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Results: G1 had 4 patients with cancer, 1 with chronic bacterial infection, 5 with diabetes; G2 had 1 with rheumatoid arthritis, 1 with cancer and 2 with diabetes. In G2 all patients developed purulent arthritis, whereas in G1 only about half (42% in hip joints and 53% in knee joints). Inflammatory level was higher in G2: during follow-up leukocytes increased by 32% and CRP - by more than 7-fold, while ESR was elevated but essentially stable (64.00 and 53.75 mm/s). Lymphocytes were only slightly greater in G2 compared to G1 (32.6% vs 24.84%), with T cells increased in G2 and G1 in relative and absolute numbers (0.78 vs 0.75 and 1529.36 vs 1125.68) but further increased in G2 up to 0.76 and 1609.74.: CD4+ cells were elevated in G2 (892.06 vs 642.43) and increased further up to 918,16, while CD8+ cells, elevated in G2 (644.18 vs 488.92), were stable (629.86). B cells were increased in G2 (184.41 vs 142.93) and increased further to 226.91. NK cells in G2 were decreased compared to G1 in relative and absolute numbers (0.12 and 222.96 vs 0.16 and 249.39) and had fallen further to 0/11 and 217.30 respectively.

Conclusion: Patients with infectious complications after implantation of joint prostheses is associated with increased inflammation involving lymphocytes with activation of specific humoral and T-cell dependent immune responses.

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Comparative profile of surface and intracellular molecule expression in 10 immortalized human T cell lines to be considered for immunomodulatory drug evaluations

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Background: Immortalized T-cell lines are widely used in immunology, oncology and immunopharmacology research, Testing of immunomodulatory drugs, especially popular in the Eastern Europe, is traditionally performed using freshly isolated human leukocytes or mice as an animal model. This investigation is to determine the T-cell line expression profiles to select cell lines suitable for drug testing.

Methods : Cells obtained from cryobanks were cultured in the RPMI-1640 medium supplied with 10% FCS, L-glutamine, sodium pyruvate, HEPES and gentamycin. The expression of the following molecules was determined: CD1a, CD2, CD3, cCD3, CD4, CD5, CD7, CD8, CD10, CD16, CD25, CD28, CD34, CD45, CD43, CD45RA, CD45R0, CD69, CD62L, CD56, CD90, CD123, CD127, HLA-ABC, HLA-DR, TCR$\alpha\beta$, TCR$\gamma\delta$. Production of major cytokines in response to non-specific stimuli (PMA) was studied, including INF-γ, TNF-α, IL-2, IL-4, IL-10 and IL-12. Expression of transcriptions factors T-bet, GATA-3, FoxP3 and ROR-γt was also assayed. K-means clustering was used to separate cell lines into clusters.

Results: 10 different T-cell lines were selected: C8166, CEM-SS, CEM.NK^R, Jurkat, Jurkat-tat, Molt-3, Molt-4, Molt-4 clone 8, MT-2 and MT-4. Cluster #1 included C8166 cell line with very early antigen differentiation profile ($CD25^{++}$ $CD123^{hi}$ $CD127^{hi}$ $CD34^{+}$ $CD2^{-}$ $CD3^{-}$ $CD7^{-}$ $CD8^{-}$). Cluster #2 included Molt-3, Molt-4, Jurkat, Jurkat-tat, CEM-SS and CEM.NK^R cell lines. This cluster is characterized by variable expression of CD2, CD5, CD7, CD4, CD8, CD45, CD45RA, HLA-ABC, CD43 and CD1a antigens and high CD3/cCD3 expression. While CD25 is required for cell activation, its expression was absent. CD28 expression was only detected on Jurkat and Jurkat-tat cells. Cluster #3 was composed of MT-2 and MT-4 cells, expressing CD2, CD4, CD25, CD123 and HLA-DR molecules, but lacking expression of CD3, CD5, CD7 and CD8. The expression of CD69 under the influence of PMA was the highest in Jurkat-tat (RFI = 130.3 $\pm$ 2.9) and Molt-4 clone 8 (RFI = 147.2 $\pm$ 5.6), while production of TNF-α was highest in MT-2 (RFI = 22.2 $\pm$ 5.2) and CEM-SS/CEM.NKR cells (RFI = 18.1 $\pm$ 2.1).

Conclusion: Jurkat-tat, CEM-NK^R and MT-2 cell lines were selected as a T-cell model for further research in the field of immunomodulatory drug testing.

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Attenuation of antigen-specific Th1 immunity by *Neolitsea hiiranensis* derived sesquiterpenes

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Background: T cells play a pivotal role in the acquired immunity that participates in a wide range of immune responses through a complicated cytokine network. Imbalance of T cell responses may be involved in several immune disorders. To seek the safe phytochemicals to regulate the functionality of T helper cells may be benefit to modulate T-cell mediated immune diseases. *Neolitsea*, one of the biggest genera in the family *Lauraceae,* possesses a great application potential in herbal medicine and contains diverse phytochemicals including alkaloids, sesquiterpenes, triterpenes, flavonoids, and steroids. As sesquiterpenes have been reported to own immunomodulatory bioactivities, in this study we characterized the effects of sesquiterpenes derived from the leaves of *Neolitsea hiiranensis* on adaptive immune responses.

Method: Dried leaves of *N. hiiranensis* were extracted three times with cold methanol for sesquiterpene and terpenoid isolation. A QSAR model for hepatotoxicity screening was applied to predict the hepatotoxicity of these compounds to filter out the compounds with higher predicted hepatotoxicity. Splenocytes isolated from ovalbumin (OVA)-sensitized BALB/c mice were applied to evaluate the immunomodulatory effects of selected compounds on adaptive immunity by determining the cell proliferation, cytokine production and mRNA expression in the presence of OVA *in vitro*. Furthermore, mice were administered with crude extracts of *N. hiiranensis* and caryophyllene oxide (CRYO) to evaluate its immunomodulatory activities *in vivo*.

Results: Five of twelve selected sesquiterpenoids including spathulenol, hiiranlactone C, hirranlactone D, neoliacionlide A and CRYO significantly inhibited antigen-specific IFN-γ production *in vitro*, while IL-2 and IL-4 were not altered. *N. hiiranensis* and CRYO attenuated several aspects of adaptive immune responses, including T cell differentiation, IFN-γ production, serum level of antigen-specific IgG_{2a} and Th1-assocaited gene expression *in vivo*.

Conclusion: As IFN-γ is the key cytokine secred by T helper-1 (Th1) cells, our results demonstrated the N. hiiranensis and its sesquiterpenoids possess potential therapeutic effects on Th1-meidated immune disorders.

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Plasma vitamin D levels at birth and the inflammatory status of preterm infants

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Background: Vitamin D has an important immunomodulatory role. We investigated whether vitamin D levels at birth may associate with immune status in preterm infants.

Poster Discussion Session PDS 7

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Synergistic collaboration of Fc-gamma receptor III and TLR-4 define the pro-inflammatory responses of nasal epithelium during re-infection with *P. aeruginosa*

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Introduction: The bacterial flora of the nasal cavity comprises of many different bacterial species, including potential opportunistic pathogens, such as *Pseudomonas aeruginosa*. It is a Gram-negative bacterial pathogen capable of causing a broad range of infections of the lower and upper airway. LPS, a major component of bacterial cell wall is a potent inducer of the innate immune responses. The innate immunity may not be sufficient for a complete eradication of an invading pathogen, therefore it is followed by the adaptive immune responses that lead to a production of antibodies and consequently elimination of the microbe. Epithelial cells play an important role within the innate responses since they are the first line of defense against microbes residing in the nasal cavity. They are able to recognize potential immunogens by pattern recognition receptors (PRRs), including Toll-like receptors. Stimulation of an individual PRR is known to induce cell responses, however the cross-talk between multiple receptors may define the ultimate amount and the profile of cytokine production. Here, we seek to investigate how a development of the adaptive immunity and, as a consequence, a subsequent presence of specific IgGs against *P. aeruginosa* contributes to responses of the nasal epithelial cells to *P. aeruginosa* challenge.
Methods: We exposed nasal epithelium to IgG-opsonized *P. aeruginosa* or to LPS co-stimulated with IgG and measured the production of cytokines and their gene expression. Selective Fc-gamma receptors were blocked to determine their contribution to epithelium responses to LPS+IgG.
Results: Despite the presence of the LPS-binding complex, nasal epithelium does not respond to challenges with *P. aeruginosa* and consequently to its major cell wall component LPS. However, when *P. aeruginosa* is opsonized with IgG or LPS stimulation is accompanied by IgG, the tolerance of the nasal epithelium is broken and a massive production of IL-6 and IL-8 can be measured. Blocking of receptors revealed that the pro-inflammatory responses to *P. aeruginosa* are mediated by the low-affinity Fc-gamma receptor III and TLR-4.
Conclusion: During a re-infection, IgG rapidly opsonize *P. aeruginosa* and only then the local pro-inflammatory responses of nasal epithelium can be triggered. The data indicate the complexity of cell responses to this pathogen and that the involvement of the adaptive immunity may be crucial for proper cell responses to *P. aeruginosa*.

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Short chain fatty (SCFA) acids induce apoptosis in peripheral blood eosinophils and promote endothelial barrier function

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Background: The increasing incidence of allergic inflammatory diseases points out the growing necessity for eosinophil-targeting therapeutics. Accumulation of eosinophils in the lung tissue is a hallmark of asthma and it is believed that eosinophils play a crucial pathogenic role in allergic inflammation. Short chain fatty acids, e.g. acetate, propionate and butyrate are produced in high concentration in the gastrointestinal tract by commensal bacteria and are readily secreted into the blood stream and thereby show various biological functions. Prompted by the observation that propionate hampers lung eosinophilia in models of allergic inflammatory diseases we hypothesize that SCFA modulate the survival and the recruitment of eosinophils.
Method: Ca^{2+} flux and respiratory burst in human eosinophils was measured with flow cytometry. Induction of apoptosis was detected using annexin V/propidium iodide (PI) double staining and caspase 3/7 activation assay. Additionally, expression of adhesion molecules on pulmonary microvascular endothelial cells was analyzed by flow cytometry. Endothelial resistance was detected via cell substrate impedance sensing (ECIS).
Results: We found that propionate concentration-dependently induces Ca^{2+} flux and respiratory burst in isolated human eosinophils. Both, propionate and butyrate significantly reduce the survival of human eosinophils starting 18 h after treatment. Similarly, activation of caspase 3/7 was induced by propionate and butyrate. Interestingly, this observation was restricted to eosinophils from allergic donors. Moreover, propionate strengthens the barrier function of human pulmonary endothelial monolayers and concentration dependently decreases the TNF-α induced expression of VCAM and E-selectin.
Conclusion: Our results suggest that propionate and butyrate induce apoptosis in human eosinophils from allergic donors, reduce the expression of adhesion molecules on pulmonary microvascular endothelial cells and, furthermore, strengthen the endothelial barrier function. We therefore propose that propionate and butyrate could serve as potential therapeutic agents in allergic inflammatory diseases.

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Human olfactory mucosa-derived mesenchymal stem cells induce a tolerogenic profile in monocyte-derived dendritic cells

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Background: Immunosuppressive activity of mesenchymal stem cells (MSC) may interact with dendritic cells (DC). This study assesses the effects of olfactory mucosa-derived mesenchymal stem cells (hOM-MSCs) on the antigenic profile of dendritic cells (DC).
Methods: Nasal mucosa samples were taken from 8 patients with non-inflammatory diseases of nasal cavity. To obtain hOM-MSCs cells explant culture methods were applied. The obtained hOM-MSCs

were $CD90^+CD105^+CD73^+/CD31^-CD45^-$. Monocyte-derived dendritic cells (mdDC) were obtained from peripheral blood monocytes cultured for 7 d in the serum-free media containing GM-CSF and IL-4. mdDC and hOM-MSCs were co-cultured in a ratio of 1:1 providing direct cell-to-cell contact (MSC-DC) and using culture insert to avoid direct cell contact (Ins-DC). Negative (DC cultured without stimuli – iDC) and positive controls (mdDC cultured with LPS – LPS-DC) were also used. mdDC were assayed for expression of CD32, CD80, CD85k, CD86, CD273 and HLA-DR antigens.

Results: LPS-DC were characterized by increased expression of CD32, CD80, CD86, HLA-DR and significantly reduced expression of CD85k and CD273. mdDC cultured in the cell insert over the monolayer of hOM-MSCs phenotypically were similar to the iDC. Only the direct cell contact led to DC differentiation towards the tolerogenic profile. Increased expression of CD85k and CD273 and reduced expression of HLA-DR, CD80 and CD86 was shown.

Conclusion: Direct cell-to-cell contact of hOM-MSCs and mdDC led to the induction of the tolerogenic profile of the DC. hOM-MSCs are a promising tool for generation of tolerogenic DC ex vivo.

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Higher expression of complement receptor 1 on monocytes and granulocytes during bacterial infection than during viral infection in children

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Background: Clinical differentiation between bacterial and viral infection is very difficult. Unfortunately, there is still lack of quick and accurate diagnostic test, which would help clinicians in establishing the diagnosis and taking a decision on a treatment. Complement receptor 1 (CD35) is involved in phagocytosis of IgG- and complement-opsonized pathogens and plays an important role in inflammatory processes. The aim of the study was to compare the expression of CD35 antigen on phagocytes during bacterial and viral infection in children.

Method: The expression of CD35-FITC, CD14-APC, CD15-V450, CD45-AmCyan in 40 blood samples from children with high fever and infection suspicion was assessed by flow cytometry (FACSCantoII). Only 100 µl of residue blood collected on EDTA (tube for CBC test) was used for analysis. 27 children were diagnosed with bacterial and 13 with viral infection. Expression of CD35 was analyzed according to mean fluorescence intensity (MFI) and antibody binding cites (ABC). Statistical analysis was performed using nonparametric Mann-Whitney test for independent samples.

Results: CD35 antigen had significantly higher MFI on granulocytes (median (25 percentile; 75 percentile): 3437 (2081; 5666), 2140 (1453; 3033), respectively, $P = 0.0104$) and monocytes (median (25 percentile; 75 percentile): 5486 (3684; 8717), 3519 (2277; 3977), respectively, $P = 0.0041$) during bacterial infection in children in comparison to viral infection. Significantly higher ABC for CD35 antigen on granulocytes (median (25 percentile; 75 percentile): 82178 (50006; 135187), 51414 (34993; 72676), respectively, $P = 0.0136$) and monocytes (median (25 percentile; 75 percentile): 130922 (88167; 207346), 84246 (54669; 95116), respectively, $P = 0.0041$) was also observed during bacterial infection in comparison to viral infection.

Conclusion: Measurement of expression of complement receptor 1 on peripheral blood phagocytes could help in distinguishing between bacterial or viral origins of infection and facilitate speedy decision on a treatment. It may help to avoid unnecessary use of antibiotics.

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Maturation of innate immune responses of the respiratory epithelium

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Background: The respiratory epithelium is critical both for the clearance of infections and the development of adaptive responses. Very little is known on the maturation of epithelial responses. The aim of the present study was to assess the maturation of epithelial responses to viral infection in relation to age.

Method: Primary nasal epithelial cells (NECs) were obtained from healthy ($n = 26$) donors of a wide age range (0–55 years). NECs were cultured and infected with Human Rhinovirus 1B (RV1B). Expression of IFNB1 mRNA was measured with RTQPCR. A large array of epithelial cytokines ($n = 25$) were measured in cell culture supernatants at 48 h with Luminex. Virus replication was titrated. Cytotoxicity levels were evaluated at 24 h, 48 h, 72 h with crystal violet staining. Age-related differences were evaluated by regression analysis.

Results: RV1B-induced IFNB1 mRNA expression, linearly increased with age ($P < 0.05$). Also, IL28A protein increases with age in healthy NECs after RV1B infection ($P < 0.05$). CCL5 protein, a downstream effect, increases with age in healthy NECs after RV1B infection ($P < 0.05$). Virus load was higher in NECs from children than adults at 8 h post infection ($P < 0.05$). Cytotoxicity levels of healthy NECs increase with age infection at 48 h and 72 h after RV1B ($P < 0.05$).

Conclusion: This is the first study investigating the maturation process of airway epithelial responses to viral infection, showing age-related evolution of antiviral responses. IFN responses upon RV1B infection increased with age.

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Specific induction of TSLP by the viral RNA analogue poly(I:C) in primary epithelial cells derived from nasal polyps

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Introduction: Chronic rhinosinusitis with nasal polyposis is an inflammatory disease that, although not directly linked to allergy, often displays a Th2-skewed inflammation characterized by elevated local IgE and IL-5 levels. The nasal cavity is constantly exposed to bacteria and viruses that may trigger epithelial inflammatory responses. To gain more insight into mechanisms by which such a biased inflammation might arise, we have investigated the epithelial expression of the Th2 skewing mediators (TSLP, IL-25, and IL-33) in relationship to disease and microbial triggers.

Methods: Epithelial cells were obtained from polyp tissues of nasal polyposis patients and from inferior turbinates of non-diseased controls. Cells were exposed to various TLR-specific triggers to study the effect on mRNA and protein expression level of TSLP, IL-25, and IL-33 and the potential regulatory mechanisms through the expression profile the transcription factors ATF-3, DUSP-1, EGR-1, and NFKB-1.

Results: The TLR3 agonist and viral analogue poly(I:C) induced TSLP mRNA 13.0