eosinophils (Eo), and T lymphocytes. Eosinophils have an important role in the pathogenesis of bronchial asthma (BA). The following of their count and mediators in peripheral blood and BAL could be employed as a marker of the inflammation in asthmatic patients.

We followed 30 patients with BA, treated with inhaled corticosteroids (IKS) (Spray Beclomethasone dipropionate) in doses of 1000-2000 µg per day.

We followed the Eo count, ECP and IL-5 in peripheral blood at the beginning of the study, after 2 and 6 months of the anti-inflammatory therapy. We found a decrease of the Eo count from $292.66 \times 10^6/l$ to $270 \times 10^6/l$, serum level of ECP from 22.92 to 19.36 mg/l, and serum level of IL-5 from 44.94 to 37.84 pg/ml at the first control (after 2 months), and Eo count to $224.33 \times 10^6/l$, ECP to 14.47 mg/l, and IL-5 to 30.44 pg/ml, 6 months from the treatment.

The analysis of variance showed statistical significance of $p < 0.05$ for this reduction, which helps us conclude that inhaled corticosteroids decrease the inflammation in BA and the eosinophils, ECP and IL-5 are just another parameters for a follow up of this effect.

P27)

AGE RELATED CHANGES OF ANTI-ELASTIN ANTIBODIES IN ICR MICE

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Introduction: Elastin is one of the long-lived connective tissue proteins and characterizes with very slow turnover. Anti-elastin antibodies were registered in human sera of healthy subjects and in the sera of patients with diabetes, connective tissue diseases, and atherosclerosis. To address the changes of the elastin with age, we investigated anti-elastin antibodies (AEAB) in sera of ICR mice at 3, 9 months. This work is a part of an international research project, targeting the aging of elastic tissue, and contributed by the European Community. ICR albino mice are used as a control group in investigation of age-related changes in senescence accelerated mice (SAM P8).

Methods: AEAB were assessed with direct ELISA in sera of mouse at 3 and 9 months.

Results: AEAB were present in all the sera tested. All the groups showed statistically significant differences. But the highest levels were observed in 9 months old mice. In 3 and 9 months old groups female showed higher level than male.

Conclusion: AEAB are in direct ratio with age. Aging is a complex process involving multiple tissue and organs. Aging of elastic tissue could be partially assessed by measuring of AEAB.

P28)

ANTI-AGE ANTIBODIES AND AGES IN ICR MICE

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Introduction: Advanced glycation is a major

pathway for the posttranslational modifications of tissue proteins and begins with the non-enzymatic addition of sugars to the primary amino groups of proteins. It has been implicated in the pathogenesis of many of the sequels of diabetes and normal aging. Excessive accumulation of AGEs on tissue proteins changes their structure respectively function and none of the last place immunogenicity. Glycated proteins form common immunological epitopes which raise formation of population of anti-AGE antibodies (AGE AB).

Methods: To address potential correlation between AGE AB and level of glycation of connective tissue protein elastin we investigated ICR mice at 3, 9, and 20 months. Determination of anti-AGE antibodies in mouse sera was done by direct ELISA with glycated KLH as antigen. AGEs in the mouse aortic α -elastin was determined as an index of advanced glycation. Fluorescence of the samples was measured in 360 nm excitation and 450 nm emission.

Results: The level of anti-AGE antibodies was in direct ratio with age of investigated animals. They increase with age. The same relation was observed for AGEs in the mouse aortic α -elastin.

Conclusion: Increasing of AGEs with age is due probably of an increasing amount of the primary amino groups involved in AGEs cross-links. In pathology or aging conditions, when non-enzymatic glycation of proteins is increased, the capacity of normal homeostasis seems to be inefficient. This way AGEs are accumulated and contribute development of long-term aging process.

P29)

FEATURES OF ULTRAMICROSCOPIC STRUCTURE OF SOMATOTROPS OF AD-ENOHYPOPHYSIS OF RATS AT EXPERIMENTAL IMMUNOSUPPRESSION

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Introduction: There are bidirectional communications between endocrine and immune systems. Hormones affect immune functions and, in turn, immune responses are reflected in neuroendocrine changes. The hormones of anterior lobe of pituitary gland are able to change activity of metabolism and function of different immune cells. Several reports support the view that growth hormone promotes proliferation and cytotoxicity by T cells. Industrial regions are most ecologically soiled on territory of many countries, that serves as reason of formation of immunosuppression.

Description of the method used: Research was carried out on 18 mature white rats-males. A single dose of cyclophosphanum (200 mg/kg intramuscular) was administered to experimental animals. Rats were taken out from experiment in 1 and 30 days. The structure of growth hormone cells of pituitary gland at experimental immunosuppression was assessed by electron microscopy.

Results: In the somatotropes of pituitary gland of rats in 1 day after administration of cyclophosphanum there are the morphological signs of increase of functional activity, such as the hypertrophy of mitochondria, rough endoplasmic reticulum and Golgi complex. Active forms prevail among them. Ultramicroscopic research of growth hormone cells in 30 days reflects the decrease of its secretory activity. Condition of nuclei, organelles, volume of cytoplasm approach such for the control animals.

Conclusion: This study shows the considerable changes of ultrastructural components of growth hormone cells of pituitary gland, that testify increase of their secretory activity in the early term of experiment, and decrease of activity in late term as a result of development of dystrophic processes of cellular structures.

P30)

EXPLOITATION RESULTS OF MISIMUNOLOG

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Systemic lupus erythematosus (SLE) is a widespread disease with unknown cause, which attacks person's immune system and injures the body's own organs and tissues.

The software solution "Imunolog", presented in this paper, is developed after winning international project between University Hospital Alexandrovska, New Bulgarian University and Barcelona Medical University.

The fundamental initiative is to localize the main predispositions for this disease based on the statistic data from Bulgarian patients, the relationship between treatment and following manifestations of this disease, sequence of results from a concrete therapy and human body systems reactions and finally adaptation. In Bulgaria there are no investigations and results, regardless of the enormous paper database.

We have addressed this problem and devised a software system for assessing both current lupus disease activity and changes in that activity since the patient's last visit. Imunolog[®] is retrospective database for explore causes, treatment schemes, manifestations and reactions of all available patients in Bulgaria since 1960.

Studies of the natural history of the disease and the effect of therapeutic interventions have been hampered by the lack of a satisfactory method of assessing disease activity.

Imunolog[®] is organized collection, for storage and presentation of medical data and other knowledge for decision making, progress reporting, and for planning and evaluation of programs.

In this paper we are going to present statistics preview of the following parameters:

1. ARA criteria
2. Activity criteria
3. Men/Women
4. Treatment scheme and medications
5. Age at the beginning of the disease

For one year period we have entered 100 patients with approximately 10 visits each, we have

developed an English version of the software and educational on-line platform for assistance from distance to the medical staff that works with the software.

P31)

EVALUATION OF ALLOIMUNIZATION TO KEL1 ANTIGEN OF THE KELL BLOOD GROUP SYSTEM

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Introduction. The Kell blood group system, especially KEL1 antigen, is very important from a clinical point of view, since the corresponding antibody is involved in haemolytic transfusion reactions and in haemolytic disease of the newborn. The KEL1 is very immunogenic and with the frequency of 9.0% in Whites.

Aim. Evaluation of KEL1 alloimmunization in transfused patients and the need of routine KEL1 typing of blood donors.

Material and method. We evaluated data from pretransfusion testing (ABO and RhD blood group typing, irregular red blood cell antibody screening and compatibility testing-cross match), antibody identification and KEL1 antigen typing in the period from January, 2005 to May, 2008.

Results. Antibody screening and/or cross match were positive in the sera from 128 transfused patients (88 - 68.7% were female and 40 - 31.2% were male). Anti-KEL1 antibodies were identified in 14 (11%) of the total of 128 patients. All of the patients with anti-KEL1 antibody were negative when typed for the corresponding antigen. 13 (10.15%) of the antibodies were identified in alloimmunized female and only 1 (0.78%) antibody in alloimmunized male.

Conclusion. Having in mind that the KEL1 is a low frequency antigen, we found high incidence of anti-KEL1 antibodies in our group of transfused patients. We also found that the

alloimmunisation to KEL1 antigen and in general was significantly higher in female transfused patients. According to the data, the routine typing of KEL1 antigen in blood donors is necessary to prevent KEL1 alloimmunization in transfused patients.

P32)

KIDNEY TRANSPLANTATION FROM LIVING –NONRELATED DONOR, SINGLE CENTER EXPERIENCE

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AIM: In order to increase the number of kidney transplantations (KTX) in our center we started with transplant program from unrelated but emotionally related-spousal donors. Objective of this study is to evaluate safety and efficacy of these transplantations.

METHOD: Since May 1996 to January 2007, we performed eight (4 males and 4 females) KTX from spousal donors, with: mean age of 51 ± 12 years and with matching of 25 to 75%. Cross match was negative except in one patient (pt) who had positive cross match in the time of hospitalization. After treatment with selective IgG immunoadsorption, she became negative prior to transplantation. One pt got kidney graft across blood group barrier (B+ on O+). Isoagglutinin antibodies were removed with original method (plasma exchange and original selective immunoadsorption), rituximab and triple immunosuppressive therapy. All patients had triple maintenance immunosuppressive therapy: prednisone+mycophenolic acid+calcineurine inhibitor. In the follow up of mean 87 ± 52 month, we noticed: surgical complications, episodes of acute rejection, reactivation of CMV infection and serum creatinine.

RESULTS: One donor postoperatively had *Serratia* sepsis, successfully treated with antibacterial therapy. 1 recipient had surgical bleeding, delayed graft function, acute rejection,

hemolytic anemia and reactivation of CMV infection. Two recipients had early steroid sensitive acute rejection and one had late rejection after conversion from tacrolimus to sirolimus. One pt due to decompensate HBV positive live cirrhosis had pre terminal graft failure. 1 patient, with matching of only 25%, have serum creatinin little above $200 \mu\text{mol/L}$. Mean sera creatinin in this pts is $120 \pm 48 \mu\text{mol/L}$.

CONCLUSION: with exception of one pt, all patients had stabile graft function in follow up including transplantations with high immunological risk.

P33)

SIROLIMUS AS A PART OF RESCUE IMMUNOSUPPRESSIVE PROTOCOL IN KIDNEY ALLOGRAFT RECIPIENTS WITH MILD OR MODERATE RENAL FAILURE - SINGLE CENTER STUDY

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This study was designed to investigate efficacy and safety of conversion from calcineurin inhibitors (CNI) to sirolimus (SRL) in patients (pts) with mild or moderate kidney allograft failure. Study included 30 pts, who were converted from CNI to SRL in the period 2003 – 2006 and followed 23 ± 10 months. We monitored: kidney function, proteinuria, lipidemia, blood cells count, antihypertensive and anti-hyperlipidemic therapy, cytomegalovirus (CMV) infection, pts and allograft survival, episodes of acute rejection (AR), cardiovascular incidents and new malignancies. To the end of follow up 10 pts continued with SRL. 20 pts ceased sirolimus due to: sudden death (2pts), reintroduction of CNI (12 pts), beginning of hemodialysis (4pts) and switching to

double immunosuppressive protocol (2pts). After conversion to SRL we haven't noticed new episodes of AR, myelotoxic effects and new malignancies. 10 pts reactivated CMV infection. Antihypertensive therapy was the same. The doses of anti-hyperlipidemic agents were increased. Pts with SRL in therapy improved graft function, but increased hyperlipidemia and proteinuria. Pts switched back to CNI compared with pts on SRL, had worse renal function and higher proteinuria at start. Conversion from CNI to SRL was safe. Better results showed pts with better allograft function and proteinuria below 500 mg.

P34)

ORIGINAL METHOD FOR REMOVAL OF ANTIBODIES IN KIDNEY TRANSPLANTATION ACROSS BLOOD GROUP BARRIER - ONE YEAR EXPERIENCE

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Background: Objective of this study is to present efficacy of our original method for removal of anti-donor blood group (BG) antibodies (abs) from the blood in ABO incompatible kidney allograft recipients. Method: From April-2006 to May-2007 eleven patients were transplanted from living donors. Titers of anti-donor BG abs were in class Ig G 4 – 128. Immunosuppressive therapy was started on day -14 with rituximab, followed by triple therapy on day -7 (prednisone + tacrolimus + mycophenolic acid) and first plasma exchange (PE) procedure in which 1 plasma volume was removed and substituted with albumins and saline. In selective extracorporeal immunoadsorption (SECIA), removed plasma is mixed with donor blood type RBC, centrifuged and supernatant separated. In the second PE procedure, removed plasma is replaced with immunoadsorbed plasma. In the follow up, we monitored titer of anti-donor BG abs, renal allograft function, rejection and infections. Results:

3 pts had an early acute rejection (AR), 1 pt early AR and hemolytic anemia, 2 pts had surgical complications and one of them lost graft function. Titer of anti-donor BG abs was below 4. Mean serum creatinine was 140±33 µmol/L. Conclusion: Our protocol for kidney transplantation across blood groups was effective and safe.

P35)

DEFICIENCY OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) IMPAIRS MURINE AUTOIMMUNE DIABETES

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Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine of the innate immune system that plays a major role in the induction of immunoinflammatory responses. Using a model of immunoinflammatory diabetes induced in susceptible mouse strains by multiple low doses of streptozotocin (MLD-STZ) we have recently shown that *in vivo* abrogation of MIF activity with pharmacological MIF inhibitor (S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxasole acetic acid methyl ester, or with neutralizing anti-MIF antibodies, markedly reduced clinical and histopathological features of diabetes. Furthermore, MIF-deficient (MIF-KO) mice were also less susceptible to the MLD-STZ-induced hyperglycemia, insulinitis and apoptosis within the endocrine pancreas than genetically matched wild type (WT) C57BL/6 mice. In all three *in vivo* approaches tested, negating the action of endogenous MIF has induced an immune deviation towards protective type 2/3 response. To further dissect the role of endogenous MIF in the pathogenesis of diabetes at the level of target tissue, we used *in vitro* approach and found that MIF-KO beta-cells or pancreatic islets were more resistant to killing by cytokines (IL-1+TNF-

alpha+IFN-gamma) or STZ (2 µg/ml) than WT counterparts. Furthermore, while cytokine-stimulated WT pancreatic islets showed significant NF-κB activation (judged by I-κB phosphorylation), MIF-KO islets had lower activity of NF-κB. Finally, in STZ-stimulated MIF-KO islets both IL-1α mRNA expression and secretion were significantly down-regulated in comparison to the elevation seen in STZ-stimulated WT islets. Our findings clearly show beta-cell protection from different diabetogenic agents, namely cytokines and STZ, in the absence of MIF activity and agreement between the *in vitro* and *in vivo* observations.

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P36)

ALLELE FREQUENCY OF HLA-DQB1 LOCUS IN MACEDONIAN POPULATION

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The aim of the study was to analyze the allele frequency of HLA-DQB1 locus in Macedonian population. METHOD: HLA DNA typing for HLA-DQB1 genes was performed on 217 samples from healthy unrelated Macedonian volunteers. The DNA samples were obtained from the Macedonian DNA Bank (genomic DNA isolated following the standard protocol for phenol-chloroform DNA extraction). HLA typing was performed using the Reverse Line Strip (RLS) method consisting of PCR amplification of exon 2 of HLA-DQB1 genes, followed by hybridization. The statistical analyses was performed by using the Arlequin Software.

RESULTS We have identified 33 different alleles of HLA-DQB1 in Macedonian population, 12 of which were unambiguous with the frequency of 40.55%, and 21 were ambiguous with the frequency of 58.53%. We did not found any geno-

typic ambiguities in HLA-DQB1 locus. The highest frequency (33.64%) was found for 05 unambiguous groups, and for 03 ambiguous groups (36.40%). The highest allele frequency in Macedonian population for HLA-DQB1 was found for allele 03NX (030101/0102/09/13) with 27.88%, followed by allele 0502 with 13.82%, and 02MN (0201/02/03) with 10.37%.

P37)

HLA COMPATIBILITY IN 200 PATIENTS WITH RENAL TRANSPLANTATION

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Introduction: 200 renal transplantations in our Dep. for Urology, Clinical Centar were prepared from 1977 till today. 183 were living donor transplantations, 175 of which were related and 8 were non-related. 17 were kadaver donor transplantations.

Many studies of the HLA system showed that the HLA antigens have the key role in the direct and indirect recognition of nonself antigens and survival of the graft.

Objective: To determine the immunogenetic characteristics of the donors and the recipients in renal transplantation.

Material and method: Determination of the immunogenetic characteristics of the donors and the recipients were prepared with ABO typing and HLA serological typing of the A and B loci. DR antigens were studied in 50 living donor and in 15 cadaveric transplantations. Presence of HLA antibodies and their specificity was identified in all recipients with microlymphocytotoxic assay. Cross-matches were regularly done using recipient's sera from different time period. Immunosupresion consisted of corticotherapy, imuran (cell cept in the last 5 years), cyclosporin A and daclizumab. All cadaveric renal transplantations were prepared from 1985 till 2006.

Results: Most of transplanted patients were

HLA haploidentical with their donors with graft survival of 1, 3 and 10 years. Pairs with 3 identical and 1 incompatible HLA antigens between the donor and recipient had 3, 5 and over 10 years of graft survival. Besides existing erythropoietin programme there were polytransfused patients with HLA alloimmunisation which could have influence on graft survival. 92 (64.79%) transplanted kidneys are still in function. The biggest number of patients (50.70%) with functional transplanted kidneys was from haploidentical group of patients with their donors. Mismatch group of patients/donors were transplanted in the last 3-4 years.

Conclusion: HLA relation and compatibility between donor and patient is of a great importance for graft survival, immunologic tolerance and longer function of transplanted organ.

P38)

DIAGNOSIS OF THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP)-CASE REPORT

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Introduction. TTP is a rare disease with the incidence among general population being 5-10 cases per year per million people. It occurs predominantly in healthy individuals as an acute idiopathic form and also the agents triggering the episode have not yet been identified.

Aim. The aim of this study is to present the difficulties involved in the diagnosis of TTP especially when there is no possibility to check the plasma levels of specific proteases. **Methods.** 56 years old, previously healthy man was brought at the hematology ward with intensive yellow color of the skin, clinical signs of anaemia and pains all over the body. Complete clinical, especially neurological and laboratory examination was performed. Lactate dehydrogenase (LDH) was closely monitored. Specific laboratory in-

vestigations such as peripheral blood smear and immunohaematologic tests (DAT-direct antiglobulin test and antibody screening tests using indirect antiglobulin test, performed with microgel column agglutination method - DiaMed). Coagulation status (PT, APTT and TT) was also screened.

Results. Clinical examination revealed signs of anaemia and neurological symptoms (motor deficit and headache). Hematogram showed reduced number of erythrocytes ($1.8 \times 10^{12}/l$ with Hb level of about 50g/l and thrombocytes about $110 \times 10^9/l$). The signs of haemolytic anaemia were as follows: peripheral blood smear revealed typical findings of mechanical fragmentation of red cells (schistocytes); the serum levels of indirect bilirubin were increased, as well as reduced levels of haptoglobin; high serum levels of LDH being 1200 U/l versus the normal range from 213 to 423 U/l. DAT and antierythrocyte antibody screening were negative. There were no alterations in coagulation and not significantly reduced number of thrombocytes. Also there were only mild changes in renal function, with moderately increased levels of serum creatinine. The patient was sent abroad for further investigation and treatment.

Conclusion. The diagnosis of TTP was not confirmed although the presence of Coomb's negative haemolytic anaemia caused by mechanical red cell fragmentation, neurological findings which prevailed over the renal and all other clinical and laboratory findings that were characteristics for TTP. The patient benefited from the treatment with plasma infusions and plasma exchange. The final diagnosis was haemolytic anaemia induced by viral infection.

The description of the key role of UL VWF (Ultra large multimers of von Willebrand Factor) in the pathogenesis of TTP and the subsequent identification of ADAMTS13 (a disintegrin and metalloprotease with thrombospondin 1 repeats) clarified the mechanisms leading to the therapeutic efficacy of plasma treatment.

P39)

IMMUNONEPHELOMETRY AND REVERSE HYBRIDIZATION GENOTYPING IN DIAGNOSIS OF ALPHA-1-ANTITRYPSIN DEFICIENCY

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Alpha-1-antitrypsin (AAT) is a protease inhibitor, deficiency of which is associated with emphysema and liver disease. Its important role is to protect the lung tissues against the proteolysis, since it is one of the few enzymes that can inhibit neutrophil elastase. The most cases of AAT deficiency are caused by homozygosis for the deficient allele PIZ or by heterozygous combination of the 2 most common deficient alleles, PIS and PIZ. A diagnosis in the case of a suspicion of AAT deficiency is carried out by measuring the alpha-1 antitrypsin level in blood and by genotyping of alpha-1 antitrypsin allele.

At Institute for Immunobiology and Human Genetics, part of the Faculty of Medicine in Skopje, in the last 7 years, total of 361 patients with suspected AAT deficiency were referred for analysis of AAT concentration using nephelometry (Dade Behring). The vast majority (88.1%) were found to be normal (range 1.37-1.41), in 8.03% lower than normal AAT levels were determined (range 0.70-0.83) and in 3.88% concentration above the normal levels was seen (range 2.28-2.48). The WHO protocol recommends subsequent AAT genotyping of individuals with AAT deficiency at protein level. Here we present a case in which homozygous presence of deficient PIZ allele was determined in a patient deficient for the AAT protein using reverse hybridization method.

The importance of early diagnosis or diagnosis early in life, resides in the possibility of smoking cessation and treatment of pulmonary disease which could significantly decrease the morbidity

associated with this chronic disease. We hope to contribute with this case report to raising the awareness of this genetic disease, and also to inform the medical community in Macedonia of the possibilities of exact diagnosis.

P40)

ASSESSMENT OF THE PHAGOCYTTIC FUNCTION AND OXIDATIVE BURST IN BLOOD LEUKOCYTES BY FLOW CYTOMETRY

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Phagocytosis by polymorphonuclear neutrophils and monocytes constitutes an essential arm of the first-line defense against bacterial or fungal infections. The phagocytic process consists of several subsequent steps such as: chemotaxis, attachment of particles to the cell surface of phagocytes, ingestion and intracellular killing. The later involves oxygen-dependent mechanisms (including production of reactive oxygen species) and oxygen-independent mechanisms (mainly due to proteolytic enzymes such as defensins, lysozyme and cationic proteins). Defective or deficient response of the phagocytes due to inborn or acquired defects can result in increased susceptibility to infection. These include chronic granulomatous disease, actin dysfunction, tuftsin deficiency and complement receptor C3bi deficiency, as well as trauma, diabetes, renal failure or infection itself.

Phagotest and Phagoburst are two functional tests, examining the phagocytic function of granulocytes and monocytes and quantitatively determine the leukocyte oxidative burst in heparinized whole blood, respectively. In the former, the percentage of phagocytes which have ingested FITC labelled E.coli at 37°C is calculated against negative control incubated at 0°C, while in the later, for quantitative determi-

nation of leukocyte oxidative burst unlabeled opsonized bacteria (E.coli) and several cell stimulants are used.

We present the results for quantitative determination of leukocyte phagocytosis and oxidative burst performed at the Institute of Immunobiology and Human Genetics, Faculty of Medicine in Skopje and the optimized protocol for the functional tests when using the CyAnADP analyzer (Beckman Coulter, formerly DAKO).

P41)

FAMILIAL MEDITERRANEAN FEVER – CASE REPORT

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Aim: Autoinflammatory disorders are a group of eight hereditary autoinflammatory syndromes characterized with recurrent self resolving attacks of systemic inflammatory reactions which are usually accompanied by associated symptoms and signs. The most common is Familial Mediterranean fever, an autosomal recessive disorder due to MEFV mutation.

Case report: 10 years old girl was admitted because of febrile attacks, erythematous rash on lower limbs, musculoskeletal pain and swelling of the left ankle joint. His father has some lumbosacral problems and her older brother recurrent inflammation of hip. The febrile attacks were one day duration with chills, recurred every day, followed by positive inflammatory answer. The girl was treated with antibiotics and non steroid anti-inflammatory drugs. The molecular diagnosis of heterozygous E148Q mutation was confirmed at the Institute of immunology and Human genetics. Specific therapy with Colchicine was started and the symptoms were resolved.

Conclusion: Familial Mediterranean fever is an inherited multisystem disease manifested with recurrent fever attacks, recurrent painful attacks affecting abdomen, joints and often accompanied with skin rash. A through diagnosis is important because of specific therapy with colchicine that can prevent development of amiloidosis.

P42)

THE EFFECT OF IL-12 FAMILY CYTOKINES ON DIFFERENTIATION AND MATURATION OF HUMAN MONOCYTE DERIVED DENDRITIC CELLS *IN VITRO*

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Introduction: Dendritic cells (DC) are antigen-presenting cells that have a prominent function in the induction of antigen-specific cell-mediated immunity. Recently, it has been known that the IL-12 family of cytokines (IL-12, IL-23 and IL-27) plays a significant role in Th polarization and function of T-cells. However, little is known about IL-12, IL-23 and IL-27 effects on DC. Therefore, the aim of this study was to investigate the effect of these cytokines on the differentiation and maturation of human monocyte derived dendritic cells (MoDC). **Methods:** Monocytes were purified from the *buffy coats* of healthy volunteers and cultured for 5 days in the presence of GM-CSF and IL-4. At day 6, IL-12, IL-27 or IL-23 was added and maturation of DC was induced by additional cultivation of the cells for 24h in the presence of LPS. The differentiation and maturation status of MoDC were determined by analysis of their phenotypical properties, allostimulatory capacity and Th profile of allogeneic CD4⁺ CD45RA⁺ T-cells. **Results:** The addition of IL-27 on the immature MoDC resulted in an increase in the expression of CD86 but not CD83, a DC maturation marker. CD4⁺ CD45RA⁺ T-cells in coculture with IL-27 treated MoDC produced higher levels of IL-2 and IL-17 and lower levels of IFN- γ , IL-4 and IL-10 compared to the corresponding control culture. In contrast, IL-12 or IL-

23 decreased the expression of CD86 by immature MoDC. Such treated MoDC decreased the production of IFN- γ and IL-10, but increased the production of IL-4 in coculture with CD4⁺ CD45RA⁺ T-cells. Finally, the pretreatment of MoDC with either IL-12, IL-23 or IL-27 lowered the effect of LPS on maturation and allostimulatory capacity of MoDC.

Conclusion: These results suggest that IL-12, IL-23 and IL-27 inhibited LPS-induced maturation of DC but differently modulated MoDC-driven Th polarization.

P43)

IMMUNOLOGICAL PROPERTIES OF PROBIOTICS: PROTECTION AGAINST EXPERIMENTAL BACTERIAL INFECTION AND INDUCTION OF CYTOKINE SECRETION

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Probiotics beneficially affect the host in different manners, among which stimulation of the immune system is of major importance. The aim of the present study was to assess the immunological properties of certain strains of probiotics using in vitro and in vivo models. The protective role of several *Lactobacillus* strains was determined against an experimental murine intestinal infection with *Salmonella enteritidis* serovar Typhimurium. BALB/c mice received viable lactic bacteria by gavage (intra-gastric intubation). After 72 h, mice were challenged by the same route of administration with a lethal dose of *S. typhimurium* (NCTC 3718). Survival was monitored for 16 days. Results indicate that mice receiving *Lactobacillus plantarum* CMGB 3, *Lactobacillus acidophilus* CMGB 16 and *Lactobacillus paracasei* CMGB 15 had a statistically significant higher survival rate than control mice ($p < 0.001$). The highest protection was noticed

for *L. paracasei*. By contrast, *Enterococcus faecium* CMGB 8 strain was pathogenic to mice. Cytokine induction in human cell cultures (PBMC) stimulated with heat inactivated lactic bacteria was assessed by Multiplex immunoassay. All the tested strains (including *Enterococcus faecium*) induced cytokine secretion; *L. paracasei* was the most potent inducer of IFN- γ while determining the lowest level of IL-10. Our results demonstrate the need for validation of in vitro results by appropriate in vivo models.

P44)

CYTOKINE INDUCING ACTIVITY OF BACTERIAL COMPONENTS ASSESSED BY MULTIPLEX IMMUNOASSAY

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CANTASTIM™ (CS) is a purified extract of *Pseudomonas aeruginosa* that induces non-specific protection against bacterial infection, enhances macrophage functions and modulates cytokine production. Most likely, it interacts with innate immunity components. Cytokines production could be used to assess the bioactivity of this product but, as they operate in vivo in a complex regulatory network with reciprocal influences, there is a need for profiling an array of cytokines rather than an individual analysis. Multiplex technology was applied to assess the cytokine pattern induced by stimulation of human leukocytes (whole blood cultures, PBMC and lymphocyte-enriched PBMC cultures) with CS. In both whole blood and PBMC cultures, stimulation with CS induced the production of pro-inflammatory cytokines (IL-1 β , TNF α , IL-6, IL-8) in a dose-response manner but at lower levels than LPS. CS also induced important levels of GM-CSF and IL-12 in monocytes. Despite great inter-individual differences, stimulation with CS led to secretion of a large panel of cytokines; ANOVA analysis displayed clear tendencies of dose dependent increase in cytokine levels for

IL-1beta, IL-6, IL-10, TNF-alpha and GM-CSF ($p < 0.05$). Stimulation experiments were repeated on THP-1 cell cultures (PMA- differentiated) using HPLC separated CS fractions. Several fractions induced cytokine production (IL-8, IL-1beta, TNFalpha, IL-6) but in a different pattern than the whole mixture. Multiplex technology proved to be an effective approach to investigate cytokine secretion.