P-08.3-57
Effect of Trefoil factor 3 deficiency in liver of streptozotocin induced Type I diabetes mice model

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Trefoil Factor 3 (Tff3) is a small protein predominantly expressed in gastrointestinal epithelial cells with a multifunctional role in the protection and repair of the mucosa through participation in an immune response. However, Tff3 is expressed in other various tissue where it exhibits different biological effects. The complete diminishment of liver Tff3 was observed in the early phase of the diabesity mice model and since then its role in metabolic processes has emerged. Downregulated expression of TFF3 is reported in the serum of Type I diabetes (T1D) patients. T1D is a complex disease characterized by absolute insulin deficiency and altered immune response. The liver, as a main metabolic organ, is one of the key factors in the pathophysiology of T1D. Hence, our goal was to analyze the impact of Tff3 deficiency on different relevant endoplasmic reticulum (ER) stress, apoptosis and inflammation markers in the liver of the T1D model. To address this issue, firstly, we generated new congenic mouse model Tff3-/-/C57Bl6/N from existing mixed background strain by speed congenics. The new model is more representative for metabolic studies due to less altered metabolic phenotype. Multiple intraperitoneal injections of streptozotocin (STZ) were administered to Tff3-/- mice and wild type control. STZ is selectively toxic to the insulin-producing β cells and it is widely utilized for inducing T1D in animal models. We have monitored different metabolic parameters and after 6 weeks of STZ-induced hyperglycemia, mice were sacrificed and relevant tissues were analyzed. DNA damage-inducible transcript 3 (CHOP), a key ER stress transcription factor involved in apoptosis was downregulated in Tff3 deficient mice. Altered ER stress, presence in different tissues and involvement in the immune response are triggering the question of Tff3 systemic action in the organism and new congenic Tff3 deficient mice represent a valuable tool in that scientific pursuit. *The authors marked with an asterisk equally contributed to the work.

P-08.3-58
Binding studies of *Hirudo medicinalis* related cationic antimicrobial peptides and human plasma proteins

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Cationic antimicrobial peptides (cAMPs) are a promising alternative to conventional antibiotics since they cause little or no microbial resistance. The interaction of cAMPs with plasma proteins is of interest because such interaction plays an important role in the pharmacokinetics of therapeutic agents. The aim of this study was to determine blood plasma proteins that bind cAMPs developed by us based on bioinformatics analysis of the genome of the leech *Hirudo medicinalis*. The cAMPs used in this work were as follows: KKGKSFKQLHIIVHLVKSWLRTILTHI and FLIGKAIKRKFCLRSVWNA. The peptides were immobilized on agarose gels via an aminocaproic acid spacer. Affinity chromatography of human blood plasma on the resins obtained revealed a 67-kDa protein to be a single protein that binds with cAMPs. The protein was identified as human serum albumin (HSA) by its molecular mass, by staining with bromophenol blue (BPB) and by reaction with monoclonal antibodies against HSA. Chromatography of blood plasma on a control sorbent (without immobilized cAMP) showed no adsorption of HSA. The complex HSA-cAMP dissociated with increasing NaCl concentration, thereby indicating the electrostatic nature of the interaction. When blood plasma was titrated with increasing amounts of cAMP, disc electrophoresis in detergent-free gels displayed a decrease in the intensity of the BPB-stained HSA band, and, conversely, a dose-dependent increase in the BPB-stained precipitate that did not migrate into the gel was observed. To avoid a possible influence of the loading dye on complexing, Cy5-labeled cAMPs were used, which also formed complexes with HSA under electrophoretic conditions. Thus, the results show that among blood plasma proteins, only HSA binds cAMPs. It is possible to assume that HSA can be a transport molecule for cAMPs in blood plasma. The study was supported by the Russian Science Foundation grant No. 20-15-00270.

P-08.3-59
Protective effects of olive-derived compounds in molecular mechanisms underlying osteoarthritis.

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Osteoarthritis (OA) is the most common disease of the joints and first cause of motor disability in the elderly. Chondrocytes, the only cell type present in cartilage tissue, are primarily affected by the disease and responsible for local inflammation and ECM remodeling, thus leading to degeneration of articular cartilage. Up to date, available treatments for OA mainly focus on symptom management. Recently, a valid alternative to restore joint homeostasis has emerged from dietary nutraceuticals with known anti-inflammatory properties. Our study aims to understand the potential protective role of two olive-derived (poly)phenols, hydroxytyrosol (HT), oleuropein (OLEU), in the pathogenesis and progression of OA. Primary chondrocytes were obtained from cartilage samples of OA patients. High density cell cultures were set up, pre-treated with HT or OLEU and then, stimulated with lipopolysaccharide (LPS). Our findings indicate that HT and OLEU decrease the gene expression of LPS-induced inflammatory markers, such as iNOS and COX-2 and significantly reduce oxidative stress after 48 h. Furthermore our data suggest the involvement of the NOTCH signaling pathway in the etiopathogenesis of OA. Of the four genes in the NOTCH family, the over-expression of NOTCH-1 in OA cartilage suggests a possible role in the onset and progression of the disease. Indeed, in

our experimental model, the silencing of NOTCH-1 resulted in the attenuation of its target HES-1 as well as of several OA markers, such as MMP13, VEGF, ADAMTS-5 and RUNX-2, and inflammation-related genes, like NFKB1, IKK-α, IL6 and IL8. HT and OLEU were able to decrease NOTCH-1 expression in LPS-stimulated-chondrocytes. Thus we can suppose an involvement of NOTCH-1 in biochemical modifications exerted by these treatments. In conclusion, these results open an interesting outlook for further investigations on the molecular mechanisms involved in OA and for future development of novel therapeutic strategies.

P-08.3-60
The gene expression of inflammatory cytokines and tight junctions proteins as a markers of impaired fish immunity

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The bacterial diseases are known to be associated with an increase in the permeability of the fish intestinal barrier, often due to decrease of the tight junctions proteins (TJ) expression. TJ such as transmembrane occludin (ocln) and claudin (cldn1) and cytosolic zonula occludens-1 (zo-1) provide intercellular contacts and thus regulate epithelial barriers, including blood-tissue barrier. Therefore, bacterial infection can cause systemic effect on structural integrity of all tissues in fish organism. In this study we compare expression of pro-inflammatory interleukin 1b and interleukin 8 (IL1b and IL8) and anti-inflammatory cytokin transforming growth factor (TGFb) with expression of TJ proteins in liver and spleen of farmed rainbow trout with vibriosis. Among fish collected from the same cage there were individuals with different extent of development of visual signs of infection progression (skin and organ color, ulcers, hemorrhages), indicating two group of fishes: sick and resistant to bacterial infection. The same, 60–100 fold difference of mRNA content of IL1b and IL8 (enchanced in sick fish) was found in the liver and spleen of fishes from these two groups by analysis of gene expression. The 4 fold increase in anti-inflammatory TGFb also was detected in diseased individuals in comparison with more healthy. Except of weak positive corrlation zo-1 with IL8 in fish spleen ($r = 0.44$), no correlation was detected between expression of TJ proteins and inflammatory cytokines in fish liver and spleen, indicating that the effect of decreased TJ expression in the intestine of infected fish, reported previously, was local. This suggests that disturbance of intestinal epithelium integrity during infection could be provoked by altered intestinal microflora, rather than cytokines, systemically circulating in the blood. The interdisciplinary research was supported by a grant from the Russian Science Foundation (No. 20-66-47012) in partnership with Irkutsk State University. *The authors marked with an asterisk equally contributed to the work.

P-08.3-61
Development of new experimental approaches to study Ca^{2+} regulation and B-cell fate *ex vivo*

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B cells play a crucial role in a humoral immune response against pathogens. Antigen (Ag)-specific B cells need to recognize Ag via B cell receptor (BCR) and then get T cell help to be recruited into long-term humoral response. The fate of Ag-exposed B cells (activation, anergy or death) is determined by the type, size and valency of Ag, duration of Ag interaction with BCR and timing of T cell help, as well as their extracellular environment. Combination of these signals stimulates intracellular calcium signaling in B cells. In the simplest case, activated by foreign Ag BCRs modulate PLCγ, IP_3 production and subsequent calcium mobilization from intracellular store. A significant increase in cytosolic Ca^{2+} can lead to mitochondrial energization and collapse. How do B cells integrate all incoming signals to decide on their activation or tolerance is insufficiently understood. Analysis of the Ca^{2+} and other signaling events in B cells *ex vivo* is complicated by relatively rapid death of B-cells. Here we aimed to develop a better experimental approach to study Ca^{2+} signaling in B-cells during activation *ex vivo*. Previous studies indicated that coculturing murine B cells with fibroblast reticular cells (FRCs) can better mimic physiological environment and support their survival *ex vivo* [1]. Therefore, we have undertaken to utilize FRC-B cells co-culture approach to study intracellular signaling and cell fate decisions in human B cells. Preliminary analysis confirmed better survival of human B cells co-cultured with FRCs from human lymph nodes *ex vivo*. By utilizing co-culture approach, flow cytometry and confocal microscopy we are planning to study various scenarios of Ag and T cell help acquisition by human B cells and dissect regulation of cytosolic and mitochondrial calcium in determining B cell fate. The study was funded by RFBR and the Royal Society of London (RS), project number 21-51-10005. [1] V. Cremasco et al., Nat.imm. (2014), vol.15, pp. 973–983

Aging stress and neurodegeneration

P-08.4-01
A new genetic drug for Hutchinson-Gilford progeria syndrome

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Hutchinson–Gilford progeria syndrome (HGPS) is a rare genetic disorder caused by a point mutation in the LMNA gene. A point