ISSN 0014-2980 · EJIMAF 49 (Supplement S4) · Vol.49 · Supplement S4 · November 2019

European Journal of Immunology

Supplement S4

MESIA
SLOVAKIA 2019

4th Meeting of
Middle - European Societies
for Immunology
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Abstracts of MESIA 2019
4th Meeting of Middle European Societies
for Immunology and Allergology
28-30 November 2019
Samorin, Slovakia

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European Journal of Immunology

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European Journal of Immunology Volume 49, Suppl. 4, November, 2019

This abstract book can be searched using the PDF search function to look, for example, for the Abstract number or author name.

To cite an Abstract, please use the following format:
Abstract title. Authors. Conference: 4th Meeting of Middle European Societies for Immunology and Allergology. Location Samorin, Slovakia. Date November 28-30, 2019. Eur. J. Immunol. 2019.49, S4, page number(s)

DOI: 10.1002/eji.201970500

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Influence of apoptotic cells on autologous T-cell culture in vitro

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It is known that defects in apoptosis of peripheral lymphocytes may cause dysregulation of self-tolerance and maintain the chronic inflammation in rheumatoid arthritis (RA). Investigation of non-autonomous effects of apoptosis as influence of apoptotic cells on adjacent tissue is of importance. The purpose of our study was to evaluate the influence of cellular and humoral components of apoptotic lymphocytes on autologous T-cells in vitro under the neighbourhood conditions in norm and RA.
At the initial stage, we optimized method to initiate cell death in «primary-induced» culture: depletion the culture medium, increased cell crowding. Then it was evaluated the influence of apoptotic components on T-cell cultured in normal condition («secondary-induced» culture). Isolated PBMCs from blood of donors and RA patients divided on 2 groups. The first part was used in primary-induced cultures (2*106 cells in 150mkl RPMI+1%FCS without stimulation and with anti-CD3 antibodies, 10-4 M dexamethasone). The second part stained with CFSE and was used in cultures under normal conditions (1*106 cells/ml, RPMI+10%FCS). On 4th day of culturing cells and supernatants from apoptotic cultures transferred to autologous cells in normal condition. After 3 days of co-culturing it was performed cytometric analysis of early and late stages of T-cells apoptosis on FACSCantoII using kit with Annexin V and 7-AAD.
It was estimated in both groups an increase in parameter of early apoptosis in primary- and secondary-induced cultures under stimulation with anti-CD3 antibodies. In Ra patients, cells and supernatants from apoptotic cultures treated with dexamethasone enhanced apoptosis level in secondary-induced cultures. In healthy individuals, dexamethasone-induced cultures had no influence on early apoptosis that implied a modulatory effect of dexamethasone. Number of T-cells in the late stage of apoptosis increased in all secondary-induced cultures.

The study was funded by RFBR and Novosibirsk region, the research project №18-44-540012

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DOI: 10.1002/eji.201970500

Interleukin-IL-1β in tumor biology and immunotherapy

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To probe the role of microenvironment IL-1β in the balance between inflammation and immunity in the tumor microenvironment, we used the model of orthotopically injected 4T1 breast cancer cells into IL-1β deficient (IL-1β KO) and wild-type (WT). In WT mice tumor progressed and induced lung metastases, while in IL-1β KO mice tumors started to regress after 14 days. Tumor regression in IL-1β KO mice is CD8+ T cell dependent, while in the microenvironment of tumors in WT mice inflammatory monocytes differentiated into IL-10 immunosuppressive TAMs, which induced progression. Based on the results, we have developed a novel immunotherapeutic approach to target the pro-inflammatory and immunosuppressive nature of the tumor microenvironment, which will also enable the development of anti-tumor cell immunity. Thus, to target the tumor microenvironment, we have used antibodies that neutralize two distinct targets, i.e., anti-IL-1β and anti-PD-1. In addition to the synergy between these agents, anti-IL-1β antibodies has potential to ablate the inflammatory effects that anti-PD-1 induces in patients. In early breast tumors, in WT mice, this combinatorial treatment leads to inhibition in tumor development. In late, breast cancer tumors, the local tumor was initially excised and then a protocol of treatment of anti-IL-1β and anti-PD-1 was applied, which lead to an increased significant survival of metastasis-free mice, as compared to only resected mice or mice treated with a single agent. Overall, our results to target in cancer patients conventional first-line therapies, to deplete the malignant cells, and then target the microenvironment to prevent tumor recurrence and metastasis.

DOI: 10.1002/eji.201970500

Associations of TNF alpha-308G>A Genetic Polymorphism with Allergic Diseases

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Background: Polymorphisms of cytokine genes are an interesting focus for association studies involving allergic diseases due to their role in immune cell communications during inflammation. Genetic studies have so far yielded inconclusive results regarding associations of TNF alpha-308G>A genetic polymorphism with allergic diseases.
Methods: This study was performed on 356 Croatian students. The diagnosis of atopic asthma (AA), rhinitis (AR) and dermatitis (AD) was based on self-reported symptoms by the modified International Study of Asthma and Allergies in Childhood questionnaire and a positive standard skin prick test (SPT) to at least one common inhalation allergen. The diagnosis of contact allergy was based on a self-reported skin symptoms and positive standard patch test (European baseline series) to at least one tested contact allergen (subgroup of 312 students was patch tested). Genetic polymorphisms were genotyped using the polymerase chain reaction- based technique. Multivariate logistic regression models were adjusted for personal and lifestyle factors (for atopic disease models: sex, history of atopy in parents, Continental vs. Mediterranean residency, and pet ownership in childhood; for contact sensitization models: sex and atopy).
Results: Compared to the control subjects, there was a significant negative association of the TNFα –308G>A polymorphism with AA, AD, and separately for skin symptoms and positive SPT. These observations were confirmed in a multivariate model only for AD and skin symptoms (atopic dermatitis: OR = 0.27; 95% CI 0.07–1.00; p = 0.050; skin symptoms: OR = 0.29; 95% CI 0.10–0.83; p = 0.021). Based on multivariate analysis, TNFα 308G>A polymorphism was confirmed as a predictor of contact sensitization to p-phenylenediamine (OR: 5.72; 95% CI 1.20-27.28).
Conclusions: Study indicates different role of TNFα –308G>A polymorphism in the development of different allergic skin reactions: a protective role regarding AD and susceptible role regarding contact allergy to p-phenylenediamine.

DOI: 10.1002/eji.201970500

Circulating microRNAs as peripheral biomarkers for aging and Alzheimer´s disease

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Alzheimer´s disease (AD) is the most common neurodegenerative disorder in elderly population manifested by progressive cognitive and memory decline. Pathological changes appear many years before the onset of the clinical AD symptoms. MicroRNAs (miRNAs), the small non-coding RNAs are the most studied group in the field of biomarker discovery for early stages of AD. MiRNAs regulate expression of genes at post-transcriptional level by initiating translational inhibition or mRNA degradation. Due to their biological function and stability in circulating biofluids, they can be indicative of AD progression as candidate biomarkers for detection of early-stages of disease. The aim of this study is to detect and validate novel miRNA biomarkers in AD linked with signalling pathways involved in neurodegeneration. Transcriptomic analysis was performed on AD individuals compared to healthy age-matched controls to define expression profiles of miRNAs. We identified panel of dysregulated miRNA profiles clearly distinguishing the AD patients from healthy individuals. Recent data suggest that panel of miRNA molecules could bring higher resolution for differential diagnostics of AD and related tauopathies.

This project was supported by research grant: VEGA 2/0118/19, 2/0076/18, 1/0240/16, APVV-17-0668, ERA-NET NEURON RepImpact.

DOI: 10.1002/eji.201970500

Effect of telomere length on spontaneous Th1 and Th2 cytokine production in asthma

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Background: Atopic asthma is associated with immune response bias from Th1 to Th2 cytokine production. Asthma is known to be accompanied by shortening of the average telomere length in leukocytes [1]. Recently, it was demonstrated that shortened telomeres in chromosomes of human cells can upregulate gene expression in the same chromosomes. This effect is referred to as telomere position effect over long distance (TPE-OLD) [2]. The main purpose was to study the association of telomere length with cytokine production in asthma.
Methods: We estimated the telomere lengths on the individual chromosome arms in groups of patients with atopic asthma (aBA, n=12), nonatopic asthma (nBA, n=6) and healthy volunteers (n=18, age and gender matched). Telomere length on individual chromosome arms in metaphase spreads obtained from peripheral blood mononuclear cells (PBMCs) was estimated by Q-FISH. The new MeTeLen software was developed to measure the telomere repeats quantity (http://www.bionet.nsc.ru/en/development/application-development/development-of-a-computer/metelen.html) in metaphase images. Spontaneous secretion of IL-4, IFN-γ and FNO-α in culture supernatants of PBMCs was evaluated by ELISA. The analysis was carried out by non-parametric statistics methods (Mann-Whitney U-test, Spearman rank correlation).
Results: We found that telomeres in certain chromosome arms (1p, 4q, 5q, 6p, 9p, 9 q, 11q, 12q, 13q, 14q, 15q, 20q) in aBA group were significantly shorter than in donor group ($p< 0.05$). While in patients with nBA, telomere length doesn't differ from donor ones. It is noteworthy that in aBA telomeres are shortened in the 5q, 12q and 6p arms where the IL-4, IFN-γ and FNO-α genes are located respectively. Moreover IL-4 level negatively correlated with 5q telomere length ($R= -0.72$, $p=0.016$) in aBA. Correlation between IL-4 level and 5q telomere length was not detected in nBA and donors. No correlation was found between 12q telomere length and IFN-γ, and 6p telomere length and TNF-α in investigated groups.
Conclusions: We have shown that there is no relationship between IFN-γ and TNF-α production and telomere length. Whereas IL-4 production may depend on 5q telomere length in atopic asthma, possibly through the TPE-OLD mechanism. Association between obtained data and the pathogenesis of Th-2 diseases remains to be determined further.

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DOI: 10.1002/eji.201970500

Is there still space for the active cell - based therapy in immunooncology?

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Active cell-based therapy involves several strategies how to induce anticancer immunity. In this context, the use of autologous monocyte-derived dendritic cells (DCs) as a means to elicit therapeutically relevant immune response in patients suffering from cancer has been extensively investigated throughout the past decades. Peripheral blood monocytes are transformed into immature DCs ex vivo, then exposed to various forms of tumor antigens (autologous tumor cell lysates, allogenic cell lines, mRNA or specific tumor-associated antigens) and after maturation (using various cytokines or TLR ligands) reintroduced into patients. Despite several encouraging clinical applications, existing DC-based immunotherapy efforts have yielded inconsistent results. In 2010, the first DC-based immunotherapy (sipuleucel-T, also known as Provenge®) has been approved by the US Food and Drug Administration (FDA) for use in humans. Reflecting the central position occupied by DCs in the immune response, the interest in harnessing them for the development of novel immunotherapeutic anticancer regimens remains high. Our group developed dendritic cell (DC)-based vaccines pulsed with high hydrostatic pressure (HHP)-inactivated allogenic tumor cells lines and matured by TLR-3 ligand, Poly-IC. These products – DCVAC/OvCa, DCVAC/PCa and DCVAC/LuCa have recently been tested in several phase II clinical trials in patients with various stages of ovarian, prostate and lung cancer with promising results. Most recent data from ongoing clinical studies will be presented in more details during the conference.

DOI: 10.1002/eji.201970500

Borrelia and complement system: an example of perfect anti-immunology

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Knowledge of host-pathogen interactions at molecular level is crucial for understanding of pathogenesis, disease prevention and cure. Here Borrelia is presented as a model neuroinvasive pathogen which employs vast immune evasion mechanisms (anti immune mechanisms) like - drastic change in antigenic proteins, complement regulatory protein binding, antigenic variation etc. It is proposed that Borrelia may employ multiple strategies to evade host's complement system by binding complement regulatory proteins like factor H, vitronectin, C4BP and CD59. Amplitude of these mechanisms, their pathogen species dependent disparity, and characterization of interacting proteins from both sides (host and pathogen) will be presented during VAS presentations. Borrelia is also capable of invading central nervous system (CNS) in hide in the immune privileged site – the CNS. To do so, it must cross the blood brain barrier (BBB). The mechanisms of the BBB crossing of this organism, like many of the other CNS invading pathogens, are still subject of on-going research. Our decade of research indicates the paracellular mechanism of the BBB penetration of Borrelia. Paracellular penetration of pathogen needs multiple protein: protein interactions between pathogen surface proteins and endothelial cells. The second part of my presentation will be dedicated to mechanisms employed by Borrelia to modulate cell signalling events in brain microvascular endothelial cells to cross the BBB. This presentation will also give sneak peak of several state-of-the-art technologies in genomics and proteomic used to understand the mechanisms of neuroinvasive and complement evasion by Borrelia.

Acknowledgment: Work presented here was supported by APVV-18-0259 and VEGA 1/0105/19.

DOI: 10.1002/eji.201970500

The influence of IL-7 on CD5 expression in CD4+ central and effector memory cells in norm and inflammatory model in vitro

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Introduction: Maintenance of T-cells on the periphery is occurred by two mechanisms: exit of new T-cells from the thymus and homeostatic proliferation. IL-7 is a key factor for survival and homeostatic proliferation of CD4+ central and effector memory cells. But it is unclear, whether the responsiveness of T-cell to IL-7 changes under the inflammation environment. Moreover, only T-cells with high affinity to self-antigens undergo homeostatic proliferation, so we investigated the expression of CD5 as a marker of TCR affinity. To provide inflammatory conditions in culture we developed inflammatory model in vitro.
Methods: The study included 10 healthy donors. Inflammatory model was based on activation of adherent fraction of PBMCs with LPC055 (10mkg/ml). CD4+45RO+CCR7+ (central, Tcm) and CD4+45RO+CCR7- (effector, Tem) memory cell was sorted with magnetic beads, columns from Miltenyi Biotec. Pure populations were cultured with non-activated and activated adherent fraction without or with IL-7 (50ng/ml) in trans-wells during 6 days. Prior to culture sorted cells were stained with CFSE (4mkM). After culturing, cells were collected and stained with monoclonal antibodies against CD4, CD45RO, CCR7, CD5 (BioLegend, USA). Analysis was performed on cytometer FACS Canto II (BD, USA) in Diva software.
Results: Under inflammatory condition responsiveness of Tcm to IL-7 did not differ from it in normal conditions. However, for Tem, the number of proliferated cells was increased in the inflammatory model compared to cells under the common IL-7 stimulation.
T-cells with high affinity of TCR to self-antigen have the high expression of CD5. Tcm characterized by a higher CD5 expression than Tem. After culturing without IL-7, expression of CD5 was downregulated on Tcm, but not Tem. In culture with IL-7, CD5 expression was similar as on cells before culturing, but there was an increasing in density of CD5. In inflammatory model in the presence of IL-7 frequency of CD5high Tcm was decreased. The absence of MHC contact led to decreasing of CD5high Tcm and Tem in both normal and inflammatory conditions.
Conclusion: IL-7 keeps maintenance of CD5high Tcm cells in normal but not in inflammatory conditions. Tcm and Tem with high affinity to self-antigen are sensitive to presence of MHC contacts.

The study was supported by RSF (project№ 18-75-00068)

DOI: 10.1002/eji.201970500

HLA analysis in celiac disease: HLA-DQ2 and HLA-DQ8 in Slovak pediatric patients with celiac disease

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Objectives: The incidence of HLA haplotypes associated with celiac disease (CD) was determined in the group of pediatric patients of the Slovak population with CD to assess the risk of antigens HLA-DQ2 and HLA-DQ8 to the development of CD, an importance of their examination in clinical practice and whether the double dose of the associated genes affects the predisposition to CD.
Patients and Methods: The group of patients included 66 patients with CD (40 females, 26 males) aged from 2 to 27 years with a histologically confirmed CD. HLA alleles/haplotypes were determined by PCR-SSP commercial kit (BAG Health Care GmbH, Germany). For statistical analysis were used chí2 test, Pcorr, Odds Ratio (OR) and validity tests.
Results and Conclusions: HLA haplotypes associated with CD had in the group of CD patients following frequencies: the haplotype with the allele DQB1*02:01 - 57.6%, the haplotype with the allele DQB1*02:02 - 36.4% and the haplotype with the allele DQB1*03:02 - 19.7%. Up to 89% of CD patients had in their genotype one of the associated HLA haplotypes. About 10% of patients with CD had no haplotype associated with predisposition to CD. The molecular immunogenetic analysis confirmed the association of the antigen HLA-DQ2 (allele DQB1*02:01+DQB1*02:02) with the CD in the Slovak population. The frequency of HLA-DQ2 in patients was 83.3% and in the control population 37.8% (OR=8.2). The antigen HLA-DQ8 was associated with CD only in DQ2 negative patients and represents an additional genetic risk with OR=7.4. The combined genetic risk for antigens HLA-DQ2+DQ8 for CD is characterized the following values in the Slovak population: the frequency in CD patients - 89.4%, frequency in the population - 44.8%, OR=10.4, specificity - 92%, predictive value of a positive finding - 89%. Patients with a double dose of the associated alleles HLA-DQ2 (composed homozygous DQ2) show a significantly higher risk of developing CD in comparison with patients which have only one dose of allele HLA-DQ2 (OR=2.7).

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DOI: 10.1002/eji.201970500

Misfolded tau protein induces activation and maturation of dendritic cells in vitro

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Objective: Recent studies suggest that the peripheral immune system plays a crucial role in imparting the "status quo" of the central nervous system. In Alzheimer's disease (AD), the peripheral immune cells, such as dendritic cells which constantly survey the CNS (Page et al., 2018), are exposed to misfolded proteins, including tau. In this study, we investigated if dendritic cells can process misfolded protein tau, one of the key proteins in the pathogenesis of AD and other tauopathies.
Methods: Confocal imaging, Flow cytometry, mixed leukocyte reaction, ELISA.
Results: We observed that dendritic cells (DCs) uptake misfolded truncated tau in a concentration dependent manner. Co-localization studies revealed that tau protein was distributed in the late endosomal and lysosomal compartments of DCs. Flow-cytometric analysis revealed the upregulation of CD40, CD80 and CD86 markers, suggesting that sensitization with tau induced activation and maturation of DCs in vitro. Furthermore, tau sensitized dendritic cells were able to activate T-cells in vitro.
Conclusion: Exposure of misfolded tau to DCs leads to its processing and activation and may lead to the infiltration of T-cells into the brain, thereby contributing to the pathogenesis in AD. On the other-hand, dendritic cells may induce the proliferation of T cells and immune response with subsequent reduction in neurofibrillary burden, which implicates the potential use of DCs in developing vaccine targeting tauopathies.

Acknowledgement: The work is supported by research grants APVV-17-0642, VEGA 2/0181/17 and Axon Neuroscience R&D Services SE.

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DOI: 10.1002/eji.201970500

Role of regulatory T and B cells in health and disease

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Basic characteristic of the immune system is its ability to distinguish self-molecules of cells, tissues and organs from non-self, to tolerate self and dispose of non-self. In the past, we concentrated on various defence mechanisms by which the immune system recognizes and destroys intruders of our integrity. Later, we paid attention to how the immune system tolerates our own antigens or regulates extent of ongoing reactions to prevent induction of autoimmune or allergic processes. Further, it was shown that the immune system played a decisive role in fighting malignant cells; however, on the other side, it was responsible for their evasion from destruction too.

Immunosuppressive mechanisms, especially those mediated by regulatory lymphocytes, play a paramount role in tolerance mechanisms. When there is an abnormal quantity and/or quality of regulatory cells, various autoimmune diseases are induced, e.g. SLE, RA, T1D, IBD, MS, and others. In recent years, a great progress was achieved in the field aspiring to profit from immunosuppressive properties of T regulatory cells ($Treg_s$) in the treatment of patients suffering from autoimmune disorders or transplantation rejections. Nowadays, several possibilities are available to up-regulate the function of patient′s Tregs or supplement their low numbers. We can up-regulate the function of Treg cells in an affected organism by using low dosage IL-2 treatment or we can treat patients by *in vitro* expanded Treg cells themselves. Induced Tregs are, however, polyspecific and therefore they have been preferentially used for the treatment of graft versus host reactions and some autoimmune disorders only. For the treatment of autoimmune diseases with known specific autoantigens, Treg cells equipped with antigen-specific chimeric T cell receptor (CARs) were introduced.

Treg cells also suppress anti-tumour immune response. There is accumulating evidence that the removal of Treg cells is able to enhance defence mechanism of anti-tumour immunity. Various cell surface molecules, such as CCR4 or CTLA-4, which are specifically expressed by effector Treg cells can be the candidates for depletion of effector Treg cells by monoclonal antibodies.

DOI: 10.1002/eji.201970500

The role of inflammation in gliomas

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Inflammation in tumors is considered one of the hallmarks of cancer. We analyzed markers of inflammatory response in peripheral blood of 63 adult Slovak patients with gliomas and 23 healthy controls. Among others, we investigated absolute number and percentage of blood monocytes, expression of TREM-1 and TREM-2 (triggering receptor expressed on myeloid cells) receptors on CD14+ blood monocytes (Mo) (by flow cytometry). The serum levels of soluble TREM-1 (sTREM-1), HMGB1 (high mobility group box protein) and plasma levels of IL-6 and IL-10 were determined by Elisa. For better specification of the state of inflammation, we determined also ratios of TREM-1 to TREM-2 (TREM-1 □ a proinflammatory, immunostimulatory and TREM-2 □ an anti-inflammatory with immune suppression activity) and IL-6/IL-10. As the TREM-2 is present only in tissues, so the detection of its level in the serum was worthlessness. The project was approved by local ethic committee and an informed consent was obtained from each patient. For statistical analysis we used GraphPad InStat and SAS programme.
Results: 1. Patients with GBM – G. IV had statistically significantly lower percentage of CD14+ monocytes than G. II gliomas (mean 7.0 vs. 4.69; P=0.001). The difference between GBM and healthy controls was also statistically significant (mean 4.69 vs. 5.99; P=0.05). 2. The range of CD14+ TREM-1+ Mo in healthy subjects was from 15.85% to 80.8% (mean 44.33%; median 41%). In the group of glioma patients, we found a subgroup of patients with a higher percentage of TREM-1+ Mo than in healthy controls. We determined the value of cut-off for TREM-1+ Mo as 55%, and 145 for the TREM-1/TREM-2 ratio. Very interestingly, patients with values above these both cut-offs survived shorter. Better surviving patients with GBM had a higher percentage of CD14+ TREM-2+ Mo (median 0.55 vs. 0.10; P=0.013). 3. The Kaplan-Meier survival curves for grade II and III gliomas shows shorter survival in patients with percentage of TREM-1+ Mo > 55% (P=0.024). The survival curves for patients with grade IV gliomas – GBM showed shorter survival with both percentage of TREM-1+ Mo > 55% (P=0.050) and TREM-1/TREM-2 ratio > 145 (P=0.022). 4. We found higher percentage of CD14+ TREM-2+ cells in better surviving patients in GBM subgroup (P=0.013). We suppose, that TREM-2+ monocytes arise as a contraregulatory subpopulation of cells with the effort to downregulate the exaggerated inflammation – both systemic and local and potentiate the phagocytosis in brain tumor. 5. The serum levels of sTREM-1 in group of all glioma patients as well as in GBM subgroup were significantly lower than in healthy controls (median 29.96 vs. 42.95, P=0.001; median 31.58 vs. 42.95; P=0.013 resp.). There was no statistically significant difference in serum levels of HMGB1 between glioma grades and between glioma patients and healthy controls. 6. We found a negative correlation of the serum levels of HMGB1 in glioma patients with both percentages of CD14+ TREM-1+ cells (P=0.036) and TREM-1/TREM-2 ratio (P=0.002). Very interesting was the finding of a positive correlation of the level of HMGB1 in glioma patients with percentage of CD14+ TREM-2+ Mo (P=0.005). These correlations were observed also in a subgroup of GBM patients; the negative correlation of HMGB1 with the percentage of CD14+ TREM-1+ cells had the P-value 0.015 and for negative correlation of HMGB1 with TREM-1/TREM-2 ratio (P=0.008).

DOI: 10.1002/eji.201970500

Modulation of the conformational ensemble of intrinsically disordered protein tau upon the interaction with the antibody against its C-terminus

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Alzheimer's disease (AD) represents with respect to the worldwide trend of population ageing, with its main risk factor being an age over 65, a serious medicinal, economic and social problem. The main hallmark of AD is the accumulation of intrinsically disordered proteins tau and amyloid- ß in the form of toxic insoluble aggregates.
The structural insights into the pathological and physiological tau protein conformations may help by the answering the key questions of the pathogenesis of AD and other tauopathies. Among tau areas that are worth of investigation there is particularly its C-terminus, because it was shown that the C-terminal part of tau molecule significantly inhibits the aggregation of tau protein into amyloid structures [1]. Phosphorylated amino acids in the C-terminal domain of tau protein (pS396, pS404, pS413, pS422) are currently being tested as targets of passive and active immunotherapy. Recently several structures of phosphopeptides from C-terminal domain of tau in complexes with phosphospecific antibodies were published [2], but the structure of tau peptide from its extreme C-terminus is still missing.
In this work we have been studying the kinetics of the interaction of an antibody DC39C, which epitope lies inside the last 12 C-terminal amino acids of tau, with various tau proteins by surface plasmon resonance. The kinetic measurements suggest the contribution of remote parts of tau molecule to its binding to DC39C antibody, what we want to explain using synergistic structural biology approaches such as X-ray crystallography, NMR and molecular dynamics simulations to investigate the conformational ensemble of tau protein in free form and in complex with DC39C antibody.
The use of several approaches of structural biology can facilitate the more complex insight into the conformational properties of intrinsically disordered protein tau and its pathological involvement in AD.

Acknowledgement: This work was supported by the Vega grant number 2/0145/19.

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DOI: 10.1002/eji.201970500

Immune profile following acute spinal cord injury

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In the spinal cord, innate immune surveillance is mainly coordinated by resident microglia and blood-borne immune cells. Their role is to monitor harmful agents that would disrupt homeostasis of spinal cord tissue. In present study, based on cytokines arrays, confocal imaging we have analyzed immune profile following acute spinal cord injury in spatial-temporal manner.
We have observed that rostral (R1) and caudal segments (C1) of spinal cord nearby the lesion express inflammatory factors whereas distant segments, i.e. R2-R3 and C2-C3, co-express factors implicated in neurogenesis. In this context, striking differences in the number and morphology of microglia cells were detected during acute SCI. The extent of microglia activation was significantly higher in gray than in white matter tracts in the C1 segments during the 3–7 days after injury. The predominance of microglia activation in caudal segments correlated with severe inflammation associated tissue damage taking place in this segment. In contrast to tissue resident microglia, neutrophils which are the first inflammatory cells to arrive at the site of injury peaked at 3days after SCI. We have detected delay in T regulators recruitment between R1 and C1 segments. Recruitment of regulatory T cells (Treg, Foxp3-positive) firstly occurred at 3 days in the rostral segment and peaked at 7 days in both rostral and caudal sites. These results are in line with the presence of CCL20 (responsible for recruitment of Treg) in both segments. Moreover, the time difference of recruitment between rostral and caudal segments of Treg together with neurites outgrowth inhibitors can also contribute to the reduced neurite outgrowth in C1 compared with R1. Present data shows dynamic segmental changes in immune cell profile following SCI and open the door of a novel view of the SCI treatment by considering the C1 as the therapeutic target.

Supported by: APVV 15-0613 (DC), Stefanik (MS), ERANET- AxonRepair.

DOI: 10.1002/eji.201970500

Late-Onset combined immunodeficiency with compound heterozygous mutation in FOXN1 gene

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Functional deficiency of FOXN1 (forkhead box N1), a transcription factor essential for the development and function of thymic epithelial cells, is associated with a nude severe combined immunodeficiency (SCID), a rare inherited syndrome with low T cells and hypogammaglobulinemia. The precise molecular mechanisms and function of this transcription factor are not completely understood. It is thought to be activated by phosphorylation, translocate to the nucleus, bind DNA through its forkhead domain and promote the transcription of genes that are critical for control of development of epithelial cells. Only few clinical cases have been described yet. FOXN1 protein is expressed in epithelial cells of the thymus, hair follicles, skin and nails. Common clinical features are absent thymus (athymia), alopecia universalis and nail dystrophy.
We present a 9-year-old boy with a history of persistent EBV infection since 2nd year of life. At age of 6 years he developed Burkitt lymphoma and underwent successful oncologic treatment. Further evaluation unveiled low T cell numbers with low TREC copies (T-cell Receptor Excision Circles) in peripheral blood. Retrospective testing of neonatal Guthrie card also confirmed low TREC copies, pointing to primary defect. Flow cytometric analysis showed near complete absence of CD45RA+ CD4 T cells, low number of recent thymic emigrants and oligoclonal T-cell repertoire. Surprisingly PHA response on separated T cells was normal. By whole exome sequencing a compound heterozygous mutations p.C82X and p.P350L have been detected in FOXN1 gene. Causality of mutation was later confirmed by reduced transcriptional activity testing. Patient underwent allogeneic thymus transplantation at Great Ormond Street Hospital in London as the first Slovak with transplanted thymus. Recently, 6 months after the transplantation, the immune reconstitution is carefully monitored.

DOI: 10.1002/eji.201970500

Asthma treatment response to inhaled corticosteroids is associated with variants in VEGFA gene

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Background: Asthma is one of the most common chronic diseases, characterized by airway inflammation and structural remodeling. Inhaled corticosteroids (ICS) are commonly used in asthma treatment, since they suppress airway inflammation. However, the response to ICS treatment is highly variable. Vascular endothelial growth factor (VEGFA) is a major regulator of angiogenesis and vascular permeability and has been shown to be elevated in asthma patients. Since inhibition of VEGFA also diminishes asthma symptoms in mice, it is predicted that variants in VEGFA gene could be associated with asthma treatment response.
Materials and methods: We genotyped two variants, rs2146323 and rs833058, in VEGFA gene in 208 adult asthma patients treated with ICSs. The percentage change in FEV1, % predicted was analyzed after short-term treatment (3 months) and long-term treatment (at least 3 years). Similarly, changes in Asthma Control Test (ACT) and Asthma Quality of Life Questionnaire (AQLQ) were determined after at least 3 years of treatment.
Results: Variant rs2146323 in VEGFA was associated with response to ICS treatment in adult asthmatics. Patients with the CC+AC genotype show higher improvement in % predicted FEV1 after three months and after at least three years, compared to AA genotype. Additionally, patients with CC+AC genotypes had a higher improvement in ACT and AQLQ scores. Genotype-dependent differences in treatment response were evident when analyzing entire group of patients as well as non-atopic patients, suggesting that treatment response was influenced by the atopy. Conversely, among smokers, the CC+AC higher improvement, compared to AA genotype was observed in % predicted FEV1 after short-term treatment and in improvement of ACT and AQLQ scores. Similarly, in variant rs833058, TT+CT genotype shows better treatment response to ICS, compared to CC genotype. However, the difference is only statistically significant in ACT and AQLQ scores.
Conclusion: Our study showed that variants rs2146323 and rs833058 in VEGFA are associated with treatment response to ICS after short-term and long-term treatment, assessed as changes in % predicted FEV1, ACT and AQLQ scores. The difference in treatment response is highly influenced by atopy and only evident in non-atopic patients.

DOI: 10.1002/eji.201970500

Association of MMP3 rs3025058 with clinical findings of Alzheimer‘s disease

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Alzheimer’s disease (AD) is a chronic neurodegenerative disease affecting mostly elderly people over 65 years of age. The disease accounts for the most common type of dementia characterised by progressive memory loss, confusion and cognitive impairment. Pathogenesis of AD is not exactly explained until now. Besides the accumulation of modified proteins (tau protein, beta amyloid) in the brain, the neuroinflammation also contributes to disease progression. Matrix metalloproteinases (MMPs) belong to zinc-dependent proteases that promote the cleavage of extracellular matrix resulting in the migration of immune cells into the brain parenchyma and induction of inflammation. The aim of our study was to analyse the association of MMP3 gene polymorphism rs3025058 affecting its expression level with susceptibility to AD and clinical findings in Slovak patients. We genotyped 139 patients with late-onset form of AD (age: 80,04 +/- 6,02 years) and 176 control subjects without neurological disorders (age: 71,46 +/- 8,62 years). The analysis of MMP3 rs3025058 in the promotor at position -1171 (5A/6A) was performed by PCR-RFLP method. Direct sequencing was used to determine the presence of ApoE-ε4 variant, which is a known genetic risk factor for AD development. The statistical significance of differences in allele and genotype frequencies between AD patients and controls was evaluated by the standard chi-square test. The linear regression analysis was used to investigate the correlation between observed MMP3 genotypes and main clinical features as the age at disease onset, MoCA score and disease duration. No statistically significant differences in allele and genotype frequencies of the MMP3 rs3025058 variant between the AD patients and control group were observed ($P \geq 0.05$). However, the analysis of investigated clinical findings with MMP3 rs3025058 genotypes revealed that 5A/6A carriers had lower age at disease onset as compared to AD patients carrying 5A/5A and 6A/6A genotypes (76.82±5.76 vs 79.69±6.34, $P = 0.024$). By contrast, homozygous genotype 5A/5A tend to protect AD patients against earlier disease onset. Analysis in APOE-ε4 positive AD group showed that 5A/6A and 6A/6A carriers tend to achieve significantly lower MoCA score as compared to AD patients carrying 5A/5A genotype (13.75±6.29; resp. 9.25±3.7 vs 17.58±2.81, $P = 0.023 – 0.043$). To conclude, our preliminary results have shown an association of MMP3 5A/6A genotypes with lower age at disease onset, thus suggesting the potential of MMP-3 to modify progression status in AD patients.

The study was financially supported by the Grant Agency VEGA (Project no. 1/0240/16).

DOI: 10.1002/eji.201970500

Molecular mimicry in ankylosing spondylitis, rheumatoid arthritis and multiple sclerosis

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The cause of ankylosing spondylitis (AS), rheumatoid arthritis (RA) and multiple sclerosis (MS) has been investigated by "molecular mimicry".
HLA-B27 occurs in over 90% of AS patients but in only 8% of healthy blood donors. HLA-B27 cross-reacts with KLEBSIELLA and antibodies to this bowel microbe have been found in active AS patients in 17 different countries.
HLA-DR4 occurs in over 80% of RA patients but in only 30% of blood donors. HLA-DR4 cross-reacts with the urinary microbe PROTEUS and the enzyme PROTEUS urease resembles hyaline cartilage. Antibodies to PROTEUS have been reported in active RA patients in 16 different countries.
MS patients suffer preferentially from lower limb paralysis compared to upper limbs and this resembles hind-quarters paralysis in cattle affected by "bovine spongiform encephalopathy" (BSE), also known as "mad cow disease".
The nasal microbes ACINETOBACTER and PSEUDOMONAS have enzymes such as carboxy-mucono-decarboxylases which cross-react with myelin. MS patients are known to have antibodies myelin. An examination of 79 active MS patients showed elevated levels of antibodies to ACINETOBACTER and PSEUDOMONAS. Similar observations were made in 157 BSE affected animals when compared to controls.
The results indicate that AS is caused by the bowel microbe KLEBSIELLA, RA is caused by the urinary microbe PROTEUS and MS is caused by the nosocomial, nasal microbes
ACINETOBACTER and PSEUDOMONAS.

DOI: 10.1002/eji.201970500

Can a standardized and evidence-based plant immunomodulator increase the effects of MEK and BRAF inhibitors with clinical benefit?

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Background: Targeting hyperactive mitogen-activated protein kinase (MAPK) signalling cascade has proven to be an effective treatment for a variety of different cancers. Using an important member of this cascade, namely MEK (mitogen activated extracellular signal regulation kinase) inhibitors, the clinical responses are often transient and complete remission is rarely observed. Outgrowth of resistant clones within progressed tumors appears to be inevitable. Recent immunological and clinical observations suggest that in background of this resistance the tumor-induced disturbance of immunoregulation at least in part may play a role. Namely, it was shown that growth factor receptor signalling pathway inhibitors can increase the immune sensitivity of tumor cells by activating the stress-related molecules (such as MICA and MICB), but they can't activate the downregulated immune effectors. Consequently, the combination of MAPK cascade signalling pathway inhibitors and the immune effectors activating immunomodulators may be a promising new strategy.
Material and Methods: In a now 59 years old patient with inoperable (BRAF-mutant) low differentiated adenocarcinoma of biliary ducts after 30GY radiotherapy and two cycles (Gemcitabin+ Cisplatin) chemotherapy a rapid progression of lung, liver and brain metastases were established by CT and MR. Thereafter, a treatment with BRAF+MEK inhibitors (2x150 mg dabrafenib and 1 x 2 mg trametinib) was started. These inhibitors were combined with daily 45 mg/kg rice bran arabinoxylan concentrate (using Biobran/MGN-3) which was shown to be a pathogenic associated molecular pattern (PAMP)-like molecule and can stimulate the type-1 innate immune cells against tumor cells in a MHC-I unrestricted manner as well as has clinical evidence too based on randomized double blind trial.
Results: After the chemotherapy and prior to the start of second line treatment, the patient had a nearly terminal state of her rapidly progressive disease. Eight months after the combination of MEK / BRAF inhibitor and immunomodulator therapy nearly complete remissions of all metastases was established in CT and MR.
Conclusion: This case report may support a hypothesis that MEK/BRAF inhibitors and type-1 immune cells activating immunomodulators together may synergistic inhibit the tumor growth. Further clinical investigations are necessary to clarify this question.

DOI: 10.1002/eji.201970500

Ragweed epidemy in Central Europe - current situation in south-west Slovakia

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Background: Despite ragweed is not natural in Europe, in last decades the contamination of environment with its pollen is alarming and the number of suffering patients is constantly increasing. In our outpatient department we monitor the changes in sensitisation profile of patients during last 20 years.
Methods: Patients with pollinosis are routinely tested by prick tests and specific IgE. In the past only extract-based diagnostics were used, in last years, we use also molecular (component resolved) diagnostics. We compared the sensitisation profile of our patients evaluated in years 1999 - 2002 and in the year 2018. More than 600 randomly selected patients were included. As clinically relevant we rate allergen which is positive in prick test and patient has symptoms during pollen season of positively tested allergen.
Results: In the year 1999 we rated as clinically relevant grasses in 72%, birch in 38% and mugwort or ragweed (Asteraceae family) in 31%. In these years only extract diagnostic was made, in all patients prick test was performed (Stallergenes diagnostic allergens). In patients sensitised by Asteraceae pollen we evaluated mugwort more clinically relevant than ragweed. The results were similar when evaluating percentage of positive specific IgE antibodies (sIgE). But interestingly we find out that the amount of ragweed sIgE (kU/L) was significantly higher than that of mugwort antibodies.
In 2018 there was no significant difference in sensitisation by grass (80%), birch (78%), ragweed (78%) and mugwort (72%) pollen according to prick test. Again, the result was similar when using sIgE diagnostics with pollen extracts. But the result was completely different when using molecular diagnostics. Main birch allergen Bet v1 was positive in 90% of patients with average value o sIgE16,65 kU/L, main grass allergen Phl p1 was positive in 83% patients with sIgE 8,15 kU/L, main ragweed allergen Amb a1was positive in 73% patients with sIgE 18,96 kU/L, main mugwort allergen Art v1 was positive only in 41% with sIgE value only 2,63 kIU/l.
The percentage of polysensitised patients increased significantly during years. In 1999 - 2002 it was 55 %, in 2018 it increased to 82,5 %.
Conclusion: We see shift to polysensitisation in pollen patients in Bratislava. We see also the increasing clinical importance of ragweed pollen in our region. According to sIgE results to main allergens we suppose higher clinical aggressivity of ragweed pollen. Following our results, we emphasise the urgent need for allergen immunotherapy preparation containing ragweed pollen extract.

DOI: 10.1002/eji.201970500

Heat shock protein 70 and anti-heat shock protein 70 antibodies in patients with chronic glomerulonephritis

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Heat shock protein 70 (Hsp-70) is an important part of the intracellular defense system, that fulfill a range of functions, including cytoprotection from damage. Moreover, the unique properties of heat shock protein Hsp70 provide an important immunoregulatory function in differentiation of specific regulatory anti-inflammatory phenotypes T cells (Foxp3 positive). Failure of this function may occur in immune diseases, including chronic glomerulonephritis (CGN).
Our study aim was to evaluate Hsp 70 levels in the urine and the anti-Hsp70 antibody levels in serum and to assess Hsp70, Foxp3 expression in renal tissue of patients with chronic glomerulonephritis.
Methods: Seventy-six patients with CGN patients were included in our study. Ten patients with mild proteinuria (median 0.48 [0.16-0.78] g/day) and 10 healthy subjects served as controls. 34 active CGN - with proteinuria (PU) ranged from 1 to 3 g/day (I group), 42 - with nephrotic syndrome (NS) (II group), including 18 pts with severe NS (PU more than 10 g/d, hypoalbuminemia < 20 g/L). Urinary levels of HSP70, interleukin-10, and serum levels of anti-HSP70 were measured by ELISA. The immunohistochemical peroxidase method was used to study the expression of HSP70 and Foxp3+ in kidney biopsies. Treg FoxP3+ cells in the interstitium was determined morphometrically.
Results: Median urinary HSP70 levels in patients with nephrotic syndrome (NS) group II [6.57 (4.49-8.33) pg/mg] and group I [5.7 (4.12-6.9) pg/mg] were higher ($p<0.05$) than in positive [3.7 (2.5-4.82) pg/mg] and negative [3.78 (2.89-4.84) pg/mg] controls. Hsp-70 levels in the urine in group II - significantly higher than in group I ($p<0.05$). HSP70 expression index in tubular cells positively correlated with urinary HSP70 ($Rs=0.948$, $p<0.05$) and proteinuria ($Rs=0.362$, $p<0.05$). The number of Treg Foxp3+ cells in the kidney interstitium and interleukin-10 excretion were decreased in patients with NS. Anti-HSP70 antibodies serum levels in patients with NS – group II [21.1 (17.47-29.72) pg/ml] and subnephrotic range proteinuria – group I [24.9 (18.86-30.92) pg/ml] were significantly higher than in positive [17.8 (12.95-23.03) pg/ml] and negative [18.9 (13.5-23.9) pg/ml] controls.
Conclusions: Hsp70 urinary and tissue levels increased in patients with active CGN, especially in severe nephrotic syndrome. However, activation of HSP70 in patients with nephrotic syndrome did not lead to an increase in tissue levels of $Treg^{Foxp3+}$ cells or release of IL-10. These data may indicate an impaired anti-inflammatory function of HSP70 in patients with a severe nephropathy.

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DOI: 10.1002/eji.201970500

High fat diet and its influence on transgenic rodent model of tauopathy

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The brain is an organ that consists of 60 percent fat and is one of the most energy intensive organs. Clinical observation studies suggest that imbalance in fatty acid intake may be associated with impaired brain performance and disease. A growing body of evidence suggests a causal role for high fat intake in the process of neurodegeneration, specifically in the pathogenesis of Alzheimer's disease1,2. Epidemiological studies show a direct relationship between high cholesterol and Alzheimer's disease. The presence of the ApoE ε4 allele contributes to the development of neuropathological changes in Alzheimer's disease and other tauopathies and increases the risk of frontotemporal dementia. Also noteworthy is the observation that impaired cholesterol metabolism in Newmann's Pick's type C leads to tau phosphorylation and neurofibrillary aggregate formation. However, the relationship between fat diet and tau pathology is least investigated. In this study, we subjected a transgenic rat model of tauopathy and age matched controls to high fat diet and monitored their status. Preliminary results show that the transgenic model of specifically preferred the high fat diet only at months 2 and 3, but not at 1 m or at 4 months of age when compared to controls. There was no difference in the levels of water consumption. Interestingly, the weight of the control rats on high fat was higher than the transgenic rodent at the age of 2 and 3 months. On the other hand, the transgenic rodent model showed consistent improvement in cat walk performance than the control rodents on high fat diet. These results suggest that high fat diet may improve the behavioural deficits in neurodegenerative tauopathies.

The work is supported by research grants: VEGA 2/0181/17.

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DOI: 10.1002/eji.201970500

Role of TFH cells in abnormal B cell distribution and disease severity in primary Sjögren's syndrome

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Background: B-cell hyperactivity and increased levels of high-affinity autoantibodies are the hallmarks of primary Sjögren's syndrome (pSS). TFH cells play a crucial role in directing B-cell differentiation toward memory B-cells and plasma cells. Since our former investigations underlined their importance in pSS development, the aim of the present study was to further investigate the functional role of circulating (c)TFH cells in B cell differentiation and determine the proportions of different cTFH subsets.
Method: We enrolled 39 pSS patients and 29 healthy controls in our study. Proportions of cTFH subsets, follicular regulatory T (TFR), and B-cell subsets were measured by flow cytometry. In functional assay, after cell isolation, B cells were co-cultured with CD4+CXCR5- T cells and CD4+CXCR5+ cTFH cell in the presence of staphylococcal enterotoxin B (SEB). In order to block T-B cell interaction, anti-human IL-21 and anti-human CD40/TNFRSF5 were used. At day 7, cells were collected and analysed by flow cytometry, and concentrations of IgG and IgM were also measured in supernatant.
Results: Compared to controls, pSS patients showed a reduction in cTFH2 subset which associated with disease severity and a shift towards cTFH1-1/17. The ratio and absolute number of cTFR cells were higher in pSS patients with extraglandular manifestations (EGMs) compared to patients with glandular symptoms only. The ratio and number of naïve and transitional B-cells were increased, while memory B-cell subsets decreased and associated with a more severe disease course. The diminished cTFH2 cell ratio correlated positively with memory B-cell percentages but showed a negative correlation with naïve B-cell proportions. Results of the functional analysis revealed that simultaneous treatment with anti-IL21 and anti-CD40 decreased the production of IgG in cTFH-B cell co-culture.
Conclusion: Imbalance in TFH subsets and TFR-cells associates with derailed B-cell homeostasis contributing to the pathogenesis and characteristic serological manifestations of pSS. Modulation of these cells could be a potentially powerful element in the novel therapeutic approaches.

The work was supported by Grants No. K124177 and PD121327 from the National Research, Development and Innovation Office (Hungary) (NKFIH).

DOI: 10.1002/eji.201970500

Familiar Mediterranean Fever – Complex Analysis of Slovak Cohort

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Introduction: Familiar Mediterranean Fever (FMF) represents the most common and famous form of monogenic hereditary periodic fever syndromes associated with the processes of autoinflammation. It belongs to the one of the 9 categories of primary immunodeficiencies – autoinflammatory diseases. Its prevalence is high in typical endemic areas, however, its frequency in the Central Europe was unclear up to now.
Patients and Methods: We analysed the clinical and laboratory characteristics of the Slovak cohort of FMF patients followed in National Centre for Periodic Fever Syndromes in Martin. We evaluated the frequency of clinical symptoms and complications and selected laboratory parameters.
Results: Altogether, 70 patients (30 males, 42.9%, age range 2 – 74 years) with genetically confirmed FMF. Regarding the genetics, 3 were homozygotes, 13 compound heterozygotes, 39 heterozygotes for pathogenic mutation (pseudo-autosomal dominant inheritance) and 15 patients the mutation with unclear or benign meaning was detected. The most common clinical symptom was recurrent severe abdominal pain (100%) followed by recurrent fever (94.3%) and intense fatigue (78.6). Among the other symptoms, the frequency was as follows: arthralgia/arthritis 68.6%, chest pain 50%, cervical lymphadenopathy 34.3%, recurrent tonsillitis 30%, headache 25.7%, and skin rash 15.7%. Pleuritis was confirmed in 18.6%, pericarditis in 10% and ascites in 22.9%. Majority of the patients were treated with colchicine (88.5%) with side effects and intolerance in 5 of them. Episodic application of anakinra was used in 32.9% and regular treatment with canakinumab was administered in 9 (12.9%) patients. Elevation of serum amyloid A between the flares was found in 37.1% and elevated IgD in 10%. Positive geographic family history for FMF was confirmed in 11 (15.7%).
Conclusions: FMF does not seem to be as rare in the Central Europe as it was previously suggested. We report the results from the biggest cohort of FMF patients in these regions and we strongly support the inclusion of FMF in the differential diagnostic algorithms of recurrent fever and abdominal pain also in the region of Central Europe.

DOI: 10.1002/eji.201970500

Foliate lymphoid aggregates – novel form of lymphoid organoids of the serosa in mice involved in the peritoneal dissemination of high-grade B-cell lymphoma

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In contrast to the mucosal side of the intestines, the immunological characteristics of the serosal surface within the peritoneal cavity are less investigated, even though they play an essential role in local inflammations and peritoneal tumor propagation. Previously omental milky spots (MS) and fat-associated lymphoid clusters (FALC) within the mesentery as two variants of lymphoadipose tissues have been identified and characterized.
Comparing to well-defined secondary lymphoid tissues, both MS and FALC appear as diffuse leukocyte congregates lacking lymphoid compartmentalization. In our study, we investigated the organization and structural characteristics of serous lymphoid tissue in the peritoneal cavity.
Analysis of the peritoneal metastatic sites of intraperitoneally injected high grade B-cell lymphoma Bc.DLFL1 in BALB/c mice (by in vivo bioimaging of XenoLight DiR near-infrared fluorescence dye-labeled or whole-mount anti-FITC immunohistochemistry of CFSE-labeled lymphoma cells) revealed selective binding sites along the mesentery and the omentum. These sites include a novel type of leukocyte congregates we termed Foliate Lymphoid Aggregates (FLAg) owing to their morphological features. Their main body consists of a flattened leaf-like part completely covered by mesothelial cells, which connects either directly to the adipose tissues or via a slender stalk to the peritoneal ligaments of omental bursa. These FLAgs demonstrate efficient binding of both B-cell lymphoma and normal B cells. Whole-mount immunohistological analysis demonstrated that FLAgs consist of tightly compacted CD45-positive cells, where a centrally positioned T-cell domain is surrounded by a B-cell rich ring, corresponding to the location of CCL21 and CXCL13-positive regions, respectively. Moreover, CD138-positive plasma cells were also identified within the FLAg. At the edge of FLAgs LYVE-1-positive macrophages reside and may act as lymphoma-binding partners sensitive to clodronate-liposoma mediated depletion. The stromal architecture of FLAgs includes a centrally positioned network of VCAM-1-positive reticular cells and extensive vasculature containing CD31-positive endothelial cells surrounded by pericytes expressing podoplanin/gp38 and fibroblast activation protein FAP, while lymphatic capillaries were absent. Furthermore, within the vasculature we also found short PNAd-positive segments, prompting us to investigate the effect of L-selectin inhibition on the serosal homing of blood-borne leukocytes. We found a significant reduction of lymphocyte lodging to the omentum, albeit to a lesser degree compared to lymph nodes. Quantitative real-time PCR analysis also revealed the presence of mRNA for several PNAd core proteins and glycosylation enzymes necessary creating of MECA-79 PNAd glycopepitope. These findings demonstrate that the serous lymphoid formations also include FLAgs as rudimentarily organized lymphoid tissues with effective seeding from the peritoneal cavity and partially L-selectin dependent homing for the blood-borne leukocytes.

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DOI: 10.1002/eji.201970500

'Silent' respiratory infections in Algerian adult asthmatics: preliminary data

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Background: Asthma is a chronic inflammatory airway disease. Many factors have been reported to aggravate asthma symptoms including respiratory infections (RIs). These latter may cause asthma exacerbations and may lead to emergency room visits and hospital admission.
Objectives: i) To investigate respiratory infections in asthmatic adults from Tizi-Ouzou city. ii) To determine antibiotic sensitivity of isolated germs.
Methods: Asthmatic patients were recruited from the pulmonary department of the Mohamed Nedir university hospital after completing an asthma questionnaire. Sputum samples from 13 adult asthmatics without apparent symptoms for RIs and 5 healthy subjects were collected and sputum cytology and culture (bacterial and fungal infection) were performed. All identified bacteria were tested for antibiotic sensitivity to 16 antibiotics.
Results: Sputum cytological analysis showed the presence of leucocytes cells including eosinophils in 7 samples (> 53%) from asthmatic patients. Epithelial cells were detected in 9 asthmatic samples (> 69%). Bacterial germs were found in 5 (> 38%) asthmatic samples including Staphylococcus aureus, Klebsiella pneumonia, and Citrobacter freundii. However, Candida albicans was the only fungal germ found in 6 (> 46%) asthmatic samples. Interestingly, bacterial infection was found to be associated with high sputum total leukocyte values and high annual rate of asthma hospitalizations. No germs were found in non-asthmatic samples. Among tested antibiotics, only second generation cephalosporins (cefoxitin), aminoglycosides (gentamycin) and tetracyclines were effectives on all isolated bacteria.
Conclusion: Our results show the presence of germs in the sputum of adult asthmatics without RIs symptoms. Moreover, RIs in adult asthmatics are associated with asthma hospitalization. Antibiotics may be a beneficial therapy to reduce asthma exacerbation and hospitalization. Sputum analysis and culture as a routine test in pulmonary department may help health professional to reduce asthma exacerbation.

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DOI: 10.1002/eji.201970500

The perspectives of use of bacteriophages in the treatment of antibiotic-resistant infections

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Global antibiotic resistance is increasing worldwidely and on the other hand only few new antibiotics were placed on the market. However, the number of infectious diseases - both local and general - is rising sharply. Some authors suggest that we have reached “post antibiotic era “, and focus must be shifted towards alternative therapeutic modalities. As an alternative to the treatment of bacterial infections with antibiotics, "natural predators" – phages or bacteriophages appear.
Bacteriophages are organisms capable of naturally attacking and killing bacteria. The advantage of their use in clinical practice is the fact that antibiotics cannot create resistance to these organisms; bacteriophages are highly specific and only infect a particular bacterial strain, which protects the natural microflora of the organism, and last but not least, the preparation of bacteriophage-containing preparations is cheaper and because of their "self-amplification" ability much faster than the development of a new antibiotics.
In fact, phages have an ability to induce a specific immune response and induce the production of specific autoantibodies directed against bacteriophage antigens. They also have non-specific immunomodulatory activity and affect the processes of phagocytosis, respiratory outbreak, but also the production of several cytokines and the production of antibodies against non-phage antigens.

Study was supported by the project of Ministry of Education, Science, Research and Sport of the Slovak Republic 2018/14631:8-26C0 “Development of new topical application systems containing specific bacteriophages as highly specific antibody”.

DOI: 10.1002/eji.201970500

Mature dendritic cells correlate with favorable immune infiltrate and improved prognosis in ovarian carcinoma patients

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A high density of tumor-infiltrating CD8+ T cells and CD20+ B cells correlates with prolonged survival in patients with a wide variety of human cancers, including high-grade serous ovarian carcinoma (HGSC). However, the potential impact of mature dendritic cells (DCs) in shaping the immune contexture of HGSC, their role in the establishment of T cell-dependent antitumor immunity, and their potential prognostic value for HGSC patients remain unclear. We harnessed immunohistochemical tests and biomolecular analyses to demonstrate that a high density of tumor-infiltrating DC-LAMP+ DCs is robustly associated with an immune contexture characterized by TH1 polarization and cytotoxic activity. We showed that both mature DCs and CD20+ B cells play a critical role in the generation of a clinically-favorable cytotoxic immune response in HGSC microenvironment. In line with this notion, robust tumor infiltration by both DC-LAMP+ DCs and CD20+ B cells was associated with most favourable overall survival in two independent cohorts of chemotherapy-naïve HGSC patients. Our findings suggest that the presence of mature, DC-LAMP+ DCs in the tumor microenvironment may represent a novel, powerful prognostic biomarker for HGSC patients that reflects the activation of clinically relevant anticancer immunity.

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DOI: 10.1002/eji.201970500

Cystatins in non-canonical NLRP3 inflammasome activation and sepsis

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Cystatins are inhibitors cysteine cathepsins and legumain that exert various physiological functions. In our study we used stefin B-deficient mice, as well as mice with an additional copy of stefin B gene, stefin B- trisomic mice and cystatin C-deficient mice and compared the signaling pathways upon induction of sepsis. Both, stefin B-deficient and cystatin C-deficient mice were significantly more sensitive to the lethal LPS-induced sepsis, due to increased caspase -11 expression.
We demonstrated that the increased expression of caspase-11 in stefin B-deficient macrophages was not due to activation of TRIF pathway and type I interferon signalling, but to NF-κB activation. Upon Nlrp3 inflammasome activation, the amount of mitochondrial ROS in stefin B- and cystatin C- deficient macrophages was greater than that in wild -type macrophages. Treatment with LPS induced autophagy in wild-type but less in stefin B deficient macrophages, as shown by immunoblot and confocal microscopy analysis. Stefin B deficient macrophages also had less p62/SQSTM1 protein upon LPS stimulation than did wild-type macrophage, that resulted in impaired mitophagy and increased ROS formation. In stefin B-trisomic macrophages, we determined diminished caspase-11 expression upon LPS stimulation, however, additional copy of stefin B gene did not protect animals form LPS induced sepsis.

DOI: 10.1002/eji.201970500

Human Leucocyte Antigens Class II Alleles in Patients with Tuberculous Pneumonia in East Latvia

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Tuberculosis (TB) is a chronic disease that still is one of the most common infectious death causes worldwide. Latvia has a relatively high TB incidence in comparison to other European countries. TB incidence in Latvia decreased during the last 30 years, but the mortality level of TB remains significant. It is defined that Human Leucocyte Antigens (HLA) Class II region of the Major Histocompatibility Complex impacts functions of the adaptive immune system. Studies confirmed that the HLA Class II alleles are among the factors impacting susceptibility for TB. At the same time, protective and risk alleles vary across populations and regions.

This study identified markers of genetic predisposition to TB in East Latvia. The sample included 40 patients of Daugavpils Regional Hospital (26 males and 14 females) aged between 18 and 85 (mean age 50) with bilateral, bacteriologically confirmed, drug-sensitive TB pneumonia and without HIV infection. HLA typing was performed in HLA-DRB1, -DQA1, and -DQB1 loci by a polymerase chain reaction with low-resolution sequence-specific primers. DNA extraction was performed by using QIAamp® DNA Blood Kit. Amplification was performed by thermocycler "DT-Lite". To determine protective and risk alleles of the HLA Class II gene, a control group of 100 adults (aged from 18 to 65, without active TB or HIV) was created.

HLA-DRB1*07, HLA-DRB1*11, HLA-DRB1*13, and HLA-DQB1*03:01 alleles were identified as risk alleles for TB. HLA-DRB1*15 and HLA-DQA1*01:02 alleles were protective. Comparative analysis with the results of other studies of different populations in Europe and Asia was performed.

The results demonstrated that the subpopulation of Latvia could have specific risk and protective HLA Class II alleles for TB. Despite a relatively high statistical significance, further study should be conducted to specify more precisely protective and risk HLA Class II alleles for TB in East Latvia.

DOI: 10.1002/eji.201970500

Levels of expression of ORAI1 and STIM1 genes in blood differ in patients with pulmonary tuberculosis and a control group

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Despite 53 million lives saved since 2000, TB is among Top 10 causes of death worldwide. One of the reasons of chronic mycobacterial persistence in the host is imperfect mycobacterial phagocytosis, which can be associated with dysfunction of Ca2+ channel of immune cells, formed by ORAI1 proteins on the cytoplasmic membrane and its activators – stromal interaction molecules (STIM1, STIM2) – located on the endoplasmic membrane. Simultaneously, there is no data regarding the significance of the levels of expression of ORAI1 and STIM1 genes in blood during infectious diseases, including TB. This study aimed at a comparison of expression levels of ORAI1 and STIM1 genes in blood between patients with TB at the beginning of the treatment and the control group without active TB.
The sample of TB patients included 40 adult HIV-negative patients aged between 18 and 85 with bacteriologically confirmed, drug-sensitive pulmonary TB. Control group included 7 adults aged from 18 to 65 without active TB. The analysis of ORAI1 and STIM1 genes included extraction of human RNA from blood and detection of the expression of ORAI1 and STIM1 transcripts by the real-time quantitative reverse transcription polymerase chain reaction. The results were analyzed by taking into account a quantification of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Mann-Whitney U-test was applied for a comparison of levels of expression of ORAI1 and STIM1 genes in two groups.
ORAI1 was higher in the control group, Mann-Whitney $U = 6.50$, $p < .001$. STIM1 was higher in the clinical group of TB patients, $U = 29.00$, $p = .004$. Despite the non-specificity for TB, expression levels of ORAI1 and STIM1 genes can provide additional information for a TB prognosis and monitoring of its treatment.

DOI: 10.1002/eji.201970500

T2-high asthma, classified by sputum mRNA expression of IL4, IL5 and IL13, is characterized by eosinophilia and severe phenotype

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Asthma is a common, highly heterogeneous inflammatory disease. Identification of asthma endotypes, that reflect highly variable response to conventional treatment, will lead to a more precise asthma management. T2 asthma is characterized by airway inflammation driven by T2 cytokines including interleukins IL-4, IL-5 and IL-13. The aim of this study was to determine whether induced sputum samples can be used for gene expression profiling and if T2-high endotype can be classified based on IL4, IL5 and IL13 profiling.
Induced sputum samples were obtained from 44 subjects, among them 36 asthmatic patients and 8 healthy controls. Immediately after collection, samples were processed with Sputolysin and cell pellets were stored in Qiazol reagent Following total RNA extraction, RNA quantity and quality measurement, mRNA expression levels of IL4, IL5, IL13, CPA3 and FCER1A were quantified by RT-qPCR.
Gene expression levels of IL4, IL5, IL13 and CPA3, but not FCER1A, were significantly increased in asthmatic patients' samples compared to controls. High correlation between IL4, IL5 and IL13 expression was also observed. We calculated T2 gene mean by combining the expression levels of IL4, IL5 and IL13 and set the T2-high/T2-low asthma cut-off value based on the expression of T2 gene mean in controls. Twenty-four (67%) asthmatic patients had T2-high asthma. Patients with T2-high asthma had significantly higher eosinophil blood and sputum counts. Furthermore, T2-high asthma was characterized as a more severe, difficult-to-treat asthma, as it was uncontrolled despite the use of inhaled and oral corticosteroids, and T2-high asthma patients more often needed biological therapy to control their asthma symptoms/exacerbations. Besides, patients with T2-high asthma had increased expression of mast cell/basophil gene CPA3, but not FCER1A.
In this study we found that interleukins transcripts can be easily detected in sputum from asthmatic patients. mRNA expression levels of IL4, IL5 and IL13 are increased in sputum cells from asthmatic patients and can be used as molecular biomarkers to categorize patients into T2-high endotype, characterized by eosinophilia, increased mast cell marker expression and severe, difficult-to-treat asthma, often requiring biological treatment.

DOI: 10.1002/eji.201970500

Detection of anti-HLA antibodies in organ transplantation

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Anti HLA antibodies are main, but not the only cause of antibody-mediated rejection (ABMR), which is associated with a poor transplant outcome. Histological findings suggesting ABMR usually demonstrate anti-HLA donor-specific antibodies (DSA). An approach to the detection of anti-HLA antibodies in immunogenetics laboratories considerably changed in the last years. The main reason was the introduction of new solid phase methods (SPM) for detection of specific anti-HLA antibodies. From a spectrum of SPM, the most reliable is Luminex. It allows identification of multiple specific alloantibodies and subsequently identification donor specific antibodies with clinical relevance. The interpretation of Luminex results requires the high-resolution HLA typing.
We have adopted these new technologies for the kidney and heart transplantation program in Slovakia. We have been using Luminex for antibody detection and SBT and, recently, NGS for the HLA typing. At first, we have used it for the detection of ABMR and monitoring of antirejection therapy in kidney and heart transplant recipients. Subsequently, we started to evaluate immunological risk for sensitized patients waiting for a living kidney donor. Now, we are at pilot phase of applying virtual cross matching to deceased kidney donors. We have to overcome some logistic problems, because the technologies are not available in all transplant centres. We are retyping donors and sensitized patients centrally and the data are accessible through a central transplant information system. Luminex is now in all transplantation centres.
The interpretation of results gave us lessons in detailed and frequent communication with clinicians because the clinical status and history of the patient are crucial for physician decisions. The new methods allow physicians to set up a more exact and quick diagnosis of ABMR and also monitoring of targeted therapy, which is not possible using classical serology methods.

DOI: 10.1002/eji.201970500

The immune system - its silent reserve repertoire

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Alacrity-based selection theory provides a unifying model for all selection processes of the immune system. The model states that specific responses in complex environments do not depend solely on the epitope-paratope interactions, but above all else on the alacrity of the engaged cells. Among the specific cells there exists a subset that is more ready to respond than the remaining population. Other specific cells remain idle and remain "second in line". The presented theory is based on the notion that in their molecular composition there are no two cells that are identical – neither within an organism nor in an entire species. Cells with the "same function" and "same specificity" differ in many components of their cellular inventory. Even cells with identical receptors and surface markers differ one from other. These differences might be small or large, nevertheless they translate into distinct eagerness and preparedness of cells to respond to a given stimulus. For this eagerness and preparedness of cells to respond we coin the term "alacrity".
If the proposed theory is correct, many processes of the immune system will have to be reconsidered: tolerance induction, breaking of tolerance, induction of regulatory cells, induction of immunological memory and recall of memory cells, preferential occurrences of immune responses in various organs (nasal, intestinal), clonal anergy or adjuvant effects.

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DOI: 10.1002/eji.201970500

Do we miss allergenic components of chickpea and other legumes in available multiplex platforms for IgE detection?

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Chickpea is a legume commonly used in healthy cooking; however, it can be an elicitor of serious allergic reactions as well. The most important allergens of chickpea are seed-storage proteins (7S and 11S globulins, 2S albumins), which are stable and heat-resistant. The less important chickpea allergens are PR-10 proteins or lipid-transfer proteins (LTPs).
Since 2018, a new multiplex platform (ALEX®, MADX, Austria) for the detection of IgE-mediated sensitization profile is available in our department. We carried out a study on frequency of sensitization to chickpea and profile of chickpea-sensitized patients.
We examined 240 patients by using ALEX platform; 23 of them were sensitized to chickpea and 18 also to lentil. Some chickpea-sensitized patients were also sensitized to the other legumes (peanuts, soy, pea, bean, lupine) and/or tree nuts and/or seeds. Since only allergenic extracts of lentil, chickpea, pea or beans are included in ALEX platform, we were not able to uncover specific molecular profile of sensitization to these foods. However, the patients, who were as well sensitized to peanuts and/or soy, were sensitized mainly to seed-storage proteins of peanuts or soy. No chickpea-sensitized patient reported an allergic reaction to this food; however, four of those patients reacted to lentil and two both to pea. Another two patients sensitized to chickpea had positive history of allergy to peanuts. No patient sensitized to chickpea clinically reacted to beans, lupine or soy.
IgE mediated sensitization to chickpea is not rare (10% in our study group) and, in some parts of the world, it is an important allergen. Although the real clinical reactivity is not very common, some patients can suffer from quite severe allergic reactions to this food or close related legumes, mainly lentil. Such patients should be appropriately diagnosed and treated. Another widely used multiplex platform (ImmunoCAP ISAC®, ThermoFisher, Sweden) do not even offer the possibility to examine allergy to other legumes than peanuts or soy.
The other legumes than peanuts or soy, including chickpea, are somewhat neglected allergenic sources. Due to its possible allergological importance, it may be suggested to consider the inclusion of major allergenic components of lentil, chickpea, and pea to multiplex platforms.

DOI: 10.1002/eji.201970500

3D culture as a model in the study of immunological changes associated to human colorectal cancer

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The structure of the tumor microenvironment may be modulated by local immunity. Cytokine are variously expressed in different sites of the bowel depending on the proximity to tumor. The tumor stroma is an important modulator of cancer cell behaviour in the tumor microenvironment. Looking by 2-photon microscopy (second-harmonic generation imaging - SHG) to the colorectal cancer specimens from patients who underwent surgery, we found differences in the stroma organization of mucosa far from the tumor (apparently normal), near the tumor border (transitional) mucosa and tumor. Analysis of mucosa proteins by qRT-PCR showed the progressive increase in expression of COL1A1, IL-1 beta, IL-13 and LOXL2, all involved in the tissue remodelling. IL-6 appeared increased especially at the transition from mucosa to tumor in correlation with higher inflammatory cell infiltrate. IL-6 participation was also immunohistochemically evident. Interestingly both check-point molecules PD-1 and PD-L1 expression was increased in transition mucosa and in the tumor. Based on this, we develop 3D cell models for better understanding how the microenvironment is going to develop and be organized. To model *in vitro* the tumor development, 3D cultures of colorectal tumor cells as spheroids are undergoing. They appear useful for investigating modality of expression of immune check-point molecules in complex cultures including e.g. fibroblasts. This will help to elucidate immunobiological evolution of the tumor and even to evaluate immune escape mechanisms in early stages of cancer development. Spheroids of colorectal cancer cells appear very promising for helping in the analysis of tumor microenvironment before to translate to animal model.

Acknowledgements: grants RVO 61388971 (CZ), Generali/Czech Insurance Foundation a.s. (CZ), UniCredit Bank s.r.o., Prague (CZ), Di Cursi Donation (CZ), AERO s.r.o. (CZ), Eurinox s.r.o (CZ) and ARPA Foundation (IT).

DOI: 10.1002/eji.201970500

Nature of BDNF response to acute and regular exercise in the elderly and young adults

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Introduction: Adaptive responses of physiological systems to exercise are crucial for homeostasis and health of the ageing body. Brain-derived neurotrophic factor (BDNF), mainly produced by brain, enhances neuronal plasticity. Importance of transient changes in peripheral BDNF for eliciting exercise benefits in humans remains relatively unexplored.
Aim was to determine effects of acute aerobic exercise and 3-month aerobic-strength training on serum, plasma and skeletal muscle BDNF in sedentary elderly and to compare acute effects of exercise with young trained individuals. Methods: Elderly individuals (n=22, 69±8yrs., 9M/13F) underwent 3-month aerobic-strength training (3x/week, 70%VO2max/1RM). Acute 40min. bicycling test (65–75% HRR) and measurements of BMI, adiposity, muscle mass, walking speed, ACE-R, VO2max, muscle in vivo mitochondrial capacity, BDNF in serum & plasma were performed before & after intervention. BDNF protein (WB) & fiber-type (ATPase) were determined in vastus lateralis biopsies. Acute BDNF response to 90min. run (75%HRR) was evaluated in young trained individuals (25±2yrs., 3M/5F).
Results: Acute exercise transiently increased serum BDNF in sedentary (p=.007) but not in trained elderly or young individuals. Resting circulating BDNF was not regulated by 3-month training. Subtle training-related changes of serum BDNF correlated with improvements in walking speed (R=0.59, p=.005), muscle mass (R=0.43, p=.04), cognition (R=0.41, p=.05). Responders, who increased muscle BDNF protein in response to training, displayed stronger acute exercise-induced increase in serum BDNF than non-responders (p=.006). Muscle BDNF protein positively correlated with type II fiber ratio (R=0.587, p=.008) and the rate of post-exercise muscle ATP re-synthesis (R=0.703, p=.005). Plasma BDNF declined 1h post-exercise in trained elderly (−34%, p=.002) and young (−48%, p=.034) individuals. Conclusion: Acute regulation of systemic BDNF by exercise was dependent on the level of aerobic fitness and correlated with training-induced improvements in metabolism and cognition. Our observations provide an indirect evidence that distinct exercise-induced changes in serum, plasma and muscle BDNF are involved in the coordinated adaptive response to exercise in humans.

Grant support: SAS–NSC Joint Research Cooperation grant 2018/10, VEGA 2/0107/18, APVV 15/0253

DOI: 10.1002/eji.201970500

Phage display as a method to discover newly BBB-shuttle peptides

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Delivery to the brain is a major challenge in central nervous system (CNS) drug development. The blood-brain barrier (BBB) prevents access of biotherapeutics to their targets in the CNS and therefore prohibit the effective treatment of neurological disorders. To find BBB- shuttle peptides that target to the brain, we performed a phage display method against a primary rat BBB cellular model which mimics the characteristics of the BBB. From the panning experiment of a 12-mer library, the specific peptide sequences were selected and their permeability validated. The permeability of peptides was measured by ultra-performance liquid chromatography – tandem mass spectrometry coupled to a triple-quadrupole mass spectrometer (UHPLC-MS/MS). Our results showed the importance of in vitro BBB model for the discovery of BBB- shuttle peptides through phage display libraries. The results indicate that the peptides identified by the in vitro phage selection approach could be useful transporters for systemically administrated biofarmaceuticals into the brain with therapeutic benefits.

Acknowledgements: This work was supported by research grants APVV-18-0302, APVV-16-0531, VEGA 2/0088/18, 2018/24-SAV-2

DOI: 10.1002/eji.201970500

Hypomethylation of IL1RN and NFKB1/p50 is associated with increased IL-1Ra and IL-1β levels in patients with type 2 diabetes mellitus

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Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance, hyperglycemia and low-grade inflammation. An imbalance between interleukin (IL)-1β and its antagonist IL-1Ra determines the outcome of islet inflammation. Epigenetic modifications, such as DNA methylation, supposed to be associated with deregulated inflammatory gene expression in patients with T2DM. Here we aimed to examine the methylation status of gene promoters of IL1RN and its transcription factor NF-kB (subunits RELA and NFKB1/p50) in peripheral blood mononuclear cells (PBMCs) from treated T2DM patients (n=36, 20 female/16 male) and healthy individuals (n=31, 16 female/15 male) of Armenian origin. Furthermore, in order to determine the influence of epigenetic alterations on cytokine production, circulating plasma levels of IL-1Ra and IL-1β were analysed. DNA methylation levels of target genes were examined using the EpiTyper MassArray (Agena Bioscience) assay and plasma levels of cytokines were measured by ELISA. The average DNA methylation status of IL1RN and NFKB1/p50 gene promoters was significantly decreased in T2DM patients in comparison with healthy controls ($P< 0.05$). An increased IL-1Ra ($P< 0.0001$) and IL-1β ($P= 0.039$) plasma levels were detected in T2DM patients compared to controls. Negative association between average methylation of IL1RN gene and IL-1Ra plasma levels were observed in female T2DM patients ($r= -0.4742$, $P= 0.046$). Methylation of NFKB1/p50 gene was negatively correlated with IL-1Ra levels in the patients ($r= -0.3345$, $P= 0.049$) and positively with IL-1β levels in female patients ($r=0.4330$, $P= 0.056$). Taken together, the findings suggest that hypomethylation of IL1RN and NFKB1/p50 gene promoters may promote the increased IL-1Ra and IL-1β production, establishing and maintaining chronic inflammation in T2DM. Further functional studies are needed to prove our findings.

Grant support: IGA_LF UP_2019_014.

DOI: 10.1002/eji.201970500

Adult-onset periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome

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Periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis syndrome (PFAPA) is an autoinflammatory disease (AID) with no known genetic defect, affecting predominantly pediatric population. In most patients the episodes of fever subside by adolescence. However, PFAPA with onset at adult age has been described.
A 37-year-old male patient presented with a 2-year history of recurrent high fever accompanied by pharyngitis, cervical lymphadenitis and infrequently a small number of oral ulcerations. The patient had a history of tonsillectomy at the age of 4, and unilateral orchiectomy followed by radiotherapy due to testicular seminoma 14 years before the onset of symptoms. He was regularly followed up by oncologist and maintained a sustained remission. Fever episodes lasted for 5 days regardless of the antibiotic treatment, and occurred at monthly intervals, with the exception of a 5-month episode-free period. Between attacks the patient was generally well. Throat swab cultures were repeatedly negative. C-reactive protein levels were consistently elevated during fever episodes (up to 88 mg/L, reference range 0-5 mg/L), and normal in between. The patient was also seen by an infectious disease specialist, and workup revealed no infectious cause for the recurrent fever. Finally, the diagnosis of PFAPA was made by exclusion, and was further supported by the prompt (within hours) and complete resolution of symptoms with oral prednisone at a single dose of 1 mg/kg. Despite the effectiveness of episodic glucocorticoid treatment, the frequency of episodes increased in the 6-month follow-up period to twice a month, and the patient was offered prophylactic therapy with colchicine.
Clinical diagnostic criteria for adult-onset PFAPA have recently been proposed. These include: recurrent fever accompanied by erythematous pharyngitis and/or cervical lymphadenitis, increased inflammatory markers during attacks and symptom-free intervals, and should be applied on patients aged 16 or older, after exclusion of infectious, autoimmune, neoplastic diseases and other AID. In addition, throat swab performed during fever should be negative and antibiotic therapy ineffective. PFAPA should be considered in adult patients with recurrent fever and pharyngitis.

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DOI: 10.1002/eji.201970500

Expression profile of serum microRNA after concussive and sub-concussive head impacts in professional soccer players

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Traumatic brain injury (TBI) is caused by an external physical force, which subsequently alters the brain function. TBI can be one of the major risk factors for development of cognitive impairment, dementia and trigger other neurodegenerative conditions, such as chronic traumatic encephalopathy. The harmful consequence of repetitive mild TBI has been described in the brains of contact sport players who suffered by repeated concussive head impacts. Due to missing immediate visible symptoms the currently used techniques have limited abilities to identify mTBI. Therefore, there is a need for a simple and reliable biofluid-based diagnostic test for TBI patients. In our study we used digital-ELISA to perform analysis of protein biomarkers (neurofilament light chain and tau protein) in blood serum of the athletes. The results indicated altered dynamics of protein markers following high intensity exercise, heading and concussion. Based on these results, expression analyses for microRNAs (miRNAs) were performed by real-time polymerase chain reaction. Specific miRNAs have been previously reported as dysregulated after TBI conditions and therefore miRNAs could possibly represent a potential peripheral biomarker. Our results showed changed levels of different miRNAs depending on time of serum collection and study group. The main aim of our project is to identify a potential molecular marker for more precise diagnostics and follow up of symptoms after TBI.

This project was supported by research grant: VEGA 2/0118/19, 2/0076/18, 1/0240/16, APVV-17-0668, ERA-NET NEURON RepImpact, Research Council of Norway and International Federation of Association Football Medical Assessment and Research Center.

DOI: 10.1002/eji.201970500

Changes of the choroid plexus barrier in tauopathies

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Tauopathies represent a heterogeneous group of neurodegenerative disorders characterized by an abnormal deposition of hyperphosphorylated form of microtubule associated protein tau into intracellular neurofibrillary tangles. Previous studies provided evidence of a close connection between neurodegenerative processes and neuroinflammation. Several neuropathological studies suggested that the neuroinflammatory responses might begin prior to significant neuronal loss. This supports the hypothesis that neuroinflammation plays an important role in the pathogenesis of most neurodegenerative disorders, including tauopathies.
Intercellular junctional complexes between the central nervous system (CNS) microvascular endothelial cells and the choroid plexus epithelial cells form the endothelial blood-brain barrier (BBB) and the epithelial blood–cerebrospinal fluid barrier (BCSFB), respectively. These barriers inhibit paracellular diffusion, thereby protecting the CNS from fluctuations in the blood. Any alteration in their function can contribute to pathophysiology of tauopathies. Tau is associated with progressive vascular alterations that may facilitate impairment of BBB in animal models and human tauopathies.
In our work we were interested in determining if neurofibrillary pathology and inflammatory processes promote changes of choroid plexus in transgenic rat model for tauopathies. We analyzed functional and morphological changes of choroid plexus in control and transgenic animals. We analyzed expression of inflammatory molecules such as intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, thickening of epithelial basal membrane and expression of tight junctions which are important in maintaining barrier integrity. Results from our study led us to speculate that neurofibrillary pathology associated with inflammatory processes lead to structural and functional changes in choroid plexus in tauopathies.

Acknowledgments: This work was supported by competitive academic grant APVV-16-0531

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DOI: 10.1002/eji.201970500

Immunomodulatory functions of enzymes matrix metalloproteinases, innate immune cells, CD 68 and heat shock protein 70 in the ethiopathogenesis of atherosclerosis

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Background: Atherosclerosis represents an inflammatory, autoimmune, and chronic metabolic disorder of the vessel wall, with genetic and environmental factors in its etiopathogenesis. Oxidized low-density lipoprotein (oxLDL) in vitro can contribute to Treg/Th17 balance in the periphery inducing the apoptosis of regulatory T cells (Tregs) and the proliferation of Th 17. Three proteins are identified as autoantigens: heat shock proteins (HSPs), oxidized low density lipoprotein (oxLDL) and beta 2 glycoprotein 1 (b2GP1). The matrix metalloproteinases (MMPs) play a key role in angiogenesis with migration and/ or invasion of endothelial cells in surrounding stroma and tissues. The CD68 molecule have an ability to bind oxLDL and points a possible role in atherogenesis and intracellular lipid accumulation.
The aim of this study: To examine the interactions between the enzyme matrix metalloproteinases, the innate immune cells and CD68+cells of patients with mild atherosclerosis (A patients) and with carotid arteries stenosis (CAS patients) who were undergoing the surgical procedure.
Patients and methods: Patients were selected from a stratified sample of the population of adult patients of both sexes with diagnosed atherosclerotic changes in medium and large-sized arteries. We used the method of enzyme immunoassay (ELISA) for the investigation of the concentration of enzyme MMPs in urine. Innate immunity was analyzed by flow cytometric method. Immunohistochemically expression of the heat shock 70 (hsp 70) protein on paraffinic atherosclerotic alterations of carotid arteries were done.
Results: patients with developed atherosclerosis (CAS group) had statistically significant increases in MMP 2 and 9 enzyme urine levels. In the peripheral blood had statistically significant increases in CD68 + molecule, NKT cells and statistically significant decrease in Tregs. The elevated values of NKT cells correlate with the elevated values of enzymes MMP 2 and 9 and are down regulated with the diminished Tregs values. Our data showed a rise in the expression of heat shock protein 70 in atherosclerotic-modified carotid arteries.
Conclusion: High concentration of MMP 2 and 9 enzymes followed by diminished values of Tregs in atherosclerotic patients highlights the importance of regulatory T immunity in atherosclerotic etiopathogenesis and indicated determination of MMPs as an easy markers for the monitoring of the development of atherosclerosis. Heat shock proteins (HSPs) act as autoantigens and stimulate cellular and humoral immune responses.

Acknowledgement**:** This work is supported by grants from the University of Rijeka (18-220, 18-146 and 18-301)

DOI: 10.1002/eji.201970500

Immunogenetics of allogeneic haematopoietic stem cell transplantation

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Allogeneic haematopoietic stem cell transplantation (aHSCT) is used as a curative treatment in severe haematological malignancies, primary immunodeficiencies and hereditary metabolic disorders. Substantial proportion of the recipients still suffer from severe immune-mediated complications including graft versus host disease (GvHD). On the other hand, in malignant diseases immune alloreactivity represented by graft versus leukaemia/lymphoma (GvL) effect prevents relapses. The favourable effect of HLA match between donor and recipient was repeatedly confirmed in aHSCT. From the immunological point of view, optimal donor for aHSCT is currently fully matched at relevant HLA loci with the patient. Because the mean probability of the identification of the HLA identical related donor is lower than 30%, the majority of patients depend on the search for an unrelated donor. In this respect, rapid development in HLA typing technology and enormous worldwide growth of the bone marrow donor registries substantially increased chance of finding an optimal donor. Furthermore, for the patients without fully HLA compatible donor, an alternative approach, namely haploidentical transplantation (HT) has been developed with promising results. Importantly, HT reminded us that loss of the HLA expression due to the tumour-specific somatic mutation is considered as the important mechanism used by tumour cells to escape from immune surveillance. Apart from the established immunogenetic factors, several "intelligent" approaches have been introduced into the transplantation protocols, such as a model of HLA-DPB1 permissive mismatches or a preference for the donor with highest killer cell immunoglobulin-like receptor (KIR)-B score among donors equivalent according to HLA match for the patients allografted for acute myelogenous leukaemia. Enormous interest in further gene variants associated with the aHSCT outcome (e.g. minor histocompatibility antigens or cytokine genes) established the field of nonHLA genetics of aHSCT. In conclusion, although clear improvement of the aHSCT approaches has been achieved in the last decades, further research in aHSCT immunogenetics supported by functional data may bring us closer to the more effective clinical strategies to the haematopoietic stem cell therapy with higher overall benefit for the patients.

Grant support: IGA_LF_2019_014 and MHCZ-DRO (FNOl, 00098892).

DOI: 10.1002/eji.201970500

MALDI-imaging mass spectrometry screening of lipid profile changes in the rat model for tauopathies

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Tauopathies are characterized by abnormal intracellular depositions of tau protein in brain cells. Tauopathies involve around 20 different degenerative disorders including Alzheimer´s disease (AD), progressive supranuclear palsy, Pick´s disease, corticobasal degeneration, frontotemporal dementia with Parkinsonism linked to chromosome-17, dementia pugilistica/traumatic brain injury/chronic traumatic encephalopathy complex and others. Currently, lipid research is gaining interest as direct involvement of lipids in the pathophysiology of neurodegenerative diseases is well acknowledged. Here, MALDI-MSI experiment was performed to identify changes in the distribution of lipids in a rat model of tauopathies and correlate the changes with neurofibrillary pathology found in brain tissue. Twelve-month-old SHR-24 transgenic rats (N = 5) expressing tau pathology were used for this experiment with SHR rats (N=5) used as controls. Perfused brains were cut sagittally, frozen in 2-methylbutane over liquid nitrogen fumes and stored at -80°C until further use. Frozen tissue sections were cut on a cryostat at a 12 µm thickness onto ITO-coated glass slides. 1,5-diaminonaphthalene (DAN) was used as matrix and applied on dried tissue sections via sublimation. Spectra were acquired by Bruker ultrafleXtreme in negative ion mode controlled by FlexControl 3.4 and FlexImaging 4.1 software packages. For visualization and statistical analysis, we used FlexImaging 4.1 and SCiLS lab 2019c software. We have successfully mapped lipids in sagittal brain sections of 12-month-old transgenic SHR-24 rats and compared it with lipid profiles of age-matched controls. Significant changes in lipid profiles were found in the brainstem which is in agreement with localization of tau pathology in our transgenic model. The presence of tau associated neurofibrillary pathology was supported by immunohistochemical staining. Features that discriminated transgenic model from control were identified by inspecting fragmentation spectra and database search. We conclude that altered spatial distribution of brain lipids can be observed in the rat model of tauopathy.

This work was supported by APVV-18-0302 grant.

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DOI: 10.1002/eji.201970500

Immunomodulatory effects of regular physical exercise in older age

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Background: Studies of the last decade revealed the important effects of physical activity on immune functions. These effects may largely depend on the type of activity, its intensity and duration. However, little information is available regarding the immunological effects of sporting activities in older age. The aim of our study was to examine the changes in a wide spectrum of lymphocyte subtypes after a period of regular workout among healthy elderly women.
Method: In our study, we enrolled 16 elderly women (between ages of 60-75 years) not engaged in regular physical activity. Additionally, as young controls, we enrolled 26 young adult women (between ages of 19-23 years), who were not engaged in regular physical activity. The group of elderly women completed a 6-week lightweight conditioning gymnastic exercise program. The percentages of peripheral natural killer (NK), NKT cells, T and B lymphocyte subtypes (early-/late activated T, naive and memory T, cytotoxic T (Tc), T-helper (Th)1, Th2, Th17, T regulatory type 1 (Tr1), CD4+CD127-CD25bright Treg, as well as naive and memory B cells) were determined by flow cytometry.
Results: Regarding the baseline values, in the elderly women, levels of CD3+6B11+ NKT cells were lower, while ratios of CD4+ Th/CD8+ Tc cells were higher compared to the values of younger individuals. At the end of exercise program, pronounced changes were observed in elderly women: percentages of IgD+ naive B cells decreased, while levels of CD27+ switched-memory B cells increased. Furthermore, proportions of CD4+IL-4+ Th2 cells increased, while levels of immunosuppressive CD4+CD127-CD25 bright Treg cells decreased following the regular exercise workout.
Conclusion: Differences observed after the regular lightweight exercise program reflect a presumably enhanced immunoreactivity and increased ability for immune responses.

The research was supported by the GINOP-2.3.2-15-2016-00062 project, which is co-financed by the European Union from the European Regional Development Fund. The work of Papp G. was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences and the ÚNKP-19-4 New National Excellence Program of the Ministry for Innovation and Technology.

DOI: 10.1002/eji.201970500

Analysis of anamnestic data in Alzheimer´s disease in Slovak population

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Alzheimer's disease (AD) is a neurodegenerative disease with multifactorial etiology. In the study of immunogenetic markers in Slovak patients with Alzheimer's disease, we obtained anamnestic data that deserve attention and have not been analysed yet. The aim of our study was to compare data of non-genetic risk factors obtained from the questionnaire (diabetes, hypertension, smoking, actual presence of inflammation, age, sex, age at onset of the disease ...) and to evaluate differences between results of the Montreal Cognitive Assessment (MoCA) test in AD patients and in controls. The group consisted of 142 (84 female, 46 male, average age: 79,08±7,08) AD patients and 76 controls (47 female and 29 male, average age 66,03±6,4) without cognitive impairment. For the diagnosis of AD or for inclusion to group of controls MoCA test was done. The criterion for inclusion in the control group was MoCA≥26 points out of 30. The most interesting result from the anamnestic data was the presence of type II diabetes in AD, which was 2.66 times higher than in controls (25.53% vs. 9.59% χ^2, p=0,0058). Hypertension was also slightly higher in patients (69.72% vs. 67.57%, n.s.). The actual presence of inflammation at the time of blood collection was observed (AD 15.5% vs. C 34.7%). The information of low number of smokers among AD patients suggests a lack of patients´ opportunities (only 4.9%) as opposed to controls, of whom 23.3% smoked. As the result of the MoCA tes, one of the classification criteria of AD, there are significant differences between patients and controls – average result 14.3/30 points (47.7%) vs. 27.78/30 points (92.6%). Within each of the MoCA category, the largest differences were between AD patients and controls in delayed word recall (11.4% vs. 75.8%). There were also differences between AD men and women. The results of our study confirm the influence of some factors on the risk of AD, which are known from the literature. In addition, they can bring interesting observations when they will be considered in further research of immunopathogenesis of Alzheimer's disease.

This study was supported by the Grant Agency VEGA, project N- 1/0240/16

DOI: 10.1002/eji.201970500

Immunotoxicity of nanoscale cavitands cucurbit[n]urils on the peripheral blood mononuclear cells of healthy donors

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Cucurbit[n]urils (CB[n]) are nanoscale macrocyclic compounds that can encapsulate a molecule or a fragment of the molecule by forming host-guest complexes. Integration of drugs with cucurbituril used for the following purposes: to control clearance, protect the drug from the biodegradation, reduce toxicity, increase the solubility, etc. Today it is known that cucurbiturils, unlike many other nanoparticles, has low toxicity in high doses. However, many aspects of the biological properties of these carriers, including immunotoxicity, remain unclear.
Materials and methods: Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood from six healthy volunteers. Cells (1million/ml) were cultured with different concentrations of CB [6] and CB [7] in 24-well plates at 37°C, in humidified atmosphere with 5% CO2 for 72 hours. The cytotoxic effect was measured using the WST assay, cell cycle analysis was evaluated using flow cytometry by staining cells with ethidium bromide.
Results and discussion. CB [6] and CB [7] did not have a cytotoxic effect on PBMCs in all used concentrations from 0.1 to 1 mM. However, there was a tendency to decrease the viability of cells cultured with CB [7] at a concentration of 1 mM. In addition, CB [6] and CB [7] did not affect the ratio of cells in different phases of the cell cycle at a concentration of 0.5 mM and below, and also did not lead to an increase in the relative number of apoptotic cells in PBMCs. These findings are consistent with the literature data that CB[n] is non-toxic to the cell lines, human and animal tissues in the concentration below 1mM [1,2].
Our study demonstrates that CB [6] and CB [7] did not have an immunotoxic effect on PBMCs.

The reported study was funded by Russian Science Foundation according to the research project №19-15-00192.

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DOI: 10.1002/eji.201970500

Allele level of HLA variation in Czech patients with sarcoidosis

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Sarcoidosis is a systemic disease in which immune and genetic factors have been implicated. HLA variation has been investigated in sarcoidosis since the 1990´s, first defining antigens by serology and later describing allelic groups using DNA typing. The recent standard of HLA typing is represented by next generation sequencing (NGS) characterising the allele level. Our laboratory has adopted NGS HLA typing for analysing sarcoidosis relationship with HLA [1] and here we report the first data from this research supported from [2].
110 patients with sarcoidosis were diagnosed according to ATS guidelines at the Brno University Hospital. The distribution of chest-X-ray (CXR) stages (I/II/III/IV) was: 36/53/19/2; 23 patients presented with Löfgren syndrome (LS), 33 patients had extrapulmonary sarcoidosis. The HLA was genotyped on 7 loci (HLA-A,-B,-C,-DRB1,-DQA1,-DQB1,-DPA1) using Omixon Holotype. The obtained frequencies of HLA alleles were compared with the allele distribution in 168 healthy unrelated Czech subjects [3].
The alleles most overrepresented in sarcoidosis patients compared with our control population were HLA-DRB1*15:01:01, -DRB1*03:01:01, -DRB1*13:02:01:02, and of the HLA-B locus the alleles -B*08:01:01:01 and -B*18:01:01. By contrast, the alleles HLA-DRB1*07:01:01:01 and HLA-DRB1*01:01:01 occurred more frequently in healthy subjects and thus could be of a protective function. The presence of LS correlated with HLA-DRB1*03:01:01. The less favourable course of disease was linked to the allele HLA-DRB1*15:01:01.
This first data from NGS assessment of HLA variation in sarcoidosis confirm and extend some previous observations. Currently, we have been expanding our cohorts in order to analyze associations with distinct disease phenotypes including remitting/progressing disease and/or treatment response. We also aim at assessing HLA-C and HLA-DPB1 loci, which have not been analysed in sarcoidosis context in detail.

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DOI: 10.1002/eji.201970500

Simple UHPLC-MS method in targeted metabolomic profiling of Crohn's disease patients

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Metabolomics as an integral part of system biology, analyze metabolites which results from metabolic processes occurring in living organisms. In general, there are two approaches by which metabolomic studies can be conducted – metabolic profiling (also known as targeted metabolomics) and fingerprinting (also known as untargeted metabolomics). The targeted approach is based on identification and quantification of preselected group of known metabolites within the biological sample and is an integral part of clinical laboratories. Metabolomic studies can lead to enhanced understanding of disease mechanisms and to new diagnostic markers as well as enhanced understanding of mechanisms for drug or xenobiotic effect and increased ability to predict individual variation in drug response phenotypes. Therefore, metabolomics is very often used as an effective tool for searching of biomarkers of various diseases – e.g. cancer, diabetes, neurodegenerative, cardiovascular, autoimmune, inflammation, liver, or kidney diseases.
Amino acids play a critical role in human biochemistry, being present in virtually all metabolic and cellular functions. Their function is bind with the regulation of gene expression, cell metabolism and signaling. Amino acids are essential in lipid transports and are implicated in several metabolic defects. Therefore, monitoring of amino acids in various biological fluids is one of the most used approach in metabolomics studies. Altered amino acids levels were found in various disorders including diabetes, breast cancer, ovarian cancer, bladder cancer, esophageal squamous cell carcinoma, systemic sclerosis, necrotizing enterocolitis, autism spectrum disorders, acute kidney injury, etc.
In the present work, we describe a modern, fast and simple UHPLC-MS approach for targeted quantitative amino acids profiling of patients suffering from Crohn's disease. The optimized UHPLC-MS method was characterized by favourable performance and validation parameters, such as time of analysis (< 6 min) , specificity, linearity ($r^2 > 0.99$), limit of detection (< 1.5 μM), limit of quantification (≤ 2.5 μM), accuracy (recovery in the range 84.60 – 115.56 %), precision (RSD in the range 0.33 – 5.81% for intra-day precision, RSD in the range 0.99 – 11.77% for inter-day precision) or stability (relative error < 13.5%). The amino acid profiling by UHPLC-MS was successfully applied to the clinical samples.

Acknowledgement: This work was supported by the projects VEGA 1/0463/18, APVV-15-0585, APVV-16-0531.

DOI: 10.1002/eji.201970500

Protein Engineering in the developments of new therapeutics

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Over the last decades, both the generation of therapeutic antibodies from synthetic antibody libraries and therapeutic proteins those of new non-antibody scaffolds, e.g. the DARPins, have progressed from the academic research lab to late stage clinical trials or even the market (e.g., www.morphosys.com, www.molecularpartners.com). Nonetheless, modern protein engineering can do much more. This presentation will show how the engineering of stealth adenovirus, not carrying any viral genes, can be made to infect arbitrary cells in vivo based on their surface markers, and thereby to produce a cocktail of therapeutics in situ. These exciting new opportunities, in conjunction with new analytical tools also developed from advanced protein engineering, may extend the possibilities beyond what is possible with classical antibody technology today.

DOI: 10.1002/eji.201970500

Human herpesvirus 6 and its connection with cell signalling

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Roseoloviruses, human herpesvirus 6 variants (HHV-6A and HHV-6B) and herpesvirus 7, are closely related viruses, which are similar with respect to genomic and genetic organization, but they differ in biological and epidemiologic features. Differences include infectivity of T-cell lines, pattern of reactivity and disease associations. Roseoloviruses primarily infect CD4 cells, though more and more research show, that human herpesvirus 6 can infect any type of cells, and able to cause clinical conditions in any organ system. HHV-6 is mainly known because of exanthem subitum (roseola infantum), but it is also connected to Alzheimer's disease, chronic fatigue syndrome, sclerosis multiplex, interstitial pneumonitis, retinitis, and many more. Not unique, but rather special feature of the human herpesvirus 6A and 6B is that they can integrate their genome into the telomeres of host chromosomes, and they may reactivate in case of immunosuppressed conditions, like after transplantation, in case of cancer, or HIV infection.
The cytokine production by Roseoloviruses have already partially described both in vivo and in vitro. One of the most important cytokines in antiviral response are the interferons, which send signals to the neighbour cells after viral infection. Data shows that one of the earliest transcripted gene of HHV-6 causes a reduced interferon release in cell culture, which eventuates a less efficient defence from host side, and supports the spread of the virus.
These data draw attention onto further studies in which cytokine and chemokine production as well as regulatory mechanisms can be investigated by gene expression, and the research of the effect of roseoloviruses on the different organs need to continue.

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DOI: 10.1002/eji.201970500

Effect of belimumab on subpopulations of lymphocytes peripheral blood in SLE patients

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Systemic lupus erythematosus is a recurrent autoimmune disease characterized by dysfunction of if activation T- and B-lymphocyte and with abnormal production of antibodies and of pro-inflammatory and anti-inflammatory cytokines such as BLyS. Belimumab is a monoclonal antibody against BLyS, used in the treatment of SLE patients.
We observed 48 patients with SLE during long-term administration of the biological preparation belimumab. Disease activity was evaluated by ECLAM score. Subpopulation of lymhocytes were measured with a BD FACSCanto II flow cytometer. Results were statistically analysed in the R-project program.
Our results showed a decreasing trend of CD19 + B cells during treatment. Significant reductions occurred after 6 months of treatment ($p < 0.05$). No statistically significant differences in naïve (CD4 + CD45RA +) Th cell were observed during treatment. The proportion of memory (CD4 + CD45RO +) Th lymphocytes showed a significant increase in values after 6 months of treatment ($p < 0.05$). However, the value was stabilized over the next two years from the start of biological treatment. Active cytotoxic (CD8 + HLA-DR +) T-lymphocytes showed a slight decreasing tendency during treatment, most pronounced at 24 months of treatment ($p < 0.05$). During three years of treatment, we observed a 61% decrease in activated cytotoxic T cells. Continuously decreased NK cells were observed during treatment but no significantly. After 6 months of treatment, we showed a 50% decrease in disease activity assessed by ECLAM score ($p < 0.05$).
Early discontinuation of treatment could underestimate delayed clinical improvements resulting from late changes in immunocompetent cells. These observations may be a potential indicator of a therapeutic response.

DOI: 10.1002/eji.201970500

Molecular mechanism of anaphylaxis: immune cells, mediators, and pathways

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Anaphylaxis is a potentially life-threatening, rapidly progressing systemic allergic reaction. Activation of mast cells/basophils, involving IgE and the high-affinity IgE receptor (FcεRI) on these cells, followed by the release of preformed mediators is postulated to have a pivotal role in anaphylaxis. Other immune cells, among others neutrophils, monocytes/macrophages, and platelets, also contribute to this disorder. Using modern laboratory methods, from multi-colour flow cytometry technics to next generation sequencing platforms, and clinically well characterized patients, who presented with anaphylaxis at the emergency department or during allergen challenge, as well as mouse samples with different severity of IgE-mediated food induced anaphylaxis, our research has been focused on characterization of mechanisms leading to anaphylaxis.

We have demonstrated that the absolute number of circulating basophils and expression of basophil specific genes significantly decreased in human blood samples during anaphylactic episode. In line with that, CCL2, a major basophil chemotactic factor, is significantly increased during anaphylaxis, and correlated with the severity of the reaction. An inverse correlation between blood basophils and serum CCL2 was observed and CCL2 from anaphylactic patients promoted the migration of human basophils in-vitro. Transcriptome analysis revealed extensive and overlapping alterations of gene expression during acute anaphylaxis in humans and mice, with cell movement and migration, acute phase response, LPS-like response, TNF, IL-6, and NF-κB, as the most important upregulated events. While, lipid-activated nuclear receptors of transcription factors being the most important downregulated pathway during anaphylaxis. Comparative analysis with expression signatures of immune cells identified changes in basophil, neutrophil, and eosinophil cell populations during anaphylaxis. Study, whether mast cell activation mutation in KIT predisposes to the severity of reaction is undergoing.

These findings improve our understanding of biological mechanisms underlying anaphylaxis, suggesting the involvement of distinct immune cells, and complex signalling changes, which reflect cellular movement and interaction during anaphylaxis.

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DOI: 10.1002/eji.201970500

Immunity and inflammation in Schnitzler syndrome

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Introduction: Schnitzler syndrome is an auto-inflammatory disease of unknown etiology, characterized by abnormalities in both innate and acquired immunity. The lecture will summarize current knowledge of the pathogenesis of Schnitzler syndrome, supported by a collection of case reports.
Patients and Methods: Over the past years, the following aspects of the immune system pathology have been investigated in a group of patients with Schnitzler syndrome (total 5): genetic background, inflammation parameters, detailed B-cell examination, B cell clonality, mitochondrial structural and functional abnormalities.
Results: In addition to polymorphism in NLRP3 gene in one patient (c.2107C> A p.Q703K) no other genetic causes were found. Inflammatory markers such as neutrophilia, CRP and SAA were increased in all patients, their reduction then reflected the effect of IL-1 blockade therapy. The intracellular increase in IL-1 also corresponded to the inflammation pattern in patients with Schnitzler syndrome. Apart from the lack of plasma cells in one of the patients, we found no significant pathologies in the B immunophenotyping. We found a small clonal population of B cells by genetic methods. We further investigated the role of mitochondria in the sub-cohort of 3 patients and found gross abnormalities in their structure and function and reduced activity of I, II and IV OXPHOS complexes. Furthermore, we observed the accumulation of neutral lipids in the cell and impaired metabolism of arachidonic acid.
Conclusion: The Schnitzler Syndrome is now a well-described autoinflammatory disorder, but its nature has not been elucidated despite intensive research, specifically in genetics. In our small cohort of patients, we show abnormalities in immune function, reflecting intense inflammation and clonal proliferation of B-lymphocytes. We also point to the important role of mitochondria in this disease, whose pathogenesis is still shrouded in mystery.

DOI: 10.1002/eji.201970500

Alzheimer's disease CD33 rs3865444 polymorphism in Slovaks

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Objectives: The most common form of dementia is late-onset Alzheimer's disease (LOAD), a genetically heterogeneous neurodegenerative disorder caused by complex interactions between environmental factors and multiple common genetic variants with low effects. Among others, single nucleotide polymorphism rs3865444 C/A located 372 bp upstream of the CD33 gene has been previously associated with the risk of LOAD; however, reported findings have been inconsistent across different populations. CD33 is a myeloid cell receptor expressed by microglia and macrophages in the brain where it is involved in modulation of microglial activation and inhibition of Aβ clearance. SNP polymorphism rs3865444 is one of the two main CD33 variants, which may alter the risk for LOAD [1].
Aim: In our study, we aimed to validate the association between rs3865444 and LOAD risk in the Slovak population and evaluate whether its effect is modified by the major LOAD risk allele APOE ε4.
Methods: The patient cohort consisted of 187 unrelated subjects (121 females and 66 males; mean age 79.4 years; mean age at disease onset 77.4 years) meeting the NINCDS-ADRDA diagnostic criteria for probable AD of late onset (≥ 65 years). The control group comprised 487 unrelated cognitively normal elderly subjects aged ≥65 years (293 females and 194 males; mean age 76.6 years). Genotyping of CD33 rs3865444 was performed by polymerase chain reaction-restriction fragment length polymorphism method and APOE alleles (ε2, ε3, ε4) were determined by direct sequencing. Association with LOAD risk and age of disease onset was evaluated by both, logistic and linear regression analysis.
Results: As expected, the distribution of the three APOE alleles was different between the two study groups ($P < 0.0001$), with allele frequencies being as follows: ε2 = 4.5%, ε3 = 68.2%, ε4 = 27.3% in LOAD group, and ε2 = 8.2%, ε3 = 82.0%, ε4 = 9.75% in controls. Logistic regression analysis revealed a statistically significant decrease in LOAD risk for the minor CD33 rs3865444 A allele in APOE ε4 carriers (AA + CA vs. CC: $P = 0.028$; OR = 0.51; 95% CI = 0.28–0.93), while no association could be found in subjects without the APOE ε4 allele ($P = 0.83$; OR = 0.95; 95% CI = 0.62–1.47). No association between rs3865444 and age of AD onset was detected in any of the inheritance models.
Conclusion: A number of genes have been highly linked to the onset and development of late-onset AD [2]. Our results suggest that CD33 rs3865444 is associated with susceptibility to LOAD in Slovaks, whereby its effect on disease risk depends on the presence of the APOE ε4 allele.

The study was financially supported by the Slovak Scientific Grant Agency VEGA under the No. 1/0240/16.

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DOI: 10.1002/eji.201970500

'ASIA' - Autoimmune (Auto-inflammatory) syndromes induced by adjuvants

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Vaccines, administered to animal and humans, are clearly one of the best achievements of modern medicine and are commonly and safely inoculated to the vast majority of subjects. However, in rare occasions vaccines may induce autoimmune or auto-inflammatory conditions both in animals and in humans (1). These conditions, either defined diseases such as Gullian Barre syndrome or enigmatic ones, have been reported following different vaccines and vaccination protocols. The susceptibility factors and the temporal association between vaccines and these rare immune mediated reactions are yet to be defined, however the similarities between vaccines and infections and the addition of an adjuvant (i.e. alum, squalene etc.) to almost every vaccine is considered major contributors to such adverse events. Perhaps the most evaluated condition is MMF, in which a cause was clearly delineated. MMF is a rare condition caused by deposition of alum, used to adjuvant different vaccines, which bring about an immune mediated muscles disease (2). The discrepancy between the wide application of aluminum hydroxide and the rarity of MMF was resolved by the observations that alum may trigger MMF in genetically susceptible subjects carrying the HLA–DRB1*01 (3). Thus, in only a minority of genetically prone patients, alum may induce this syndrome.

A common denominator to each of these four syndromes as well as to various infectious agents is the trigger entailing adjuvant activity. Adjuvants are commonly used in medicine to augment an immune response to treatments such as the protective response induced by vaccines and were considered to be inert. However, it was shown they induced NALP3 inflammasome system. Moreover, adjuvants enhance adaptive immune responses via inducing Th2 cell activation as well as chemokines and cytokines secretion thus helping the recruitment of B and T cells (4-6) as well as inducing the secretion of Il-17 (7). Alas, studies of animal models and humans demonstrated the ability of some of these adjuvants to inflict an immune mediated disease and even autoimmunity by themselves (8).

Thus, herein we will describe the syndrome entitled the "Autoimmune (Auto-inflammatory) Syndrome induced by adjuvants" (ASIA) (9). We will also delineate who is at risk of developing autoimmune conditions upon exposure to adjuvants (10).

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DOI: 10.1002/eji.201970500

The Kaleidoscope of Autoimmunity – *primum non nocere*

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Close to 30 years ago we (1) came up with the notion that autoimmune diseases share a common background which they had termed the *mosaic of autoimm*unity. The various features of this background known already at that time were genetic, hormonal, environmental and immune system defects which are shared by many of the autoimmune diseases and were thus termed as the *mosaic of autoimmunity* meaning that re-arranging factors known to be involved in the induction of autoimmune diseases, will lead to different patterns of diseases or different diseases. These various common factors involved in the induction of autoimmune diseases, obviously require a trigger mechanism in order for the disease to break out. Such triggers could be a drug, various environmental exposures both physical and chemical, with the major cause related being to various infections (2-3). These are believed to trigger autoimmunity *via* few mechanisms such as molecular mimicry in the case of rheumatic fever, or *via* the induction of polyclonal activation or *via* changes in the gene function in the case of EBV or CMV related autoimmunity. Later on, some 25 years ago we further described the phenomenon of shifts in autoimmune diseases, which we had termed the *Kaleidoscope of Autoimmunity* (4-5). One of the long-known examples is that of Rhupus described by Panush who described the coexistence of features of both rheumatoid arthritis (RA) along with those of systemic lupus erythematosus (SLE) (6). At times these patients make a full transition from one disease to the other. For example, primary antiphospholipid syndrome (APS) which was induced in a patient with myasthenia gravis two years following thymectomy (7), or two cases of immune thrombocytopenia (ITP) which developed chronic active hepatitis following a successful splenectomy which led to a complete recovery from the ITP (5,8). This phenomenon of switching from one disease to another autoimmune disease in the same patient has since been described by many others, using the same terminology (9-10).
We will discuss the impact of the Kaleidoscope of autoimmunity related to organ resection, drug manipulating the immune system and the effect of microbiome.

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DOI: 10.1002/eji.201970500

Cross-linking mass spectrometry: a tool for investigation of protein interactions and structure

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Mass spectrometry-based methods have been developed as powerful tools in proteomic workflows for protein identification, quantitative proteomics, analysis of protein complexes, protein-protein interactions, etc. Protein cross-linking mass spectrometry (XL-MS) which uses chemical linkers to covalently link two side-chain moieties of proteins in near proximity and mass spectrometry identification of yet formed cross-links represents a challenge. In last decade, new techniques have been developed using highly sensitive and accurate mass spectrometry and software processing of measured data aimed to identify cross-linked peptides. Here we present the workflow for identification of cross-linked peptides originated from enzymatic/chemical cleavage of complex protein samples. Using Waters Synapt G2-Si mass spectrometer it is possible to obtain high accurate data which are analysed by the MeroX – the software for identification of cross-linked peptides. Identification of cross-links provides a high reliable data for determination of exact interaction site within or between proteins. Furthermore, implementation of distance restraints of individual cross-linkers through Xlink Analyzer and their visualization in 3D protein model serves as useful tool for solving structure of proteins or protein complexes.

This project was supported by: APVV-16-0531; VEGA 2/0148/18; VEGA 2/0150/19; VEGA 2/0147/19; ERDF CZ.02.1.01/0.0/0.0/15_003/0000441

DOI: 10.1002/eji.201970500

A synthesized new model of extracellular ATP - adenosine danger cycle

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Purinergic signalling comprises the processes induced by extra- or intracellular adenosine triphosphate (ATP) and adenosine (Ado) via P2 (ATP specific) and P1 (Ado specific) receptors.
In danger(stress) situations this system forms a functional cycle with augmented cellular activity serving defence, then the regeneration of energy (ATP) pools.
Our hypothesis contains four new elements:
1. In all types of stress/danger situations the first cellular reaction can be the increase in the intracellular Ca^{++} level inducing a release of ATP into the extracellular space, which provokes the secretion of a lot of proinflammatory mediators from the highly activated cells.
2. Extracellular Ado derived from extracellular ATP has main dual effects:
    a.) It prevents the over stimulation of hyperactivated cells serving their survival;
    b.) Later, it also contributes to the regeneration of ATP pools.
3. Plasmapheresis can be useful clinical model to study the biochemical elements of a stress situations.
4. A synthesized new ATP-Ado model is created to demonstrate these processes.

DOI: 10.1002/eji.201970500

Polymer drug delivery systems for targeted chemotherapy and immunooncotherapy

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Polymer drug delivery systems represent a modern strategy for tumor treatment, which possess the ability to potentiate the treatment effect of the parent drug without significant systemic toxicity. Conjugation of a low-molecular weight drug to a synthetic polymer carrier enables targeted delivery of the drug to tumor tissue or cells, while limiting the exposure of normal tissues. Water-soluble *N*-(2-hydroxypropyl) methacrylamide (HPMA) is one of the most promising drug carriers, enabling creation of variable carrier architecture, controlled drug release, and solubilisation of hydrophobic drugs. The conjugates show significantly extended circulation time and preferentially accumulate in solid tumor tissue by Enhanced Permeability and Retention (EPR) effect. In experimental tumor models, the HPMA copolymer conjugates carrying cancerostatic drugs have proven reduced systemic toxicity, high antitumor efficacy, and even capability to induce complete tumor regression with subsequent development of anti-tumor immunity. Indeed, the immune system of the host is co-responsible for the curative effect of the treatment. Specific anti-tumor immunity, chiefly mediated by $CD8^+$ cells, guarantees resistance to further tumor re-challenge. The treatment with the polymer cytotoxic drugs also appears beneficial in combination with checkpoint inhibitors. The T cells, namely cytotoxic $CD8^+$ cells of mice treated with polymer cytotoxic drugs, showed elevated levels of PD-1 as compared with the mice treated with the parent low-molecular weight drug. Moreover, the HPMA-based drug delivery system has also showed capacity to deliver agents, which could reduce activity of suppressor cells in the tumor microenvironment, in order to achieve synergistic effect of targeted chemotherapy and modulation of the host anti-tumor immune responses.

Supported by Czech Science Foundation (17-08084S), Ministry of Health (16-28600A), and Ministry of Education Youth and Sports (LTAUSA18).

DOI: 10.1002/eji.201970500

Dyslipidemia and its association with cytokines in patients with SLE

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Cardiovascular disease (KVCH) is one of the major causes of morbidity and mortality in patients with systemic lupus erythematosus (SLE). Accelerated atherosclerosis is associated with traditional and untraditional factors associated with the disease. Risk factors for KVCH in SLE are markers of inflammation (increased levels of acute phase reactants and TNF-α), dyslipidemia (increased levels of low density triglycerides and low density lipoprotein), anti-phospholipid antibodies; lupus anticoagulants, low-density oxidized lipoprotein antibodies, and high homocysteine levels. In adult patients with SLE, dyslipoproteinaemia occurs in the range of 30-73%.
In our study, 196 patients (24 males, 172 females) with SLE with an average age of 46 years and measured total cholesterol levels of 5.23 mmol / L, HDL 1.55 mmol / L, LDL 3.28 mmol / L and triglyceride 1.47 mmol / L. According to dyslipidemia criteria, the prevalence of dyslipidemia in our study was up to 58.16% of patients (18 males, 96 females). Triglyceride levels were increased in 79 women (28%) and 8 men (33%). Dyslipopretinaemia, together with elevated TNF levels, is closely related to cardiovascular and renal manifestations in SLE. We did not observe increased mean values of IL-6, IL-10, IL-1β and TNFα. We found only a negative correlation between IL-10 and triglyceride and LDL levels. IL-10 levels were elevated in patients with lower TG levels.
Interleukin 10 is a cytokine with an inhibitory effect on proinflammatory T helper cells, endothelial cells, monocytes / macrophages, and can also be an immune stimulator by promoting antibody production and B-cell activation. It was shown that he was positively associated with KVCH in SLE (1,2).

Work supported by the VEGA project 1/0244/17

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DOI: 10.1002/eji.201970500

Genetic background influences the propagation of tau pathology in transgenic rodent models of tauopathy

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Alzheimer's disease (AD) is age dependent progressive neurodegenerative disease. Epidemiological studies implicate the role of genetic background in onset and progression of AD. In AD, there is a sequential and hierarchical spread of tau pathology to other brain areas. Studies have strived to understand the factors that influence this characteristic spread. Using transgenic rat models with different genetic background, we reported that genetic background may influence the presence of neurofibrillary pathology. In this study we investigated if genetic background has an influence in the spread of tau pathology using hippocampal inoculations of insoluble tau from AD brain in rodent models of tauopathy with either spontaneously hypertensive (SHR72) or Wistar-Kyoto (WKY72) genetic background. Our results showed that insoluble tau from human AD induced AT8 positive neurofibrillary structures in the hippocampus of both lines. However, there was no significant difference in the amount of the neurofibrillary structures in hippocampus. On the other hand, we observed significantly higher levels of AT8 and pT212 positive structures in the parietal and frontal cortical areas in W72 when compared to SHR72. Interestingly, we also observed that the microglia (IBA1 immunostainings) in these brain areas in W72 were predominantly phagocytic in morphology, while in SHR72, the microglia were either reactive or ramified. The microglia in hippocampus and occipital cortex in both lines were mixed with reactive or ramified structures. Put together, our results, for the first time, show that immune response modulating genetic variability is one of the factors that influence the propagation to tau neurofibrillary pathology.

The work is supported by research grants APVV-17-0642.

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DOI: 10.1002/eji.201970500

Mononuclear phagocyte subpopulations in kidney transplantations

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Mononuclear phagocytes represent heterogeneous population of phagocytic cells differentiated from blood monocytes into tissue macrophages and dendritic cells. They are involved in multiple immune responses including reactions to the transplanted allograft. Peripheral blood monocytes can be categorized into specific subpopulations according to the expression level of CD14 and CD16 antigens. In addition to prevailing "classical" (CD14+CD16-) monocytes, there are also "intermediate" (CD14+CD16+) and "non-classical" (CD14lowCD16+) monocytes with exaggerated inflammatory responses. Tissue macrophages may be divided into pro-inflammatory M1 type and anti-inflammatory population M2 expressing CD163 and CD206. The aim of our study was to characterize the expression of M2-related molecules in different subsets of monocytes and in relationship to occurrence of acute rejection. Simultaneously, other myeloid functional antigens such as CD209 (DC-SIGN), CD47, and HLA-DR were measured on blood monocytes before and at 1 week, 1 month, and 1 year after the transplantation. The expression of CD163 was markedly induced during the first week after transplantation in all three subpopulations of peripheral monocytes with higher pre-transplant levels in patients developing acute rejection. CD206 as an alternative M2 marker was expressed only on a limited number of blood monocytes without any correlation with CD163 but its expression at one month after the transplantation was lower in patients with acute rejection. Serum levels of IL-10, an anti-inflammatory cytokine characteristic for M2 macrophages, did not correlate with the expression of either CD163 or CD206 on blood monocytes. The absolute number of pre-transplant CD209 positive peripheral monocytes as potential precursors of dendritic cells was higher in acute rejection patients. The expression of CD47 showed almost constitutive high expression comparable to HLA-DR.
We assume from our data that peripheral blood monocytes can change their phenotypic pattern after kidney allograft transplantation and combination of several membrane markers might be helpful in assessing potential risk of acute rejection.

Supported by Ministry of Health of the Czech Republic, grant No. 15-26883A and by MH CZ-DRO (Institute for Clinical and Experimental Medicine - IKEM, IN 00023001).

DOI: 10.1002/eji.201970500

Astrocytes and Stem Cells in Pathophysiology of Neurodegenerative Diseases

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Stem cells have been investigated for their therapeutic potential in spinal cord injury (SCI), stroke amyotrophic lateral sclerosis (ALS) and Alzheimer's disease. We used animal models and compared human mesenchymal stem cells (MSC), conditionally immortalized human stem cell line from fetal spinal cord (SPC-01) and human induced pluripotent stem cell-derived neural precursors (NP-iPS) for their capacity to migrate towards lesion sites, differentiate and induce regeneration. SCI in rats was evoked by a balloon-induced compression lesion and ALS was studied in SOD1 G93A transgenic rats. We compared transplantation of MSC, SPC-01 or NP-iPS labelled in culture with iron-oxide nanoparticles for MRI tracking. In vivo MRI proved that MSC, SPC-01 or NP-iPS migrated into the lesion and survived for several months. Implanted animals showed functional improvement, but MSCs rarely differentiated into neurons. SPC-01 and NP-iPS differentiated in the host tissue to motoneurons, astrocytes and oligodendrocytes.
In the ALS model, we used multiple intraspinal grafting into asymptomatic and early symptomatic SOD1 G93A transgenic rats. NP-iPS transplantation preserved motoneuron numbers (MNs), slowed disease progression, and extended the survival of all cell-treated animals. We found that SOD1 G93A rats have dysregulation of some components of ECM such as versican, has-1, tenascin-R and hapln-1 and spinal chondroitin sulphate proteoglycans (CSPGs). NP-iPS grafting led to normalized host gene expression, (versican, has-1, tenascin-R, ngf, igf-1, bdnf, bax, bcl-2 and casp-3) and to a restoration of perineuronal nets around the preserved MNs. In the host spinal cord, transplanted cells adopted a glial or MN phenotype. Based on our preclinical data, a prospective, non-randomized, and open-label clinical trial (phase I/IIa, EudraCT No. 2011-000362-35) has been designed to assess the safety and efficacy of autologous bone marrow MSCs in treatment of ALS patients. We found a reduction in ALSFRS decline at 3 and 6 months after application that, in some cases, persisted for 9 months. In about 80% of the patients, FVC values remained stable or above 70% for a time period of 9 months. These results demonstrated that the intrathecal application of BM-MSC in ALS patients is a safe procedure, and that it can slow down progression of the disease.

Supported by: APVV-17-0642.

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DOI: 10.1002/eji.201970500

Alteration in follicular T helper cell subsets and cytokine production contributes to dysregulated humoral immune response in systemic lupus erythematosus

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Background: Follicular T helper cells (TFH) orchestrate physiological B-cell response and are required for formation of germinal centres and generation of long-lived plasma cells. In systemic lupus erythematosus (SLE) TFH could promote aberrant B-cell development and autoantibody production. Our aim was to determine the ratio of peripheral TFH cells in lupus and investigate their relationship with B-cells and autoantibody production.
Methods: Circulating (c) TFH subsets and follicular regulatory T (TFR) cells and certain B-cell subpopulations were identified by multicolour flow cytometry in the peripheral blood of 35 SLE patients and 32 sex and age-matched healthy individuals. To elucidate the role of costimulation in B-cell development, recombinant anti-human IL-21R His-tag protein was used to neutralize the interaction between TFH and B-cells which previously separated with magnetic beads. At day 7, cells were harvested, the ratio of plasmablast differentiation was analysed by flow cytometry while concentrations of IgG and IgM were measured with ELISA.
Results: Decreased frequency of cTFH17 subset was found in SLE patients compared to controls. The ratio and absolute number of cTFR cells were significantly elevated in lupus. Regarding B-cell subsets, the proportions of mature-naïve, transitional B cells and plasmablasts were higher, while memory B-cell subsets were decreased in SLE patients compared to controls. Blockage of IL-21R decreased both IgM and IgG antibody production in cTFH-B cell co-culture system but no difference was found between patients and controls, however IgG production was more affected indicating that IL-21 is critical for TFH-cell dependent B-cell responses.
Conclusion: This study suggests that the imbalance in the frequency of cTFH subsets and cTFR cells in SLE patients is associated with disproportionate B-cell help and autoantibody production. The interruption of TFH-B cellular cross-talk via IL-21R may lead to disease amelioration. These results indicate that TFH cells and their molecules play a key role in the disease pathogenesis and could contribute in designing new therapeutics in lupus.

This work was supported by Grants No. K124177 and PD121327 from the National Research, Development and Innovation Office (Hungary) (NKFIH).

DOI: 10.1002/eji.201970500

Influence of urban (city of Zagreb) and rural (National park Lonjsko polje) environment in allergic diseases

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Increasing trends in the prevalence of allergic diseases of affluent countries, encouraged worldwide studies on environmental influence in immunoregulation. Some factors have been identified as high risk, e.g. maternal smoking and respiratory viruses in the pathogenesis of asthma, while some other, like breastfeeding, are known as protective. The most consistent findings of protective lifestyle are those of rural environment, especially for asthma and atopic dermatitis, while this is less clear for allergic rhinitis and allergic sensitization. The underlying mechanisms are not completely understood, but it has recently been demonstrated that rural environment has a role in innate immunity. Due to genetic differences, it is very important to perform national epidemiological studies, in order to plan preventive strategies.
During the school year 2017/2018, we completed a huge epidemiological study on allergic diseases; asthma, atopic dermatitis, allergic rhinitis and allergic sensitization, in two regions of Croatia: urban (city of Zagreb) and rural (National park Lonjsko polje). More than 1700 school children aged 6-14 years old participated in the study. We used standardised ISAAC (International Study on Allergy and Asthma) questionnaires on symptoms, as well as skin prick tests with standard set of inhalant allergens. Results showed protective role of biodiverse environment on allergic diseases, which was strongly expressed for allergic rhinitis and atopic dermatitis symptoms. Odds ratios for developing these two entities during a 12-month period were lower for all age groups of the children from the Lonjsko polje area. Children from Lonjsko polje also used medications and visited pharmacists less often than those from the city of Zagreb. Regarding asthma symptoms, there was no difference in odds ratios between the two groups, except for a variable wheezing ever in a life for a 13-14 years-old age group. A number of positive skin prick tests was also higher in children from the city of Zagreb. Further research should be focused on genetic-environmental interplays in this region and microbiome analysis.

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DOI: 10.1002/eji.201970500

Nanoparticles and nanomaterials, unique modulators of the inflammatory response

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Various nanomaterials (NMs) of natural or industrial origin are presented in the environment. The development of new nanomaterials for various medicinal applications is driven by pharmaceutical industry and the term "biocompatibility" is required as tie-on label for any new nanomaterials intended for medicinal application. The special class of NMs is represented by carbon-based nanomaterials (C-BNM) which have been recently attracted an increased attention as the NMs with the perspectives of various applications in industry and medicine. Chemical industry is capable to produce C-BNM in large quantities and, therefore, their impact on environment and hence on human health is inevitable. C-BNM, predominantly graphene oxide (GO), carbon nanotubes (CNT), and nanodiamonds (ND) were tested for their potential use in nanomedicine as drug carriers. Enthusiasm of material scientists often do not reflect ability of immune system to recognise foreign structures and eliminate them by various mechanisms including inflammation.
Bio-resistance and proinflammatory potential of C-BNM are the main obstacles for their medicinal application which was documented in several toxicological studies *in vivo* and *in vitro*. The favourable physico-chemical properties of C-BNM for construction of drug delivery systems and diagnostic preparations for *in vivo* imaging must be viewed from the perspective of biocompatibility. Engineered nanoparticles are inevitably recognized by the immune system and are internalised by various mechanisms. Any persistence of NMs in tissues can lead to sustained activation proinflammatory mechanisms such as inflammasome resulting in chronic inflammation. Therefore, ability of NP to activate inflammasome can be assumed to be a main factor responsible for the toxicity of various NMs observed *in vivo*. Induction of inflammation is a key event that has an impact on the whole organism, and it may represent the general mechanism behind the observed adverse effects caused by NMs.
In this presentation we will discuss the proinflammatory effect of various types of NMs and mechanisms of action with respect to their size and shape, surface modification, bio-resistance and chemical reactivity. Inflammasomes, NOD1/2 and TLR receptors will be in the focus. Inflammation represents important factor for understanding the complex mechanism(s) of action of NMs and can explain the ambiguity of the existing toxicological data obtained in various in vivo models.

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Grant support: OPVVV PO1 project "FIT" (Pharmacology, Immunotherapy, nanoToxicology) CZ.02.1.01/0.0/0.0/15_003/0000495; Strengthening interdisciplinary cooperation in research of nanomaterials and their effects on living organisms" [reg. n. CZ.02.1.01/0.0/0.0/17_048/0007421], Pro-NanoEnviCz" [reg. n. CZ.02.1.01/0.0/0.0/16_013/0001821], project PROGRES Q40/10 and PROGRES Q40/09).

DOI: 10.1002/eji.201970500

Re-thinking the tumor immune microenvironment

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The elicited expression of stress molecules and release of alarmins from a transformed cell clone in expansion starts the early immune reaction and tissue changes. This can lead to either tumor clone ablation or its establishment and progression, involving the collagen scaffold that appears particularly sensible to the local immune changes. In our animal models we simulate various conditions of immune activation (chronic inflammation, carcinogenesis) involving the colon mucosa and the relationship immunity-structure and its high dynamism appear evident. The evidenced changes in the collagen scaffold, by 2-photon confocal microscopy, appear linked to the changes of pro-inflammatory signals and their regulation in the tissue microenvironment. This was seen under the various conditions of enhanced immune response in the colon (DSS-induced colitis in the rat and in the mouse, AOM-induced carcinogenesis in the rat and in the bacterial colonization of germ-free mice) The integrated role of IL-6, IL-1, IL-10 and TGF-β, depending the type of inflammatory process involving the microenvironment, appears since early period. The comparison with human samples from colon cancer surgical specimens - including normal mucosa, near tumor mucosa and cancer tissue, showed the morphological changes were paralleling the immunological features in the mucosa. The condition of progression to tumor can be hypothesized as consequent to a downregulation or deficiency of regulatory response cytokines in the initial phases of tumor microenvironment establishment.

Acknowledgements: grants RVO 61388971 (CZ), UniCredit Bank a.s. (CZ), Generali CEE Holding B.V. (CZ), CAMIC CZ, Michael Cukier Fund (CA), Achitectural Consulting s.r.o. (CZ), Italinox a.s. and Eurinox a.s. (CZ), Tristano Testa Fund (CZ), Manghi Czech Republic (CZ), Paul's Bohemia (CZ) Elso Giacomucci Fund (IT) and ARPA Foundation (IT).

DOI: 10.1002/eji.201970500

Immunotherapy of sepsis

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Sepsis, life-threatening organ dysfunction caused by a dysregulated host response to infection, is a leading cause of death in critically ill patients. Treatment relies on control of the underlying infection, adequate hemodynamic resuscitation and organ support. Despite decades of sepsis research and identification of multiple potential targets within the complex immune response pathways, no specific immunotherapeutic treatments, except moderate doses of hydrocortisone in severe septic shock, are currently available. One of the main reasons for this is the heterogeneous nature of sepsis with the immune response varying among individuals according to different patient- and infection-related factors, making it unlikely that any one intervention will be effective in all septic patients. Moreover, although previous attempts to modulate the immune response have largely concentrated on reducing or blocking the pro-inflammatory response, patients with sepsis also have an early anti-inflammatory response. Hence, some may benefit more from immunostimulatory agents. New techniques are being developed that will help identify individual immune profiles, enabling new immunomodulatory treatments to be trialled in patients most likely to respond. And repeated characterization of immune status will help determine the most appropriate treatment for individual patients at different stages in the course of their disease as we move towards more personalized sepsis management. Some of the immunomodulatory agents that show promise and are being evaluated in phase III trials include thrombomodulin, which may be of benefit in patients with sepsis-induced coagulopathy; intravenous immunoglobulins, currently being tested in patients with community-acquired pneumonia with low IgM levels; and gelsolin, which may be beneficial in patients with community-acquired pneumonia and low gelsolin levels. We could also consider anti-programmed cell death protein 1 (PD1) antibodies, which may be beneficial in patients with sepsis-induced immunosuppression and increased T-cell PD-1 expression; and interleukin-7, which could be of use in patients with sepsis and severe lymphopenia. Which patients could benefit from corticosteroids needs to be better defined by other criteria than only severity.

DOI: 10.1002/eji.201970500

The importance of being Earnest: The conversation of the brain immune system and degeneration

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Alzheimer's disease (AD) – the major cause of dementia, is characterized by the aberrant folding of the tau protein, leading to its intracellular and extracellular accumulation and formation of neurofibrillary lesions and to β-amyloidosis seen as extracellular deposits of β-amyloid (Aβ) in the brain parenchyma. It is well-documented that both tau and Aβ deposition are considered to be an important inducer of the chronic inflammatory response driven by activated microglia, astrocytes and leukocytes. There is bidirectional communication between neurodegeneration and neuroinflammatory cascade. We have previously hypothesized that the type of neuroinflammation may influence the progression of spreading of neurodegenerative lesions.
In order to stimulate the desired immune response targeting misfolded proteins in AD brain several immunotherapeutic approaches have been proposed. The most advanced strategy for the treatment of human AD remains active immunization and passive immunotherapy, which have already reached the clinical stage of drug development. Tau and amyloid vaccines or humanised antibodies target a variety of pathogenic protein species either in the intracellular or extracellular spaces. Clinical studies demonstrated that the immunotherapy is able to remove pathological lesions from the brain. These findings support the notion that targeted immunotherapy might represent effective treatment with disease modifying effect.

The work is supported by research grant: APVV - 18-0515

