ABSTRACTS

CARDIOMYOCYTE INFLAMMATION MODEL IN SIRT 3 /- /- MOUSE

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Background: SIRT3, as a NAD+ dependent protein deacetylase, controls several enzyme activities, through which it might became a critical regulator of different pathways. SIRT3 substrates are involved in metabolism and ATP production (respiratory chain, Krebs cycle, fatty acid β-oxidation, urea cycle and ketogenesis), adaptive thermogenesis. Its role in defense against oxidative stress and in protection of the heart has also been described.

Aims: The goal is to get a better perspective of molecular variations caused by SIRT3 deficit, and to define the place of this gene in inflammatory regulation processes, using SIRT3 /-/ mouse model.

Methods and results: Relative levels of mRNAs / proteins, from wild-type (fed with standard Chow diet - SCD / high fat diet - HFD) SIRT3 /- (fed with SCD / HFD), were assessed by real-time reverse transcription-polymerase chain reaction (RT-PCR) / Western blotting. The absence of SIRT3 gene significantly induced the expression of pro-inflammatory genes such as ICAM-1, IL-6 and MCP-1 (p<0.05) compared to their wild-type littermates. High fat diet mostly increased ICAM-1, MCP-1, SOD2 and PDK4 gene expression. pAMPK staining intensities were slightly decreased in animals fed by simple HFD (12,55%), and intensively decreased in SIRT3 deletion with SCD and HFD (34,48% and 38,76%). At the level of pERKs a straight increasing expression trend from WT CT through SIRT3 /- /- HFD animals has been observed. IκB showed overtly higher levels in HFD conditions especially in the KO group.

Conclusion: SIRT3 deletion might activate pro-inflammatory cytokines, such as ICAM-1, IL-6, MCP-1, SOCS3) gene expression, suggesting a possible inflammatory profile of the heart.
Correlation of MACC1 and c-Met expression in HeLa+18 cells and infection with HPV16/HPV18

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Cervical cancer is a worldwide medical problem with a very disproportionate global distribution. Recent studies indicated that over 99% of cervical tumors contain HPV DNA; approximately 65% of them contain the most common high-risk types HPV 16 and 18. Currently beside Pap screening, only HPV testing has been assessed as a primary screening marker for cervical cancer. Despite HPV infection has been proposed as a indispensable factor for cervical cancer development, only a subset of neoplastic lesions with HPV infection persist and progress to invasive cancer. This suggests us that other molecular events are also involved in cancer progression. Recently identified protein metastasis-associated in colon cancer-1 (MACC1) has been shown to play an important role in growth and progression of cervical cancer cells. MACC1 induces pro-survival and anti-apoptotic responses in cancer cells, through transcriptional upregulation of c-MET proto-oncogene encoding a protein known as Hepatocyte growth factor receptor (HGFR). Previous studies have shown that HGF and c-MET proteins gradually increase with the severity of cervical intraepithelial neoplasia (CIN) containing oncogenic HPVs. Since MACC1 is a key regulator of HGF-MET pathway aim of this study is to evaluate and correlate the expression of MACC1 and c-Met transcript in HeLa+18 cervical cancer cell line and in HPV+18/HPV+16 patient samples. The methodology will include isolation of total cellular mRNA followed by reverse transcription polymerase chain reaction (RT PCR) and real-time polymerase chain reaction (Real-Time PCR).

Keywords: HPV, MACC1, c-MET, HGF
Changes in complement activation provoke dual effect on the joint destruction in zymosan-induced arthritis

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Objectives: Zymosan-induced arthritis (ZIA) is an experimental model of rheumatoid arthritis used to analyze cells and molecules that mediate the pathogenesis of inflammatory joint diseases. Although, the alternative pathway of complement is involved in the pathogenesis of arthritis, many issues are not well elucidated. The present studies were designed to evaluate the effect of complement depletion on the late stage of arthritis.

Methods: ZIA model was produced by intraarticular injection of zymosan particles which are able to activate complement by the alternative pathway and to induce enzyme secretion from macrophages. Different schemes of decomplementation were performed using cobra venom factor (CVF), a peptide fragment of cobra C3 component which is capable of activating the alternative pathway (AP) complement activity. In one of the schemes CVF was injected before induction of ZIA, and in the other scheme - during ZIA development. Joint destruction was evaluated by histological examination after H&E and safranin O staining. The amount of CD11b and Ly6G neutrophils and C5a receptor (C5aR) positive cells was analyzed by flowcytometry. C5aR STAT1/3, BMP-2 and TGF-β3 expression intensity was estimated by immunohistochemistry.

Results: Complement depletion before ZIA initiation reduced cartilage and bone erosion associated with a decreased expression of STAT1/3, BMP-2 and TGF-β3 in joints along with limited numbers of C5aR and CD11b/Ly6G positive cells in synovial fluid and CD11b/Ly6G positive cells in peripheral blood of mice. On the contrary, the activation of complement several times during arthritis aggravated the disease.

Conclusion: Present data extend our understanding on the influence of complement system on the joint destruction in ZIA and we argue that it may be a promising therapeutic target in important clinical conditions such as rheumatoid arthritis.
Parasite-elicited Gr1+ cells suppress proliferation of CD4+ lymphocytes in mice

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Objectives: Immunosuppression during helminth infections can be mediated by multiple mechanisms. These include regulatory T cells, regulatory B cells and alternatively activated macrophages and a regulatory cytokine milieu. Recently, new population of suppressor cells is suggested to play a role in parasite escape from host immune response. Myeloid derived suppressor cells (MDSCs), observed widely in cancer, are a population of immature progenitors that suppress activated T and B cells. Murine MDSCs are characterised by monocytic morphology, immature state and presence of CD11b and Ly6G/Ly6C (recognised by Gr-1 monoclonal antibody) markers. Cells with such characteristics are present in steady state in the bone marrow, where they constitute a reservoir and differentiate into granulocytes, monocytes or dendritic cells when needed. In pathology, these cells migrate to secondary lymphoid organs, their differentiation is blocked but activation occurs. They mediate their tolerogenic effects by cytokine production, reactive oxygen species secretion and manipulation of L-arginine metabolism that alter functions of cells of both adaptive and innate immunity. Aim of the study was to establish whether *H. polygyrus* induces MDSCs in mice.

Methods: Mice infected with *H. polygyrus* were studied at 6 and 21 days post infection (dpi) and cells positive Gr-1 marker were isolated by magnetic beads from the peritoneum, spleen, small intestine and mesenteric lymph nodes (MLNs). As shown by flow cytometry, 95% of these cells were double positive for CD11b and Gr1. Then, cells were co-cultured with naive splenic CD4+ cells stained with CFSE and stimulated with anti-TCR antibodies. Using flow cytometry, proliferation of lymphocytes was measured.

Results: We observed that Gr1+ cells from mice infected with the parasite had an ability to decrease T lymphocyte proliferation measured by CFSE test. Gr1+ cells isolated from the peritoneum and MLNs of parasitized animals had the most efficient suppressive potential. Contrary, Gr1+ cells obtained from naive mice stimulated T cell proliferation, what confirms immunosuppressive properties of *H. polygyrus*. However, Gr1+ cells isolated from the small intestine, where the parasite resides, did not inhibit proliferation. Conclusion: We showed that both life stages of the parasite, L4 larvae (at 6 dpi) and adults (at 21 dpi), mediated immunosuppression by Gr1+ cells. Studies to confirm that these Gr1+ cells are MDSCs are in progress.

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The comparative effect of Der p1 on monocyte-derived DCs from allergic and non-allergic patients

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**Objectives:** Allergic diseases are a complex and heterogeneous disorders characterized by chronic inflammation. The orientation of the immune response depends on the antigen nature, costimulatory molecules expressed by dendritic cells (DCs) and the polarizing cytokines environment during DC-T cell interaction. The aim of study was to evaluate the *in vitro* effect of Der p1, the major allergen of *Dermatophagoides pteronyssinus* (Der p), on Th2 cytokines production by monocyte-derived DCs and by naive CD4+ T cells cocultured with autologous DCs from Der p allergic patients in comparison with healthy donors.

**Methods:** CD14+ monocytes were isolated from the venous peripheral blood of house dust mite allergic patients and healthy donors and differentiated to immature DCs using GM-CSF and IL-4. Generated DCs were pulsed for 24 hours with Der p1 or LPS, as a control of DC maturation. Pulsed DCs were then cocultured with the autologous naive CD4+ T cells for 24 hours. Supernatants removed after each phase were assayed for IL-10, IL-12, IL-4, IL-13 and IFN-γ production.

**Results:** Der p1 is associated with an increased expression of CD86 and CD83 on DCs from house dust mite allergic patients and a higher production of IL-10, a promoter of Th2 response. Naive T cells from allergic patients stimulated by autologous Der p1 pulsed DCs preferentially produced IL-4 rather than IFN-γ.

**Conclusions:** The modulation of immune response and the establishment of the balance between allergy and tolerance against an allergen are close dependent on immune status of the individual.
SUPPRESSION OF dsDNA SPECIFIC B LYMPHOCYTES REDUCES DISEASE SYMPTOMS IN SCID MODEL MOUSE OF LUPUS

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Objectives: Self-specific B cells play a main role in the pathogenesis of lupus. This autoimmune disease is characterized by the generation of autoantibodies against self antigens and the elimination of B and T cells involved in the pathological immune response is a logical approach for effective therapy. We have previously constructed a chimeric molecule by coupling a DNA-mimotope peptides to an anti-CD32 antibody. Using this protein molecule for treatment of lupus-prone MRL/lpr mice we suppressed selectively the autoreactive B-lymphocytes by cross-linking B cell receptors with the inhibitory FcγRIIb receptors. This approach was limited by the development of anti-chimeric antibodies in MRL mice. In order to avoid this problem, we established a murine SCID lupus model, allowing a long-term chimera therapy.

Methods: SCID transfer models, Cell separation, FACS, histological staining, ELISA, Proliferation, Sygnal transduction.

Results: Elimination of the double-stranded DNA-specific B cells by chimera therapy in MRL-transferred immunodeficient mice resulted in inhibition of T cell proliferation, suppression of anti-dsDNA B cell activity and prevented the appearance of IgG anti-DNA antibodies and of proteinuria.

Conclusions: In the present study we report a possible way to restrict the communication between autoimmune B and T cells, leading to suppression of lupus symptoms in MRL/lpr cell-transferred SCID mice.
Metallophilic Macrophages in the Thymus of XCL1-Deficient Mice

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Objectives: To investigate morphology and positioning of metallophilic macrophages in the thymus of XCL1-deficient mice.

Methods: Thymuses from normal and XCL1-deficient mice were impregnated with ammoniacal silver according to Weil-Davenport method and photographed. A computer-aided image analysis software Analysis 3.1 was used to determine the number of metallophilic macrophages in cortex, medulla and cortico-medullary zone.

Results: In XCL1-deficient mice numerous metallophilic macrophages are aberrantly positioned in the thymic cortex, instead of their normal location in the cortico-medullary zone. These cells keep their normal morphological features.

Conclusion: XCL1 signaling plays a role in correct positioning of thymic metallophilic macrophages, but it is not involved in their morphological development.
IL-6 AND IL-11 - A LINK BETWEEN INFLAMMATION AND CANCER

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Several studies showed that mediators and cellular effectors of inflammation, including IL-6 cytokine family, are important constituents of the local environment of tumours, being involved in tumour progression. Inflammatory conditions can be present before a malignant change occurs or, in other cases, an oncogenic change induces an inflammatory microenvironment that promotes the development of tumours.

Objectives: Our study intended to establish the connection between inflammation / cytokines and gastric cancer development. The association between Helicobacter pylori infection and the levels of proinflammatory cytokines, was also analyzed in tumoral and adjacent non-malignant tissue fragments.

Methods: Blood, tumor tissue and adjacent normal tissue samples were obtained from 56 patients with gastric adenocarcinoma. ELISA technique was used to analyze IL-6 levels in proteins and plasma. In order to find out if H. pylori presence is responsible for the elevated levels of IL-6 and IL-11 detected in patients’ samples, PCR was performed on DNA samples, using primers specific to H. Pylori urease A (ureA) and cagA genes.

Results: Proteomic analysis regarding the level of cytokines (IL-6, IL-11, LIF), in serum and mucosa of patients with gastric adenocarcinomas, showed a positive correlation between IL-6 and IL-11 tissue level and tumor progression (AJCC/TNM classification). A similar tendency was noticed for plasma IL-6 but not for IL-11 or LIF. An increased level of IL-6 in plasma of patients with gastric adenocarcinoma was associated with tumors showing no gastric H. pylori infection.

No statistically significant differences were observed between IL-6 level in tumor positive for H. pylori compared with tumors H. pylori negative.

Conclusion: Our results suggest that IL-6 high level seems to be mainly associated with the presence of tumor epithelial cells rather than inflammation due to Hp infection, and could be a valuable marker for tumor progression and prognosis.

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Human epidermal melanocytes cells proliferation: In vitro effects of corticosterone, epinephrine and norepinephrine

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An interesting recent approach of the skin is being referred as a "neuro-endocrine-immune organ". (Nejati et al., 2013) The aim of our study was to investigate the in vitro effects of corticosterone, epinephrine and norepinephrine on human epidermal melanocytes (HEM) cells. HEM cells were cultured using DMEM F12 medium supplemented with 10% serum fetal bovine and 1 % antibiotic. 1x10^4 cells/ml were seeded in 96-well tissue culture plates and allowed to attach for 24 hours at 37°C, in a humidified atmosphere with 5% CO2. The dynamic cell proliferation of HEM cells was monitored for 160 h before running the experiment. Cells were treated with different concentrations of corticosterone (80µm; 8 µm; 0,8 µm) epinephrine (40 µm; 4 µm; 0,4 µm) and norepinephrine (8 µm; 0,8 µm; 0,08 µm). The cellular viability was determined using CytoTox 96 Non-Radio Cytotoxicity Assay, based on the release of lactate dehydrogenase (LDH) and the cell proliferation was determined using CellTiter96 Aqueous One Solution Cell Proliferation assay based on MTS reduction. Both viability and proliferation were investigated at 24 h, 48 h and 72 h after cell-exposure. The dynamic response of HEM cells to the corticosterone, epinephrine and norepinephrine exposure was investigated using the impedance-based xCELLigence system.

Norepinephrine and epinephrine, but not corticosterone, inhibited growth of HEM cells in a time and concentration dependent manner. The impedance-based results correlated with both cellular proliferation and cellular viability assays.

These results bring valuable targets for new therapies in skin diseases. A better understanding of the complex communication between immune responses of the skin, and the neuro-endocrine axis could provide better treatments in dermatology.
THE IMPACT OF TNF REVERSE SIGNALING ON THE EXPRESSION PATTERNS
OF INFLAMMATION-RELATED EFFECTOR MOLECULES ON MONOCYTES
AND MACROPHAGES

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Objectives: Professional (i.e. monocytes, macrophages) and semi-professional (i.e. fibroblasts, endothelial cells) immune cells are able to produce both the soluble and membrane-bound forms of TNF. Beside the pleiotropic effects of the TNF (i.e. cell proliferation, cell activation and cell death) reverse signaling of TNF (intracellular signaling elicited by interaction with receptors) has essential role in the regulation of the innate and adaptive immune response. It is known that the N-terminal domain of TNF contains a putative nuclear localization signal. The treatment of the TNF producing cells with agonistic anti-TNF antibody (i.e. infliximab, IFX) results in the liberation of a 10 kDa fragment which is translocated in the nucleus. Moreover, the induction of reverse signaling in human monocytes together with LPS challenge resulted in the down regulation of the LPS induced production of inflammation-related cytokines.

Methods: In order to analyze this phenomenon more accurately, simultaneous induction of reverse signaling was carried out by TNF neutralizing monoclonal antibodies (namely infliximab and certolizumab pegol; IFX and CZP, respectively) on human and mouse monocytic cell lines. To mimic inflammatory conditions, Escherichia coli-derived LPS challenge was performed. Experiments were carried out on bone marrow derived primary mouse macrophages as well. Investigations were performed at relative gene expression and secreted protein level by quantitative real-time PCR (QRT-PCR) and proteome profiler, respectively.

Results: Simultaneous induction of reverse signaling by IFX or CZP stimulation resulted in robust relative gene expression down regulation in inflammation-related cytokines (i.e. IL-1β, IL-8, etc.) in human and mouse cell lines as compared with LPS treated samples. Moreover, the regulatory effect of TNF reverse signaling is independent from the presence of the Fc fragment, as IgG stimulation has no dramatic impact on the gene expression pattern of the previously mentioned genes. In case of PMA induced primary mouse macrophages, LPS stimulation massively enhanced the relative gene expression of the previously mentioned effector molecules, however IFX and CZP treatment resulted in marked relative gene expression decline. In accordance with the results obtained from cell lines, the induction of reverse signaling resulted in significant relative gene expression decline.

Conclusions: Although TNF blocking antibodies are widely used in the clinical practice in many autoimmune diseases (i.e. rheumatoid arthritis, Crohn’s disease, etc.) little is known about their exact mechanism of action. Our results strongly suggest that reverse signaling has role in the beneficial effects of TNF-neutralizing monoclonals by utilizing this pathway to interfere with cytokine production. Furthermore, as increased apoptotic tendency of macrophages is induced by reverse signaling, this phenomenon may have therapeutic potential in clinical oncology by affecting on M2 polarized tumor-associated macrophages.
Functional labeling of hepatitis B virus middle protein

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Hepatitis B virus (HBV) is a small enveloped DNA virus, member of the Hepadnaviridae family, with tropism for liver cells and which can lead to hepatocarcinoma. There are not many data concerning hepatocytes HBV receptors and early steps of viral infection. However, great progress has been made regarding HBV replication and viral genome. The viral DNA is packed in a nucleocapsid, surrounded by viral envelope, which contains three proteins: large (L), medium (M) and small (S). All proteins have a common S domain but they exhibit different functions. The S protein is important in subviral particles (SVPs) formation and secretion. The M protein which contains both S domain and a preS2 extension is known to be dispensable for secretion and infection. The L protein which includes M protein and preS1 has a dual function, being important in both virion assembly and infectivity.

The challenge of this work is to label the virus proteins without impairing the envelope functionality. Therefore, the aim of this study is to design and then investigate the infectivity features of HBV fluorescently labeled at M envelope protein. In our previous experiments, results showed that, under non-reducing conditions, enhanced green fluorescent protein (EGFP)-M fusion viral protein (EGFP.M) revealed a normal intracellular glycosylation and dimerization pattern, but was poorly secreted. We evaluate the incorporation of EGFP.M into the viral envelope and its subsequently secretion by separation of virions from empty envelope particles using isopicnic CsCl gradient centrifugation. HbsAg-specific ELISA and spectrofluorimetry data indicated fluorescence signal in fractions containing SVPs as well as in more dense fractions containing virions.

A second tagging method was also employed, making use of a newly developed labeling technique PRobe Incorporation Mediated by Enzyme (PRIME). Coumarin PRIME labeling on M protein will be further investigated.

Since M protein is dispensable for infectivity and the tag required for coumarin PRIME is as short as 13 aminoacids length the new labeling method will not alter the infection properties of the virions and therefore could help in investigating HBV endocytosis and intracellular motility in living cells.

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Synergic antiproliferative effect of Epigallocatechine-3-gallate and menadione in human leukemia Jurkat T cells

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Objectives: The goal of this study was to explore the chemotherapeutic potential of Epigallocatechine-3-gallate (EGCG) and menadione (vitamin K3; MD), and to determine whether a synergic interaction exists between the two agents that could enhance significantly their antitumoral effect in a cellular model for acute lymphoblastic leukemia (ALL). To this end, we investigated the antiproliferative effect of EGCG and MD, applied alone or in combination on human leukemia Jurkat lymphoblasts. Some underlying cellular mechanisms were also scrutinized.

Materials and methods: Cell suspensions of Jurkat lymphoblasts were treated at various concentrations of EGCG and/or MD. Cell cycle and apoptosis/necrosis were determined by flow cytometry, using the fluorescent indicators propidium iodide and Annexin V-FITC/7-AAD, respectively. Clonogenic survival was evaluated as the colony forming capacity in 96-well plates. Determination of oxidative stress and mitochondrial polarization was performed by spectrofluorimetry, using the fluorescent probes CM-H2DCFDA and JC-1, respectively.

Results: EGCG did not affect the cell cycle distribution, but decreased clonogenic survival in a dose-dependent, highly cooperative manner. MD and the combination EGCG:MD induced cell cycle arrest in G2/M and S phases, respectively. At high doses of menadione, a strong synergic inhibitory effect on viability and clonogenic survival was evidenced, which was associated with a very good combinatorial index (0.25). Both agents induced oxidative stress, which was synergistically augmented by their combination. On short term (1 h), EGCG and MD exerted a depolarizing effect at the mitochondrial level, most likely via the opening of the mitochondrial permeability transition pore. On a longer term (up to 6 h), MD induced mitochondrial depolarization, whereas EGCG generated a mitochondrial hyperpolarized state which was blocked by rotenone, an inhibitor of mitochondrial respiration, hence suggesting that EGCG can stimulate the activity of the respiratory electron transfer chain.

Conclusions: These results support the notion that the combination EGCG:MD exerts a strong synergic antiproliferative effect in human leukemia Jurkat cells and encourage further studies to test the clinical utility of this combination in ALL therapy.

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The expression of CD38 molecule on activated cytotoxic T-lymphocytes in HIV infection and EBV-induced infectious mononucleosis

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OBJECTIVES: Both viral diseases, the acute HIV infection and infectious mononucleosis exhibit similarities in the clinical presentation of the disease as well as in the distribution of lymphocyte subpopulations in the peripheral blood. In HIV infection, the inversion of the CD4+ T-lymphocytes/CD8+ T-lymphocytes ratio is caused by the massive helper T-cells depletion caused by virus-induced cytolysis whereas in infectious mononucleosis there is an antigen-driven clonal expansion of cytotoxic T-lymphocytes in response to, in most cases, Epstein-Barr virus (EBV) infection. The activation of the immune system is detected in both diseases. In infectious mononucleosis, immune system activation is an important factor leading to the suppression of the initiation of lytic EBV infection. Mature T-cells express low levels of CD38 molecules on their surface, but upon activation, the expression of this molecule increases and this property makes it an excellent T-cell activation marker. The aim of this study was to analyze the differences in the percentages CD8+CD38+ T-cells in the peripheral blood of patients with acute and chronic HIV infection as well as with EBV-induced infectious mononucleosis and compare the levels of cytotoxic T-cell activation in these infections.

METHODS: This study enrolled 25 patients with EBV-induced infectious mononucleosis and 49 HIV-infected adults; 24 patients in the acute phase of HIV infection and 25 treatment-naive HIV-infected patients in the chronic phase of the infection. Whole blood was stained with monoclonal antibodies specific for CD45-FITC/CD4-RD-1/CD8-ECD/CD-3PC-5 and CD38-PC-7 (Beckman Coulter) and the percentage of double positive CD8+CD38+ T-cells was determined on Cytomix FC500 cytometer (Beckman Coulter). Kruskal Wallis and Man-Whitney tests were used for statistical analysis.

RESULTS: Median percentage of activated cytotoxic T-lymphocytes (CD8+CD38+ T-lymphocytes) in the whole blood of patients with acute HIV infection was in 30% (range 12%-74%). In patients with chronic HIV infection, percentages of activated cytotoxic T-lymphocytes ranged between 11% and 63% (median 30%). Median percentage of CD38+CD8+ T-cells in patients with infectious mononucleosis was 65% (range 35%-78%). There was a statistically significant difference in the percentages of CD38+CD8+ T-cells between both groups of HIV-infected patients (acute and chronic phase of the infection) compared to the infectious mononucleosis group (p<0.001). Percentages of CD38+CD8+ T-cells in acute versus chronic phase of HIV infection were not significantly different (p=0.56).

CONCLUSION: EBV-induced infectious mononucleosis causes higher expression of CD38 on CD8+ T-cells compared to HIV infection, in both acute and chronic stage of infection.
Treatment with tyrosine kinase inhibitor tyrphostin AG490 causes tissue specific response in models of acute and chronic inflammation

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Objectives: The janus kinase (JAK)-signal transducer and activator of transcription (STAT) cascade plays a principal role in the signaling of a vast array of cytokines and growth factors which stimulates diverse cellular functions and immune responses. The present studies were designed to evaluate the effects of Jak2 inhibitor, tyrphostin AG490 in murine models of acute (lypopolysaccharide, LPS-induced shock) and chronic (collagenase-induced osteoarthritis, CIOA) inflammation.

Methods: CIOA was provoked by intraarticular (i.a.) injection of collagenase in mice and then intraperitoneally (i.p.) treated with AG490 under different schemes. Shock was induced by i.p. injection of 500 mg/kg LPS and then treated with 5 mg/kg of AG490. The levels of chemokines and cytokines were measured by ELISA. The frequencies of CD11b and Ly6C neutrophils, F4/80 macrophages and RANKL (Receptor activator of nuclear factor kappa-B ligand) were analyzed by flowcytometry. Histological examination was performed after H&E, toluidin blue and safranin O stainings. The expression of STAT1/3, pSTAT1/3, BMP2 and TGF-β3 were determined by immunohistochemistry.

Results: Tyrphostin AG490 reduced cartilage and bone erosion associated with a decreased expression of pSTAT1/3 and TGF-β3 in joints along with limited numbers of CD11b, F4/80 and RANKL positive cells in synovial fluid of mice with CIOA. AG490 ameliorated liver injury but not that of spleen in LPS-induced shock. AG490 down-regulated STAT1/3 expression in liver, and pSTAT1/3 and TGF-β3 expression in joints.

Conclusion: Present data extend our understanding on the application of AG490 in acute and chronic inflammation and we argue that it may hold promising therapeutic potential against important clinical conditions such as osteoarthritis.
The *in vitro* effect of bone-like micro patterned titanium coated biomaterials on adhesion properties and cytokine release of THP-1 derived macrophages

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**Objectives:** Inflammation plays an important role in host’s response and regeneration after biomaterial implantation, thus substrate surfaces have been modified for improved osseous integration. The present study aims to investigate the *in vitro* effect of micro and nano scale grooved titanium-coated biomaterials on human monocytic THP-1 cell viability, proliferation and morphology and to examine the proinflammatory cytokine secretion modulated by the surface.

**Methods:** THP-1 cells PMA-differentiated to macrophages stimulated or not (for 18 hours) with bacterial endotoxin (LPS) were cultured on laser-tailored titanium covered biomaterials with bone-like cavities ranging from 250 nm to 6 µm in depth. Macrophage viability and proliferation was evaluated by MTS colorimetric non-radioactive cell proliferation assay. Morphological changes and adhesion behavior of differentiated cells were visualized with immunofluorescent microscopy techniques and the proinflammatory cytokine TNF-α released from macrophages was measured using an ELISA-type method.

**Results:** No cytotoxic effect onto THP-1 cells treated or untreated with LPS for 18 hours was revealed, but endotoxin addition led to a decrease of viability irrespective of surface patterning. Further at 72 hours the viability remained unchanged for macrophages adhered on biomaterials with pits depths below 3 µm, but increased for those cultured on pattered material with 4-6 µm cavities. Next, actin and vinculin proteins involved in cellular adhesion show that cells adhere and exhibit spread morphology on engineered bone-like patterned biomaterials. Cells cultured on structures with 6 µm cavity-depths although show reduced spreading area, more circular morphology and tend to orient toward the groove, still retain the adherence properties. TNF-α secretion from THP-1 cells revealed no detectable levels of proinflammatory cytokine compared to the cells treated with lipopolysaccharide used as a control. Cytokine release is observed only in the case of endotoxin treatment for 18 hours, TNF-α levels increasing in general with the depth of the cavity from the biomaterial.

**Conclusion:** Our data show that laser – engineered substrates coated with titanium examined, modulated adhesion, cell behavior and cytokine secretion *in vitro* especially those with cavities with greater depths compared to planar controls. These pit structured biomaterial similar to bone transversal section could be of interest for improving bone healing and repair.
Application of 3D image based assessment for studying the impact of innate immunity signaling on global methylation status

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Objectives DNA methylation is a crucial epigenetic modification of the human genome beyond the DNA sequence level that is involved in the regulation of many cellular processes. DNA methylation also occurs beyond promoter regions at non-CpG islands within the heterochromatin. In this study we questioned whether the innate immunity signaling elicited in Sertoli cells is DNA methylation dependent. For that purpose we challenged mouse Sertoli cell line 15p-1 by the Toll-like receptor 4 (TLR-4) ligand bacterial lipopolysaccharide (LPS).

Methods Bacterial lipopolysaccharide (LPS) treated and untreated (control) mouse Sertoli cells were subjected to confocal scanning microscopy and dedicated 3D image analysis for the following features: differential nuclear 5-Methylcytosine (MeC)/4',6-Diamidino-2-phenylindole dihydrochloride (DAPI) load and co-distribution patterns, cell similarity based on these patterns, and corresponding differences in the topology of MeC and DAPI sites.

Results Using 3D DNA methylation image based analysis we assessed the bacterial lipopolysaccharide (LPS) induced global methylation status at cellular level resolution.

Conclusion We conclude that this method is favorable to research innate immunity signaling induced 3D DNA methylation phenotype. Epigenetic modulators could affect Sertoli biology under stress impairing fertility.
EXPRESSION OF IMMUNOREGULATORY PROTEINS BY MESENCHYMAL STEM CELLS - DOES THE SOURCE MATTER?

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Introduction: Mesenchymal stem cells (MSCs) are powerful sources not only in regenerative medicine, but also for therapy of autoimmune diseases. MSCs can be virtually isolated from each adult organ, as well as from fetus-associated perinatal tissues. MSCs possess stemness properties in vitro, they are able to differentiate towards different mature cell types and express molecules involved in immune modulation. MSCs have immunosuppressive effect on T-cell activation and proliferation and generally inhibit the development and function of nearly all immunocompetent cells; including NK cells and monocytes-derived dendritic cells. They also have immune avoidance mechanisms that reduce immunogenicity. Several immunosuppressive mechanisms have been described, among these immunosuppressive factors expression. Human leukocyte antigen-G protein (HLA-G) and progesterone induced blocking factor (PIBF) are key immunoregulatory molecules found in MSCs.

Aim: We examined and compared the expression of immunomodulatory proteins HLA-G and PIBF of MSCs, isolated from different sources.

Methods: Isolation and culturing of MSCs, flow cytometry analysis and confocal microscopy.

Results: Mesenchymal stem cells have been successfully isolated from adult and perinatal tissues. All examined types of MSCs have plastic adherent properties, fibroblast-like morphology and express MSCs marker panel – CD 29+, CD 73+, CD 90+, CD 105+ and CD 45-.

Immunomodulatory proteins, PIBF and HLA-G, were not equally expressed in different kinds of MSCs. Our hypothesis is that perinatal tissues isolated MSCs have higher expression of immunoregulatory proteins and are more potent regulators of immune functions when used for therapy of autoimmune diseases.
Characterisation of Mesenchymal Stromal Cells (MSC)

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Over the last decade, human term placenta has been described as a source of mesenchymal stromal cells (MSC), which represent a particular cell type, for both their immunomodulatory properties and multilineage differentiation potential. These unique features of MSC make them the focus of intensive studies to apply in cell-based therapies.

Aim of the study: The aim of the study was to characterize amnion- and chorion-derived MSC based on morphology, phenotype, and immunomodulatory properties.

Materials and methods: Human placentas (n ≥ 10) were obtained from healthy woman after delivery and processed immediately. Isolated cells were designated as human amniotic mesenchymal stromal cells – hAMSC, and human chorionic mesenchymal cells – hCMSC. Morphology of cells was assessed during subsequent passages. Expression of CD90, CD73, CD45, HLA-DR was assessed on freshly isolated and cultured up to P2/P3 cells by flow cytometry. Immunomodulatory properties were evaluated by lymphocyte 3 proliferation test with peripheral blood mononuclear cells (PBMC) stimulated with anti-CD3. Lymphocyte proliferation was assessed by incorporation of [3-H]-thymidine after 3 days of co-culture with hAMSC or hCMSC at a 1:1 ratio.

Results: hAMSC and hCMSC were successfully isolated from fetal membranes of human placenta and presented typical elongated, spindle shape, fibroblast-like morphology during culture. Cells cultured up to P2 presented following phenotype: CD90 ≥ 90%, CD73 ≥ 90%, CD105 ≥ 80%, CD45 ≤ 2%, HLA-DR ≤ 2%. Immunomodulatory potential of freshly isolated fetal membranes-derived MSC was confirmed by inhibiton of T cells proliferation after anti-CD3 stimulation in comparison to the control.

Conclusion: hAMSC and hCMSC presented morphology and phenotype characteristic for MSC and were able to inhibit T cell proliferation.
SELECTIVE ELIMINATION OF ALLERGEN-SPECIFIC B LYMPHOCYTES WITH CHIMERIC PROTEIN-ENGINEERED MOLECULES

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Objectives: Der p1 is allergenic molecule of Dermatophagoides pteronyssinus (Dpt) which causes house dust allergy. The pathological Der p1-specific B cells produce allergen-specific IgE antibodies that mediate most of the hypersensitivity allergic reactions.

Aim: It may be possible to influence Der p1-specific B cells by administrating to them a chimeric molecule, containing a monoclonal antibody against the inhibitory B-cell receptor CR1 coupled to a B and a T cell epitopes from the Der p1 allergen. Co-crosslinking of the immunoglobulin receptors and CR1 by this molecule is expected to deliver suppressive signal selectively silencing these B cells only.

Methods: A synthetic peptide, Der p1 p52-71, and anti-CD35 monoclonal antibody 3D9 were used for the construction of Der p1 chimera. We analysed the effects of the chimeric molecule in vitro using PBMC from allergy patients. We measured Der p1-specific IgE and IgG antibody production by ELISA and determined the B-cell proliferation by ELISpot. We studied the effect of the constructed chimeric molecules on apoptosis by flow cytometry using AnnexinV-FITC/PI staining.

Results: We observed significant inhibition of allergen-specific cell proliferation and reduction of specific IgE antibodies. Expression of phosphatidylserine on the outer layer of the cell membrane was changed in CD19+ and CD3+ cells from patients.

Conclusion: The constructed chimeric molecule binds Der p1 specific B-lymphocytes via their BCR and suppresses selectively their proliferation and the production of anti-Der p1 IgE antibodies by co-crosslinking of the inhibitory CR1. This way we could alter the allergic immune response towards a milder outcome.
Antimelanoma effects of isoxanthohumol

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Objectives: Isoxanthohumol (IXN), a natural prenylflavonoid from the hop plant (Humulus lupulus), have potent antiinflammatory effects. Since it is often reported that many natural as well as synthesized compounds with an antiinflammatory properties can also exhibit strong anticancer activity, the aim of this study was to investigate possible anticancer potential of IXN on melanoma cells and to explore cellular mechanisms involved.

Methods: Two cell lines of different origin and intracellular characteristics, mouse (B16) and human (A-375) melanoma cell line, have been used. Cell viability was estimated by MTT and CV assays. Proliferation was measured by carboxyfluorescein succinimidyl ester staining. Presence of apoptotic/necrotic cell death was determined by annexin V/propidium iodide double staining assay while autophagic process was evaluated by acridin orange supravital dye. After all procedures applied, flow cytometric analyses were performed. Potential morphological transformation of the treated cells was evaluated using light microscopy and differentiation process was characterized by measurement of enzyme tyrosinase activity (colorimetric assays). Finally, intracellular signaling pathways responsible for the observed phenomenon were estimated using Western blot.

Results: IXN decreased dose-dependently viability of melanoma cells. Analysis of cell proliferation revealed strong decrease in the rate of division of melanoma cells treated by IXN. In parallel with this, minor contribution of different types of cell death was found. The process of autophagy, defined by the increased presence of authophagosomes in cells exposed to IXN, was not in correlation with cell viability decrease. Oppositely, the inhibition of autophagy by specific inhibitor 3-methyladenine, remarkably provoked cytotoxic effect of IXN, indicating citoprotective role of this process in IXN antimelanoma action. Microscopic evaluation of cells exposed to IXN showed remarkable morphological transformation. This effect was accompanied with increased tyrosinase activity, indicating enhanced melanin synthesis as a sign of differentiation toward melanocytes. This non-aggressive antimelanoma action is in correlation with the inhibition of p70-S6 Kinase and S6 ribosomal protein activity, well-known regulators of cell proliferation.

Conclusion: Results demonstrate that IXN possesses strong anticancer potential realized through the induction of melanoma cells differentiation toward melanocytes, followed by the decrease of their malignant properties.

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**In vitro** effects of undoped and Fe$^{3+}$-doped TiO$_2$ nanoparticles on HepG2 hepatocytes

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The photostability of drugs represents one of the major concerns in the field of photomedicine, the science concerning the interactions of drugs, light, and biomolecules. Such interactions are especially important in the human skin, which is one of the most important sites of the immunological processes in the body. In this context, the widespread use of TiO$_2$ nanoparticles for the stabilization of photolabile drugs raised concerns regarding the potential health risks induced by the accumulation of such nanoparticles in the human body. The accumulated nanoparticles preferentially affect the function of vital organs such as liver, which is one of the most active organs involved in the processing of exogeneous compounds (including nanomaterials) and detoxification.

With the present study we bring new insight regarding the combined **in vitro** effects of TiO$_2$ nanoparticles and DMSO (used as a generic hepatotoxic agent and compound that facilitates the penetration of plasma membrane by TiO$_2$ nanoparticles) on liver cells.

We investigate the cytotoxicity and intracellular ROS production induced in HepG2 cells (human hepatocarcinoma) by two types of TiO$_2$ nanoparticles (the commercial Degussa P25 TiO$_2$ (P25) and anatase nanoparticles (undoped and Fe$^{3+}$-doped) synthesized under hydrothermal conditions). The experiments were such designed to elucidate the following aspects:

- effects of the studied TiO$_2$ nanoparticles on hepatocytes that were “already damaged” – the cells were first treated with DMSO and two hours later were exposed to the action of nano-TiO$_2$;
- viability and intracellular ROS production in case of HepG2 cells simultaneously treated with DMSO and nano-TiO$_2$;
- response of hepatocytes to the action of DMSO, administered two hours after the cells were exposed to nano-TiO$_2$.

The cell viabilities and intracellular ROS productions were determined using the MTT assay and the DCF-DA (2',7'-dichlorofluorescein-diacetate) test respectively.

The obtained results were analyzed with respect to specific physicochemical properties (structural, photocatalytic and hydrophobic) of the tested nanomaterials and the known hydroxyl radical scavenger properties of DMSO.

For all the tested materials, the most prominent viability reductions were observed in the case of cells post-treated with DMSO (TiO$_2$-DMSO), the highest cell killing effect being induced by Degussa P25 titania nanoparticles. The hydrothermal materials (HT and FeHT) induced only weak or insignificant cytotoxic effects. No significant cellular effects were detected in the case of cells simultaneously exposed to TiO$_2$ and DMSO (TiO$_2$+DMSO).

The observed viability variations did not depend on the treatment time (24, 48, 72 hours), for none of the tested TiO$_2$ types.

Small or moderate increases in the intracellular ROS levels of the treated cells were observed in all experimental cases, being more pronounced in the case of DMSO post-treatment (TiO$_2$-DMSO). Although the highest ROS production was determined for Degussa P25 TiO$_2$, corresponding to the TiO$_2$-DMSO case, no clear distinction can generally be made between the pro-oxidative effects of the commercial and the hydrothermal samples.

The determined ROS productions were generally directly proportional to the tested TiO$_2$ concentration.

The fact that both, the highest viability reduction and the highest intracellular ROS production, were associated to the TiO$_2$-DMSO case, suggested that the effects determined in this case were dictated by the early action of TiO$_2$, most probably by means of oxidative stress. The attenuated cellular effects observed either in the case of co-treatment (TiO$_2$+DMSO) or pre-treatment with DMSO (DMSO-TiO$_2$) may indicate a possible interaction between TiO$_2$ and DMSO, leading to the attenuation of the damaging effects of TiO$_2$. Such effects may be related to the known hydroxyl radical scavenging activity of DMSO.

The observed differences between the biological effects of P25 and the other two TiO$_2$ types may be related to their photocatalytic properties and hydrophilic/hydrophobic character.

The conducted study offered first information regarding the **in vitro** effects of the combined action of TiO$_2$ nanomaterials and DMSO on human hepatocytes. The studied effects were shown to depend on the properties of TiO$_2$ and the characteristics of the exposure.
Impact of Ambrosia pollen on allergic airway inflammation in the Banat area

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**Background:** Allergic rhinoconjunctivitis is a Th2-type inflammatory disease of the nasal mucosa and the conjunctiva, mediated by immunoglobulin E (IgE) antibodies. It is the most common allergic disease, affecting between 10 and 25% of the general population. The prevalence of asthma in patients with rhinitis varies from 10 to 40%. Ragweed (Ambrosia artemisiifolia) pollen represents the main cause of late-summer allergic rhinoconjunctivitis in the Western region of Romania (Banat area). Our study evaluated the impact of ragweed pollen on allergic patients from the Banat area.

**Methods:** 97 subjects (84 patients with positive skin prick test to ragweed pollen extract and and 13 negative controls) were recruited prospectively between August and November 2013, in an observational cross-sectional study. Patient evaluation was performed by skin prick test to a panel of 18 standard allergens. Specific serum IgE to 176 allergens was determined by using the ImmunoCAP ISAC microarray.

**Results:** Out of the 84 patients with positive skin prick test to ragweed pollen extract, 90% also had increased specific serum IgE levels to the major ragweed allergen, Amb a 1 (5% class 1, 35% class 2, and 45% class 3). However, the IgE class did not correlate significantly to the skin prick test class. Most patients (74%) had moderate-severe intermittent allergic rhinoconjunctivitis, and 25% of them also had allergic asthma. All patients with a disease history older than 15 years had developed allergic asthma.

**Conclusion:** There is a small fraction of patients that are allergic to minor ragweed pollen allergens and they are not identified by standard in vitro diagnostic procedures. Component-based diagnosis using microarrays is needed for the selection of the appropriate allergen source for specific immunotherapy. Appropriate therapeutic measures need to be taken in order to prevent progression of allergic inflammation from rhinoconjunctivitis to bronchial asthma.
Expression and prognostic relevance of MACC1 in breast cancer cells

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Breast cancer is the most common type of cancer in woman worldwide. Although progression that is made in management of breast cancer to decline the mortality rate by combining different treatment modalities that includes different combination of surgery, chemotherapy and radiation, breast cancer represents a challenge for early diagnosis and treatment. The variations in morphologies and differences in metastatic behavior and in the response to therapeutic treatments make breast cancers hard to treat. Due to these facts it is necessary to elucidate possible signaling pathway that could serve as potential therapeutic target and at the same time also be a biomarker for early detection no matter which subtype patients are diagnostic with.

The newly identified proto-oncogene MACC1 gene (Metastasis-Associated Colon Cancer 1) is a prognostic marker for the detection of metastases in primary tumors of colon cancer. The MACC1 was first identified to be overexpressed in primary and metastatic tumors tissue of colon cancer compared to healthy tissue. MACC1 stimulates proliferation, motility and invasion in colon cancer cells through transcriptional upregulation of c-MET. MACC1 binds to 60 bp proximal fragment endogenous MET promoter; binding to a specific site that is essential for MACC1 it induces activation of c-MET and the HGF/Met signaling pathway. Once activated, c-MET can result in activation of several downstream signaling cascades, such as MAPK and PI3K/Akt pathways. Studies have shown that MACC1 gene is overexpressed in different types of tumors such as; lung cancer, adenocarcinoma, gastric cancer and hepatocarcinoma. MACC1 gene could play a key role in differentiation, migration and invasion of breast cancer cells and serve as potential therapeutic target.

In past years a large number of clinical studies have describe c-Met receptor overexpression and pathway hyper-activation in tissue derived from breast cancer patients and have found a strong relationship with tumor progression. To our knowledge no previous studies have shown a correlation between MACC1 and c-MET expression in breast cancer cells. Furthermore there are no studies addressing activation pathway of MACC1 in breast cancer.

Recent studies showing that these signaling pathways play a key role in carcinogenesis, cell survival, anti-apoptotic effect, invasion, metastasis and angiogenesis in malignancies including breast cancer it is of interest to elucidate the role of MACC1 gene in this cascade and the correlation with c-MET in breast cancer cells.

Key words: breast cancer, MACC1, biomarker
Regulatory T lymphocytes in evaluation of the local protective cellular immune response to *Mycobacterium tuberculosis* (MTB)

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**Background**

Active suppression by Regulatory T lymphocytes (Tregs) plays an important role in the down-regulation of T cell responses to foreign and self-antigens, including *Mycobacterium tuberculosis* (MTB).

**Objective:** Our aim was to assess local cellular immune response to MTB by evaluating regulatory T cells in tuberculous pleural fluid samples compared to peripheral blood from patients with MTB infection and healthy control subjects.

**Methods:** The percentages of CD4⁺CD25⁺ T cells in pleural fluid and peripheral blood from patients with tuberculous pleurisy and peripheral blood from healthy control subjects were determined by flow cytometry before and after enrichment using MACS CD4⁺CD25⁺CD127dim/- (Miltenyi Biotec). The expression of forkhead transcription factor Foxp3 was also examined both in CD4⁺CD25⁺ and CD4⁺CD25⁻ T cells from pleural fluid and blood.

**Results:** There were increased numbers of CD4⁺CD25⁺ T cells in tuberculous pleural fluid compared with peripheral blood from both patients and normal subjects, and these cells also expressed Foxp3. In pleural fluids from patients infected with MTB, a purity of 85.7±4,2% CD4⁺CD25⁺ was obtained even after first separation using MACS CD4⁺CD25⁺CD127dim/- strategy, compared to 80,6± 7,5% for the cells from peripheral blood of patients and 55,7 ± 27,1% for the cells from peripheral blood from control subjects.

**Conclusion:** The increased level of Treg cells in tuberculous pleural fluid explains the relatively effective local immune response against MTB infection by suppressing the proliferation of CD4⁺CD25⁻ T cells.
IMMUNOTOXICITY OF SUBCHRONIC ORAL CADMIUM EXPOSURE IN RATS: EFFECTS ON SPLEEN, LUNGS AND INTESTINE

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Objectives: Cadmium (Cd) is one of the most toxic metals in the environment that adversely affects a number of organs and tissues. Numerous studies have shown that oxidative stress and inflammation are the underlying mechanisms of Cd toxicity. Cd can induce the formation of reactive oxygen species (ROS) and triggers the antioxidative response represented mainly by enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST). The influence of subchronic oral low dose of Cd, relevant to human exposure, on the systemic immune response of Albino Oxford (AO) rats, was examined in this study.

Methods: To investigate the subchronic effects of cadmium intoxication on antioxidative defense and the inflammatory responses, male AO rats were administered with 5 ppm CdCl2 in distilled water, for 30 days, while control group received distilled water solely. Cd tissue content (determined by atomic absorption spectrometry), the activity of antioxidant defense enzymes (spectrophotometric measurements) and Interleukin-6 (IL-6), and interleukin-1ß (IL-1ß) contents (ELISA) in spleen, lung, and intestine were evaluated. Serum aspartate aminotransferase (AST) and alanine transaminase (ALT) activity were measured as markers of systemic response to cadmium administration.

Results: Administration of cadmium resulted in significantly increased values of AST and ALT compared to controls. Cadmium exposure resulted in significant elevation of cadmium content the most notably in intestine, following with lungs and spleen respectively. In the lung homogenates the levels of IL-1ß were increased compared to controls while the levels of IL-6 remained the same as control. SOD and GST activities were decreased and CAT was unchanged. In the spleen homogenates, IL-1ß was unchanged while IL-6 was elevated in Cd treated group. SOD activity was elevated while CAT and GST remained without changes. Cytokine contents in intestinal homogenates were below the level of detection. SOD and GST activities were elevated and CAT activity was without change.

Conclusion: Besides liver and kidneys, the most intense examined tissues in subchronic Cd intoxication, the oral administration of Cd induced the oxidative stress and triggers proinflammatory immune response in spleen, lungs and intestine. The activity of antioxidant defense enzymes and proinflammatory immune response were differentially affected in different tissues.
ABSTRACTS

REACTION AND ACTIVATION OF PERIPHERAL BLOOD LEUKOCYTES FROM PREGNANT WOMEN

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Objectives: Pregnancy is a state of “immunological paradox”, when the maternal immune system doesn’t reject the semi-allogeneic embryo. Allusively inactivated, maternal immune system recognizes pregnancy occurrence and tolerates the implantation of the embryo. T-lymphocyte activation is directly regulated by the Immunoglobulin factor CD83, widely considered to be a marker for mature dendritic cells. Accumulating data report the controlled expression of CD83 in activated immune cells, such as Tand B-lymphocytes, monocytes and macrophages, probably related to their effector and antigenpresenting functions. As a rule, effector leukocytes recirculate in the peripheral blood until they reach the target non-lymphoid tissues. In the present study we investigate the functional activation of peripheral leukocytes from pregnant women.

Methods: PMBC from healthy, non-pregnant and pregnant women are isolated form blood samples using density-gradient separation. After labeling with CFSE, cells are polyclonally activated with the mitogen phytohemagglutinin (PHA-P) for 5 days. Cell proliferation is examined by FACS. Additionally, total RNA is isolated from patients’ PBMC and CD83 expression is quantified by qRT-PCR.

Results: PBMC mRNA quantification doesn’t reveal differences in CD83 expression between pregnant (first trimester) and non-pregnant women. Moreover, the ex vivo PHA-P stimulated T-cells from all analyzed groups show identical proliferation kinetics.

Conclusion: These observations demonstrate the lack of activated, characterized by de novo CD83 synthesis, leukocyte subpopulations in the peripheral blood of first trimester pregnant women. They also show normal functional T-cell reactivity in the pregnant women from the investigated early and advanced pregnancy groups.

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Anti-cancer properties of gastropodan hemocyanins in murine model of colon carcinoma

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Various immunotherapeutic approaches have been used for the treatment of cancer. A number of natural compounds are designed to repair, stimulate, or enhance the immune system response. Among them are hemocyanins (Hcs) - extracellular copper proteins isolated from different arthropod and mollusque species. Hcs are oxygen transporter molecules and normally are freely dissolved in the hemolymph of these animals. Hemocyanins are very promising class of anti-cancer therapeutics due to their immunogenic properties and the absence of toxicity or side effects. KLH (\textit{Megathura crenulata} hemocyanin) is the most studied molecule of this group setting a standard for natural carrier protein for small molecules and has been used in anti-tumor clinical trials. The Hcs isolated from marine snail \textit{Rapana thomasiana} (RtH) and the terrestrial snail \textit{Helix pomatia} (HpH) express strong \textit{in vivo} anticancer and antiproliferative effects in the developed by us murine model of colon carcinoma. The immunization with RtH and HpH prolonged the survival of treated animals, improve humoral anti-cancer response and moderate the manifestation of C-26 carcinoma symptoms as tumor growth, splenomegaly and lung metastasis appearance.
Differentiation of Mesenchymal Stromal Cells

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Fetal membrane-derived cells has become an attractive source of mesenchymal stromal cells (MSC), alternatively to bone marrow derived MSC. In this study, differentiation of amnion- and chorion-derived MSC in vitro and on hybrid polyurethane scaffold toward osteogenic lineage was evaluated and their immunomodulation properties were assessed before and after differentiation.

Materials and methods: Human placentas (n ≥10) were obtained from healthy woman after delivery and processed immediately. Isolated cells were designated as human amniotic mesenchymal stromal cells –hAMSC, and human chorionic mesenchymal cells –hCMSC. Phenotype and morphology was assessed as in other studies. Freshly isolated and cultured cells were plated on plastic dishes and on polyurethane foam coated with calcium phosphate, culturing them in osteogenic medium. Cytochemical staining and gene expression were used to assess the differentiation. A proliferation test was performed as previously in our laboratory to evaluate immunomodulatory properties of 3 placenta-derived cells, both before and after differentiation.

Results: hAMSC and hCMSC were successfully isolated from fetal membranes of human placenta. Fibroblast-like shape and phenotype confirmed that cells obtained were mesenchymal. Alizarin Red and von Kossa stainings revealed presence of calcium deposits in cell cultures indicating osteoblast-like features of hAMSC and CMSC. Placenta-derived cells presented capacity to down-regulate T cell proliferation. It remained to be determined the osteogenic differentiation on hybrid scaffolds and immunomodulatory properties after osteogenic differentiation.

Conclusion: Fetal-membrane derived MSC showed ability to differentiate into osteogenic lineage and to inhibit the proliferation of activated T cells when co-cultured.
The role of IL-33/ST2 pathway in changes of macromineral concentration in acute inflammation

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Objectives: Homeostasis of macro- and microminerals in acute inflammation is important. The role of IL-33/ST2 pathway in pathogenesis of acute inflammation is not completely revealed. The aim of this study was to examine effects of IL-33/ST2 pathway on macro- and micromineral concentration changes, at the site of acute inflammation.

Methods: Wild-type and ST2 knock-out mice, were divided into the groups: WT-C (wild-type control group), KO-C (ST2 knock-out control group), WT-I (wild-type inflammation group), and KO-I (ST2 knock-out inflammation group). In order to induce acute inflammation, animals received intramuscular injection of turpentine oil into the right and left hind limb. Control animals received intramuscular injection of saline, in the same way. After 12 hours, mice were sacrificed, and treated muscles were collected for pathohystological analysis, and determination of concentration of macrominerals, magnesium, sodium and calcium, and microminerals, manganese and cooper.

Results: Induction of acute inflammation in WT-I and KO-I was confirmed by pathohystological analysis of the treated muscles. Magnesium concentration in the muscle was significantly lower in WT-I when compared to WT-C and KO-I. In contrast to this result, concentration of magnesium in the muscle did not significantly change in KO-I when compared to KO-C. Concentration of sodium, calcium, manganese, and cooper, in the muscle did not significantly change in acute inflammation.

Conclusion: Results of this study showed that IL-33/ST2 pathway could have a role in changes of magnesium concentration at the site of acute inflammation. Concentration of sodium, calcium, manganese, and cooper, did not change at the site of acute inflammation.

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Molecular analysis of MACC1 and c-Met expression in HPV-associated cervical cancer

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**Introduction:** Cervical cancer (CC) displays notably increased or decreased expression of a large number of cellular oncogenic or tumor suppressive. Cervical cancer is the second leading cause of death among female patients with cancer in the world. Human papillomavirus (HPV)-associated cervical cancer has a significantly more favorable outcome compared with HPV-negative cervical cancer. The newly identified proto-oncogene MACC1 gene (Metastasis-Associated Colon Cancer 1) stimulates proliferation, motility and invasion in colon cancer cells through upregulation of c-Met. Once activated, c-Met can result in activation of several downstream signaling cascades (MAPK and PI3K/Akt pathways). The MACC1 was first identified to be overexpressed in primary and metastatic tumor tissue of colon cancer compared to healthy tissue.

**Objectives:** Aim of this study is to analyze the expression of MACC1 and C-Met at protein level in HPV-associated cervical cancer. Main objectives of this study will be to determine whether MACC1 and C-Met expression at protein level differed as a function of HPV status and to assess whether MACC1 and c-Met provide prognostic value beyond HPV status.

**Material and methods:** For the purpose of this study swab samples will be collected from 30 HPV+ females with different HPV genotyping and swab samples from healthy individuals as control. MACC1 and c-Met expression will be evaluated and correlated with potential development of HPV associated cervical cancers. Methodology will include following basic molecular biology techniques such as isolation of proteins, Western blot and statistical analysis.

**Results:** Expected outcome of this study is an overexpression of MACC1 and c-Met at protein level in swab samples, and also a molecular correlation between c-Met and MACC1 expression patterns, and HPV-associated cervical cancers.

**Conclusion:** MACC1 could be an excellent candidate for developing new therapies and management of HPV-associated cancers.
Impact of allergens on allergic patients

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Allergic rhinoconjunctivitis is the most common allergic disease, affecting up to 25% of the population. It can greatly affect patient quality of life, giving rise to indirect economic costs which demonstrate the need to improve management of these patients. Our study assessed the impact of outdoor allergens on the quality of life of allergic patients from the Western part of Romania (Banat area).

Methods: 97 subjects (83 patients with positive skin prick test to ragweed pollen extract and 14 negative controls) were recruited prospectively between August and November 2013, in an observational cross-sectional study. Patient evaluation was performed by skin prick test to a panel of 18 standard inhaled allergens and quality of life was assessed by using a standardized questionnaire.

Results: Out of the 84 patients with positive skin prick test to outdoor inhaled allergens, 93% had moderate-severe symptoms (74% intermittent and 19% persistent). 42% of the 64 polysensitized patients were sensitized to several pollens. The symptoms were more prevalent in August and September, due to the highly deleterious impact of ragweed pollen. The average intensity of symptoms on a scale from 1 to 10 was 8.1 before treatment, and this only decreased to 4.1 even under therapy with several classes of medication (inhaled corticosteroids and antihistamines). Nasal obstruction and sneezing were the most common symptoms. 25% of the allergic patients also had bronchial asthma.

Conclusion: The quality of life of patients with allergic rhinoconjunctivitis is greatly impaired, and this burden is not alleviated properly even by standard therapy.
B7 COSTIMULATION AND INTRACELLULAR INDOLEAMINE 2,3-DIOXYGENASE (IDO) EXPRESSION IN UMBILICAL CORD BLOOD AND ADULT PERIPHERAL BLOOD

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Objectives: Alterations in the expression of B7 costimulatory molecules and their receptors as well as differences in the tryptophan catabolic pathway may influence immunological reactivity of umbilical cord blood (UCB) compared to adult peripheral blood (APB) T lymphocytes.

Methods: We determined the frequency of activated (CD11b+) monocytes expressing B7-1, B7-2, B7-H1, and B7-H2, and that of T cells and CD4+ T helper cells expressing CD28, CTLA-4, PD-1, and ICOS in UCB and APB samples using flow cytometry (BD FACS Aria). We also examined the intracellular expression of indoleamine 2,3-dioxygenase (IDO) applying flow cytometry and plasma levels of tryptophan (TRP), kynurenine (KYN) and kynurenic acid (KYN) using high-performance liquid chromatography.

Results: The level of CTLA-4 expression on CD4 cells was higher in UCB compared to APB, indicating that the possibility of CD28-mediated costimulation may be decreased. The level of the corresponding costimulator molecule, B7-2 was also elevated. Therefore, this inhibitory relation may function to a higher extent in UCB than in APB. The plasma KYN to TRP (K/T) ratio was two-fold higher in UCB compared to APB. However, the capacity of UCB monocytes compared to APB monocytes was lower to produce IDO, and reverse signalling via B7-2 in UCB monocytes was found to be immature, which suggests that the observed increase in K/T ratio may be due to placental rather than fetal overexpression of IDO in competent cells.

Conclusion: These factors may all contribute to the previously observed reduced reactivity of UCB T lymphocytes compared to APB T cells.
Inflammatory cytokines modulate adipose tissue mesenchymal stem cells properties

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Objectives: Increasing evidence suggests that inflammatory conditions can affect mesenchymal stem cells (MSCs), their self-renewal and multipotent differentiation potential, as well as their immunomodulatory properties. In our previous studies we have shown that gene expression of various molecules related to the immunomodulatory functions of adipose tissue mesenchymal stem cells (AT-MSCs) can be influenced by inflammatory cytokines, such as IFN-γ and TNF-α. The present study attempts to evaluate the effects of IFN-γ and TNF-α on the proliferation, adipogenic differentiation, migration and pluripotency factors (Nanog and Oct-4) expression in AT-MSCs. Additionally, we analyzed the mitogen-activated protein kinases (MAPK) signaling pathways (ERK1,2, p38, JNK1,2) triggered by IFN-γ and/or TNF-α in AT-MSCs.

Methods: Human AT-MSCs were exposed to IFN-γ and/or TNF-α and their proliferative and migratory capacities were assessed by MTT assay and wound healing assay, respectively. AT-MSCs were also induced to differentiate into adipocytes in the presence or absence of these cytokines. The influence of IFN-γ and/or TNF-α on the expression of Nanog and Oct-4 mRNA and proteins was determined by RT-PCR and indirect immunofluorescence, respectively, while MAPKs induction by these cytokines in AT-MSCs was analyzed by immunoblotting.

Results: TNF-α stimulated both the proliferation and migration of AT-MSCs, while IFN-γ exhibited no significant effect on both cell functions. In the same time the combined usage of IFN-γ and TNF-α inhibited both the proliferative and migratory capacity of AT-MSCs. The adipogenic differentiation of AT-MSCs was inhibited in response to both cytokines, used either alone or in combination. On the other side, the gene and protein expression of transcriptional factors Nanog and Oct-4 in AT-MSCs were increased after exposure to both IFN-γ and TNF-α and their combination. However, treatment of AT-MSCs with IFN-γ and TNF-α activated different MAPKs, since IFN-γ enhanced the phosphorilation of p38 and JNK1,2, while TNF-α activated pERK1,2.

Conclusion: Data obtained demonstrated the importance of inflammatory microenvironment, especially the pre-conditioning with IFN-γ and TNF-α, for the expression of human AT-MSCs properties and biological functions.
Reduced frequency of nTregs in patients with reproductive failures

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**Aim:** Regulatory T cells (Tregs) represent the effective arm of immune tolerance. They consist of two populations- natural (nTregs) and inducibles (iTregs). Treg cells play an important role in the control of immune responses, autoimmune and allergic reactions and for the successful pregnancy. The aim of the present study was to analyse nTregs in the peripheral blood of women with reproductive failure.

**Materials and Methods:** PBMCs isolated from 7 healthy women (HW) with no history of pregnancy complications (26-58 years) and 8 patients with unsuccessful pregnancies (21-44 years) were stained with anti-CD3, -CD4, -CD45RA, -CD25, -FOXP3 antibodies. The FACS-analysis was done using FlowJoV10 software. Statistical analysis was performed using GraphPad software.

**Results:** Considering the importance of FOXP3+ T cells for the development of immune tolerance, the population of FOXP3+CD45RA+ nTreg-cells was evaluated. No significant difference between study groups was found (p>0.05) The more detailed examination of CD25 expression showed that in HW CD25+FOXP3+ cells represented the majority of nTregs, which was not the case in the patient group (p< 0,05).

**Conclusion:** The results obtained showed that in patients with reproductive failure the percentage of nTregs is lower, as compared to the controls. Further investigations are needed to clarify the impact of this cells for the successful pregnancy.
Methanol extract of *Origanum vulgare* – anti-inflammatory and cytoprotective effect during diabetes pathogenesis

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**Aim:** Type 1 diabetes (T1D) is an autoimmune disease which results from progressive destruction of pancreatic beta cells. Plants and their extracts are promising candidates for new treatment of T1D. The aim of this study was to determine the effect of methanol extract of *Origanum vulgare* (MOE) on development and progression of T1D.

**Methods:** MOE was prepared from oregano leaves by sequential extraction of four solvents of gradually increasing polarity (hexane, ethyl acetate, dichloromethane and methanol). T1D was induced with multiple low doses of streptozotocin (MLDS) in C57BL/6 mice and MOE was given i.p. for 10 days. Evaluation of MOE effect on T1D was performed by monitoring glycemia and insulin (ELISA) and *ex vivo* assessment of insulitis (histochemistry) and immune response (flow cytometry and real-time PCR) within mononuclear cells that infiltrate pancreatic islets. *In vitro* anti-apoptotic effect of MOE on pancreatic beta cells was determined by histone-DNA ELISA and caspase-3 assay.

**Results:** MOE treatment significantly reduced diabetes development in mice and preserved insulin secretion. This was further confirmed by the absence of severe insulitis in MOE-treated mice. However, the infiltration of immune cells within the pancreas of MOE-treated mice was the same as in diabetic mice and could be explained by the high percentage of islets with benign peri-insulitis. The composition of pancreatic islets infiltrates suggest that the number of pathogenic Th17 cells is increased in MOE-treated mice as well as the expression of their key cytokine IL-17. However, the numbers of Th2 and Treg cells were also elevated after MOE treatment suggesting that these cells were able to control autoimmune response and ameliorate diabetes. Furthermore, MOE exerted a cytoprotective effect since it preserved beta cells from *in vitro* induced apoptosis via blockade of caspase-3.

**Conclusion:** The observed immunomodulatory and anti-apoptotic effect of MOE provide a solid basis for potential implementation of this extract as a therapeutic or a supplement for the treatment of T1D.

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