2016 ANNUAL MEETING OF THE

CROATIAN IMMUNOLOGICAL SOCIETY

OGULIN, OCTOBER 14th-15th 2016

ORGANIZED BY

THE CROATIAN IMMUNOLOGICAL SOCIETY
University of Rijeka Faculty of Medicine
University of Zagreb School of Medicine

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13:00-14:45	**REGISTRATION**
14:45-15:00	**OPENING** **Danka Grčević**, president Croatian Immunological Society
15:00-15:30	**SESSION I** *Chairs*: Sabina Rabatić and Felix Wensveen **INVITED LECTURE** **Antonio Inforzato** Istituto Clinico Humanitas IRCCS, Milan, Italy **Crosstalk between pentraxins and complement in cancer and infection immunology: new insights from the long pentraxin PTX3**
15:30-16:00	**SELECTED ORAL PRESENTATIONS** **Branka Popović**: IL-33 drives regulatory T cell suppression of severe liver damage upon mouse cytomegalovirus infection **Tamara Gulić**: Purification and characterization of the motogenic properties of Migration Stimulating Factor, a genetically truncated onco-fetal isoform of human fibronectin 1 **Ilija Brizić**: Perinatal cytomegalovirus infection drives NK cell hyporesponsiveness characterized by downregulation of T-box transcription factor
16:00-16:30	**COFFEE BREAK**

FRIDAY October 14th 2016

SESSION I (continued)

Chairs: Sabina Rabatić and Felix Wensveen

16:30-17:00

INVITED LECTURES

Stipan Jonjić
University of Rijeka Faculty of Medicine, Rijeka, Croatia

Cytomegalovirus evasion of DNAM-1 dependent immune control by inflammatory monocytes and NK cells

17:00-17:30

Boris Turk
Institut "Jožef Stefan", Ljubljana, Slovenia

Cysteine cathepsins in inflammation: targets for noninvasive whole body imaging

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17:30-18:30

CROATIAN IMMUNOLOGICAL SOCIETY GENERAL ASSEMBLY

18:30-19:30

DINNER

19:30-22:00

POSTER SESSION

Chairs: Gordana Blagojević Zagorac, Jelena Tomac, Dora Višnjić, Ivo Kalajzić

SATURDAY October 15th 2016	
	SESSION II *Chairs:* Tomislav Kelava and Vanda Juranić Lisnić
08:30-09:00	**INVITED LECTURE** **Ivo Kalajzić** Center for Regenerative Medicine and Skeletal Development, UConn Health, CT, USA **Mesenchymal stem cells and Notch signaling regulation of bone regeneration**
09:00-09:40	**SELECTED ORAL PRESENTATIONS** **Antonio Markotić**: Protective effect of LPS-induced inflammation on Fas-mediated hepatocyte apoptosis **Vilma Dembitz**: The role of autophagy in the effects of AMP-kinase modulators on acute myeloid leukemia cells **Lovro Lamot**: From symptom to genes: applicability of functional genomic methods in discovering the mechanisms of newly described disease entity **Felix Wensveen**: Memory CD8 T cell formation requires induction of Bcl-2 by Eomes in response to low-affinity T cell receptor ligation
09:40-10:05	**INVITED LECTURES** **Dora Višnjić** University of Zagreb School of Medicine, Zagreb, Croatia **AMPK/mTOR, autophagy and differentiation**
10:05-10:30	**Miroslav Harjaček** University of Zagreb School of Medicine, Zagreb, Croatia **From genes to bedside: the current view on pathophysiology of juvenile idiopathic arthritis**
10:30-11:00	**COFFEE BREAK**
11:00-13:00	**TOUR TO IVANA BRLIĆ MAŽURANIĆ MUSEUM WITH SIGHTSEEING**

SATURDAY October 15th 2016	
13:00-14:00	**LUNCH**
14:00-14:30	**SESSION III** *Chairs:* Stipan Jonjić and Astrid Krmpotić **INVITED LECTURE** **Ennio Carbone** Università degli Studi di Catanzaro "Magna Graecia", Catanzaro, Italy **Solid tumor's therapy new opportunity: NK cells**
14:30-15:10	**SELECTED ORAL PRESENTATIONS** **Jelena Železnjak**: Who wins the fight? A game of cat and mouse between Ly49 receptors and MCMV encoded immunoevasins **Marko Šestan**: CMV infection enhances development of glucose intolerance and insulin resistance in obesity **Daria Kveštak**: NK cells persisting in the brain following MCMV infection induce polarization of microglia toward proinflammatory phenotype and delay in cerebellar growth via interferon γ **Kristina Vuković**: CMV vector expressing RAE-1γ ligand serves as a highly efficient anti-tumor CD8 T cell vaccine
15:10-15:35	**INVITED LECTURES** **Bojan Polić** University or Rijeka Faculty of Medicine, Rijeka, Croatia **The role of NKG2D in development and education of NK cells**
15:35-16:00	**Jelena Tomac** University or Rijeka Faculty of Medicine, Rijeka, Croatia **Multiple overlapping mechanisms of ovarian follicle resistance to CMV infection**
16:00-16:30	**COFFEE BREAK**

SATURDAY October 15th 2015

	SESSION IV *Chairs:* Bojan Polić and Danka Grčević
16:30-17:00	**INVITED LECTURE** **Matija Rijavec** University Clinic of Respiratory and Allergic Diseases, Golnik, Slovenia **The novel role of basophils in anaphylaxis**
17:00-17:30	**SELECTED ORAL PRESENTATIONS** **Ivan-Christian Kurolt**: Urinary microRNAs as new early indicators for diseases severity in hemorrhagic fever with renal syndrome **Beata Halassy**: Native elution in immunoaffinity chromatography of viruses – a step toward high-purity virus particle purification **Ljerka Karleuša**: Disruption of proteasomal function, endosomal acidification and actin integration influence IE1 expression in MCMV infected cells
17:30-17:55	**INVITED LECTURES** **Gordana Blagojević Zagorac** University or Rijeka Faculty of Medicine, Rijeka, Croatia **Chasing recycled molecules by monoclonal antibody-based recycling assays**
17:55-18:20	**Janoš Terzić** University or Split School of Medicine, Split, Croatia **Role of IL-6 in cancer development**
18:20-18:30	**CLOSING REMARKS AND AWARDS** **Danka Grčević**, president Croatian Immunological Society

LECTURES

CROSSTALK BETWEEN PENTRAXINS AND COMPLEMENT IN CANCER AND INFECTION IMMUNOLOGY: NEW INSIGHTS FROM THE LONG PENTRAXIN PTX3

Antonio Inforzato

Humanitas Research Hospital, Rozzano, Italy

Traditionally regarded as the first line of defense against pathogens, innate immunity plays key roles in a number of additional processes, including tissue remodelling, inflammation and cancer development. The innate immune system comprises a cellular and a humoral arm, the latter encompassing soluble pattern recognition molecules (sPRMs) that cooperate in the recognition of and response to pathogen and danger associated molecular patterns (PAMPs/"non-self" and DAMPs/"modified-self", respectively). The complement system is a major component of the humoral arm comprised of a cascade of more than 30 proteins, which is activated via three distinct pathways (alternative, classical and lectin). Recognition and disposal of invading pathogens are the canonical biological commitments of complement, however it is now appreciated that this system is actively involved in adaptive immunity, cancerogenesis and cancer-related inflammation. In this regard, the traditional paradigm describes complement as "good", in that it recognizes, when not deceived, the cancer cell, and either directly (via membrane attack complex-mediated lysis) or indirectly (via complement-dependent cell toxicity) kills or disposes of it. New ideas, however, are emerging that challenge this dogma and point to the complement system as a component of the tumor promoting inflammation.

Amongst other sPRMs, pentraxins are a superfamily of highly conserved proteins with distinctive quaternary structures. C-reactive protein (CRP) and serum amyloid P component (SAP) collectively form the short pentraxin arm of the superfamily, and share a typical cyclic pentameric symmetry. Both proteins are mostly produced in the liver in response to IL-6, and are major acute phase reactants in humans and mice, respectively. Pentraxin 3 (PTX3) is the prototypic member of the long pentraxin arm; as such, it contains an amino-terminal region linked to a C-terminal pentraxin domain, and differs from the short counterparts in chromosomal localization, gene expression, cellular source and ligands. PTX3 is not expressed by hepatocytes, but it is rather produced by a number of other somatic and immune cells at sites of inflammation and infection. The locally made protein acts as a non-redundant protective factor in the host defence against selected microorganisms, most notably the opportunistic fungus Aspergillus fumigatus (AF), the major etiologic agent of invasive aspergillosis (IA), a lethal infection amongst immunocompromised individuals. This property relies on a tight molecular crosstalk with the complement system. Indeed, PTX3 can be regarded as a functional ancestor of antibodies: it has opsonic activity towards AF, and enhances recognition, phagocytosis and killing of fungal conidia by immune cells, mainly polymorphonuclear neutrophils, via complement and Fc receptor pathways.

Additional functions have been reported for this long pentraxin in several processes and mechanisms of innate immunity, inflammation and tissue remodelling, including a novel activity as extrinsic oncosuppressor gene in cancer-related inflammation, where PTX3 exerts a protective role, once again through regulation of complement activation. Here I discuss the most recent findings on the complement/PTX3 crosstalk with major regard to cancer and infection immunology. Novel vistas will be proposed on this two-sided system, based on a critical revision of current literature and original data from my own work.

CYTOMEGALOVIRUS EVASION OF DNAM-1 DEPENDENT IMMUNE CONTROL BY INFLAMMATORY MONOCYTES AND NK CELLS

Stipan Jonjić

University of Rijeka Faculty of Medicine, Rijeka, Croatia

The poliovirus receptor (PVR, CD155) is a highly conserved and ubiquitously expressed glycoprotein involved in cellular adhesion and immune recognition. PVR is constitutively expressed on the majority of somatic cells under physiological conditions and its expression is modulated as a consequence of viral infections and oncogenesis. Interestingly, while tumors frequently exhibit abnormally high PVR cell surface levels, some viruses (for example HCMV or HIV) downregulate PVR surface expression presumably to avoid immune cell recognition. PVR serves as a ligand for three receptors: DNAM-1 (CD226), an activating receptor expressed on the majority of immune cells; TIGIT, receptor that inhibits NK and T cell cytotoxicity and CD96 (Tactile), receptor with both activating and inhibitory functions on NK cells. Therefore, the precise mechanism that balances activating and inhibitory signals gathered through these receptors, as well as consequences of the PVR modulation in vivo are important unresolved issues of the PVR biology that might explain differences in tumor and viral modulation of PVR.

To assess the effect of balancing mechanism mediated by PVR receptors in vivo, we took advantage of murine model of cytomegalovirus infection. Our results showed that similar to human cytomegalovirus (HCMV), mouse cytomegalovirus (MCMV), downregulates the surface PVR. We have also characterized the molecular mechanism of this viral regulation that includes PVR retention in endoplasmic reticulum and proteasomal degradation. In addition, using a panel of MCMV deletion mutants it was possible to attribute this function to a novel MCMV protein, within the predicted m20 gene region that we named m20.1. Viral mutant lacking this regulator was severely attenuated in vivo, and this attenuation was reduced or abolished in DNAM KO mice, indicating the dominance of the activating receptor DNAM-1 in deciding the outcome of the modulation of PVR levels. The early attenuation of mutant viruses lacking the PVR inhibitor was only partially dependent on NK cells, which can be explained by the fact that these cells induce both activating and inhibitory PVR receptors upon infection. However, depletion of mononuclear phagocytes abolished the virus control which correlates with dramatic upregulation of DNAM-1 and the absence of inhibitory PVR receptors on these cells, even upon infection. In particular, we identified CCL2 dependent inflammatory monocytes as the major subpopulation controlling virus lacking PVR inhibitor via induction of iNOS. Overall, our data provide the strongest evidence so far for CMV control by mononuclear phagocytes and NK cells in which DNAM-1–PVR pathway plays an essential role and demonstrates novel mechanism of viral regulation of paired receptors. Hence, these results may be instrumental to identifying novel intervention targets and in designing novel vaccines and vaccine vectors.

**This work has recently been published: Lenac Rovis et al, Journal of Experimental Medicine, 213(9):1835-50, 2016*.

CYSTEINE CATHEPSINS IN INFLAMMATION: TARGETS FOR NONINVASIVE WHOLE BODY IMAGING

Boris Turk

Jozef Stefan Institute, Department of Biochemistry and Molecular Biology, Jamova 39, 1000 Ljubljana, Slovenia
Center of Excellence CIPKEBIP, Jamova 39, 1000 Ljubljana, Slovenia
Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

Inflammation plays an important role in disease onset and progression in a vast number of diseases, called also inflammation-associated diseases including various cancers, psoriasis, dermatitis, inflammatory bowel diseases, pancreatitis, various forms of arthritis, osteoarthritis, osteoporosis/bone resorption, septic shock, atherosclerosis, ischaemia-reperfusion injury, coronary heart disease, vasculitis, amyloidosis, pulmonary fibrosis, viral infections, systemic lupus erythematosus, and asthma. Proteases play a major role in a number of these diseases. However, understanding the precise role of an individual protease in a disease remains a major challenge for successful therapeutic applications. There are several ways how to address this issue, including the chemical biology approaches including small molecule inhibitors and activity-based probes. The latter approaches, especially those based on activity-based probes, offer a major potential for noninvasive optical imaging by monitoring protease activities in situ, i.e. on disease site. Moreover, the approach enables also validation of proteases as drug targets, in vivo validation of drug candidates and evaluation of the diagnostic potential of the target proteases. Among the proteases found to be tightly linked with inflammation-associated diseases are also cysteine cathepsins that can be found at the sites of inflammation. Furthermore, since they are heavily upregulated in a number of inflammation-associated diseases, they are therefore perfect targets for such approaches. There is increasing evidence that monitoring cathepsin activity in vivo may be applicable to diagnostic imaging, such as demonstrated primarily for cancer, arthritis and inflammatory bowel diseases. Moreover, cathepsins can be also used as targets for targeted drug delivery approaches combined with diagnostics, thereby offering a theranostic potential.

MESENCHYMAL STEM CELLS AND NOTCH SIGNALING REGULATION OF BONE REGENERATION

Ivo Kalajzić

Center for Regenerative Medicine and Skeletal Development, UConn Health, CT, USA

Notch signaling has been recently identified as a key player during bone and cartilage development. Notch inhibits the differentiation of osteoprogenitor cells, but has an osteogenic effect in mature osteoblasts. We have previously shown that alpha smooth muscle actin (αSMA) is a marker of mesenchymal progenitor cells that make a significant contribution to fibrous, osteoblast, and chondrocyte lineages within a fracture callus. Gene expression analysis of isolated αSMA-labeled progenitor cells revealed that a number of components of the Notch signaling pathway, including receptors Notch 1, 3 and 4, and target genes Hes1 and Hey1, were significantly decreased during the early stages of fracture healing. We hypothesize that a decrease in Notch signaling could regulate the expansion, migration and the differentiation of periosteal cells in the fracture callus. In this context, inducible Cre-expressing transgenic models enable precise definition of when Notch signaling is required and in which cell population during the fracture healing process.

We are using an inducible mouse model overexpressing the Notch1 intracellular domain (NICD1) in osteoprogenitor cells: αSMACreERT2/Rosa-NICD1. Periosteal progenitor cells (PPC) were isolated from the periosteum of 8-9 week old mice and cultured. The targeted overexpression of NICD1 results in increased expression of Notch downstream targets Hes1 and Hey1. PPC overexpressing NICD1 had increased proliferation and migration compared to tamoxifen treated cultures from Cre negative littermates. Notch overexpression reduced osteogenic differentiation, evidenced by reduced von Kossa staining and lower expression of osteocalcin.

The influence of NICD1 overexpression on the fracture healing process was assessed in 8-9 week old αSMACre/NICD1 mice after 3 tamoxifen injections at D0, D2 and D4 post femoral fracture. Histological analysis was performed 1-3 weeks after fracture. Mice with targeted NICD1 overexpression showed a trend towards a smaller callus displaying first less cartilage and then less mineralized content than the control mice.

Appropriate regulation of Notch signaling appears to be important for osteogenic differentiation of PPCs and bone fracture healing. It constitutes a potential target to improve and accelerate fracture healing by inhibiting its effect in specific cell populations responsible for the bone repair at specific stages of the process.

AMPK/mTOR, AUTOPHAGY AND DIFFERENTIATION

Dora Višnjić

Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia

Recent studies suggest that drugs targeting metabolism may have some role in differentiation therapy of leukemia. Adenosine monophosphate (AMP)-activated kinase (AMPK) is an evolutionary conserved serine/threonine kinase that is activated in response to any decrease in AMP/ATP ratio. Among many substrates, activated AMPK inhibits the activity of mammalian target of rapamycin (mTOR), which decreases protein synthesis and cell growth. Physiologically, mTOR is activated downstream of phosphoinositide 3-kinase (PI3K)/Akt pathway, and our previous studies demonstrated the activation of PI3K and Akt in nuclei of leukemia cells during differentiation. PI3K/Akt inhibitors reduce the number of viable cells, but negatively affect their differentiative capacity. In contrast, use of rapamycin, an mTOR-inhibitor, potentiates differentiation along granulocytic pathway. To further investigate the role of upstream regulators of mTOR in leukemia differentiation, we tested the effects of two AMPK-modulators, metformin and AICAR (5-amino-1-β-D-ribofuranosyl-imidazole-4-carboxamide). Our results demonstrated that AICAR alone induced the expression of cell surface markers associated with mature monocytes and macrophages in U937 cells. However, no significant increase in the expression of differentiation markers was observed in U937 cells treated with metformin alone, although both modulators had similar effects on proliferation and survival. Although we detected time and dose-dependent increase in the level of Thr phosphorylated AMPK, a significant decrease in AMPK expression that was achieved by using commercially available siRNA sequences in U937 cells had no significant effects on the AICAR-mediated effects on the number of viable cells or the expression of differentiation markers. Therefore, present studies are aimed to determine the mechanism responsible for beneficial effects of AICAR in AML cells and to further elucidate signaling mechanisms and metabolic changes responsible for monocytic and granulocytic differentiation of AML cell lines in response to other inducers.

Our results show that AICAR and other differentiation agents induce autophagy flux, as measured by the level of LC3II in the presence and absence of bafilomycin A. No increase in the level of autophagy was observed in the presence of metformin. PI3KC3 inhibitor, 3-methyladenine, inhibited the expression of differentiation markers, but increased the level of LC3-II suggesting that 3-MA cannot be used as a specific autophagy inhibitor under nutrient-rich conditions. siRNA experiments showed that ATG7-dependent autophagy pathway is necessary for AICAR-mediated effects on the expression of differentiation markers. The role of autophagy in differentiation of leukemia cells and peripheral blood mononuclear cells will be discussed.

FROM GENES TO BEDSIDE: THE CURRENT VIEW ON PATHOPHYSIOLOGY OF JUVENILE IDIOPATHIC ARTHRITIS

Miroslav Harjaček

Department of Pediatrics, Division of Clinical Immunology and Rheumatology, Clinical Hospital Center "Sestre milosrdnice, Zagreb, Croatia

Juvenile idiopathic arthritis (JIA) is the most common childhood rheumatic disease. JIA is not a single disease entity, but rather a group of seven 'genetically heterogeneous' and 'phenotypically distinct' disorders (subtypes). The early diagnosis of new-onset JIA has become a major objective for pediatric rheumatologists in order to identify a management strategy able to change the natural history of the disease and to prevent joint damage and functional impairment. The term undifferentiated arthritis (UA) is applied to the most common type of arthritis at the early stage when, in the absence of current recommended diagnostic criteria, it cannot be classified into the clinical subtypes of JIA. Patients with UA may progress towards JIA; however in some cases arthritis may completely resolve. JIA is a multi-factorial disease that is influenced both by environmental and genetic factors. The fundamental process in JIA is chronic inflammation, in which the immune system understandably plays a critical role. Both innate and adaptive immune systems have been implicated in the pathogenesis of various subtypes of JIA. In addition, many studies have established the magnitude of the genetic basis of JIA. JIA is a complex genetic condition and the multiple genes that influence susceptibility are actively being sought. Current dogma supports the concept that the expression of a disease-inducing signature cytokine phenotype is important to the maintenance stage of chronic synovitis. This cytokine phenotype has been characterized as a polarization toward type TH1/TH17 cytokines, which are proinflammatory. Traditionally, JIA has been viewed as "autoimmune" in nature although it has been increasingly clear that particular subtypes of the disease are predominately "autoinflammatory" in nature (e.g. systemic onset JIA) or shows overlapping features of both (e.g. enthesitis-related arthritis- ErA). More recently, the inappropriate responsiveness to various "stressors" like viruses, bacteria, prolonged antibiotic use, diet, trauma or mechanical stress, as well as psychogenic stress have been recognized as initial trigger in activation of the orchestrated, complex crosstalk between various parts of the immune system and CNS leading to "danger" response. Stressors activation of the various neuroendocrine pathways and oral/gut dysbiosis are triggering simultaneous activation of the crucial transcription factors like NF-κB, inflammasomes like NLRP-3, and due to failure of negative immune regulation, ultimately create a pro-inflammatory milieu leading to chronic synovitis. However, we are still far from having a clear picture of the molecular network that predisposes a child to develop the disease, to worsen the symptoms, or to successfully respond to a specific treatment. By using our own data on biomarkers, genetic, gene expression studies, and epigenetic control of key master genes affecting the pathophysiology of ErA (spondyloarthritis), the most common subtype of JIA, I would attempt to challenge current dogma and propose the working platform for the further research of the JIA pathophysiology.

SOLID TUMOR'S THERAPY NEW OPPORTUNITY: NK CELLS

Ennio Carbone

Department of Experimenal and Clinical Medicine University Magna Graecia of Catanzaro, Italy
Department of Microbiology and Tumorbiology (MTC), Karolinska Institutet, Stockholm, Sweden

The talk will discuss recent data showing new biological property of NK cells: a) NK cells capability to efficiently target the Cancer Initiating Cells (CIC) tumor compartment of solid tumor b) New NK cells subset identified in the melanoma metastatic lymph node exerting a robust autologous cytotoxicity against tumor b) NK cells potential role in the anti immune check point therapy prognosis.

In the first part of the talk data showing the in vitro and in vivo NK cells recognition of human colon adenocarcinoma derived CSC and murine breast adenocarcinoma lesion will be presented (Tallerico et al under revision Oncoimmunology 2016).

The second part the NK cells recognition and elimination melanoma metastasis will be discussed in the context of NK cells anatomic localization, NK subsets differentiation, cytokines and chemokines tumor environment and disease stages.

The last section the anti immune check points era take shape in a study where we investigate the potential role of NK cells in melanoma patients treated with anti CTL-4 (Ipilimumab).

The lack of knowledge on predictive biomarkers that could assist the anti immune check points cancer therapy remains a limiting factor. We speculate that, along with additional markers, the immunoscore is fundamental as prognostic and predictive marker for response to immunotherapies in metastatic melanoma. Our previous data demonstrate that NK cells control the melanoma progression in the infiltrated lymph nodes [Ali et al Nature Comm 2014]. Therefore we have analyzed both T cells and NK cells subsets frequencies and receptors repertoire in the peripheral blood of 63 Ipilimumab treated patients with Stage IV metastatic melanoma. The study was performed in one Italian and one Swedish melanoma patient's cohort (Tallarico et al Submitted 2016). The role of IL-15, TIM-3 and NK cells subset will be discussed in depth.

THE ROLE OF NKG2D IN DEVELOPMENT AND EDUCATION OF NK CELLS

Bojan Polić

Department of Histology & Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

NKG2D is an activating receptor expressed on all NK cells early in NK cell development and have important role in the cellular stress-surveillance. 'Stressed' cells due to viral infection, oncogenic transformation, metabolic or other reasons up-regulate NKG2D ligands („induced self") which can engage the receptor and activate NK cells. MHC class I like-molecules (Rae1 and H60 family, MULT1) are well characterized ligands for NKG2D (encoded by Klrk1). Previously, our group has shown that NKG2D plays an important role in NK cell development (Zafirova et al. Immunity, 2009). NKG2D-deficiency resulted in hyper reactive phenotype of NK cells which caused better control of MCMV infection. However, Klrk1-/- NK cells still kept impaired ability to kill tumor targets expressing NKG2D ligands.

In this study, we investigated whether and how NKG2D-deficient mice can control tumors which do not express NKG2D ligands, but express ligands for other NK receptors. As a tumor model we used syngeneic melanoma cell line (B16, clone F10). Subcutaneous tumor inoculation resulted in smaller tumor size in Klrk1-/- mice, while intravenous inoculation resulted in prolonged survival of Klrk1-/- mice compared to C57BL/6J littermates. The survival differences as well as differences in tumor size between the groups were lost upon depletion of NK cells, showing NK-cell dependence of the phenomenon. Using NCR1-/- and IFN□-/- mice we were also able to show that the tumor control is NCR1- and IFN□- dependent. Also, in vitro stimulation of Klrk1-/- NK cells via NCR1 and CD16, but not other activating NK receptors, resulted in higher production of IFNγ. However, conditional deletion of Klrk1 at the stage of NCR1 expression (Klrk1flox/flox NCR1Cre mice) resulted in absence of NK cell hyper reactivity in vivo and in vitro.

In conclusion, our findings for the first time show that NKG2D sets specifically activation thresholds for NCR1 and CD16, two important activating receptors expressed on all NK cells. This regulatory role of NKG2D takes place early during the NK cell-development, before the NCR1 expression, which implicates its role in NK cell education.

MULTIPLE OVERLAPPING MECHANISMS OF OVARIAN FOLLICLE RESISTANCE TO CMV INFECTION

Jelena Tomac

Department for Histology and embryology and Center for proteomics, Faculty of Medicine, University of Rijeka, Croatia

Human cytomegalovirus (HCMV) is a widespread herpesvirus that causes life-long persistent infections in its host. Although relatively harmless to immunocompetent individuals, it can cause grave disease in patients with weakened or immature immune system. Infection during pregnancy can cause pregnancy-loss or numerous long-term developmental disabilities.

HCMV is highly species specific and only infects humans. Murine cytomegalovirus (MCMV) is biologically similar and related to HCMV; therefore, the infection of mice with MCMV became the most commonly used model for studying the biology and pathogenesis of CMV, especially in research that is difficult to conduct in humans. Although CMV's ability to pass the placenta and cause devastating congenital disease is well established, very little is known about the CMV infection of reproductive organs and its consequences on fertility and pregnancy outcome. We have performed a detailed analysis of CMV pathogenesis in the ovary and observed that CMV very successfully infects the ovaries. The virus is cleared by day 8 PI, indicating a strong role of innate immune system in virus control. Moreover, the infection was completely excluded from ovarian follicles, even in strongly immunosuppressed mouse strains in which nearly whole ovarian stroma and corpora lutea were infected. Since MCMV is natural pathogen infecting the majority of wild mice, development of strategies that can act immediately or very early to prevent infection of follicles in order to preserve reproductive potential is a necessary evolutionary strategy. We have uncovered several layers of protection that preserve ovarian follicles: physical barriers preventing the infection and cells of the innate immune system. We show that ovarian follicles are protected from the infection by a ring of macrophages that rely on NK cells. While the depletion of NK cells does not result in increased viral titers in the ovary, we could observe infected follicles and absence of macrophage rings.

THE NOVEL ROLE OF BASOPHILS IN ANAPHYLAXIS

Matija Rijavec

University Clinic of Respiratory and Allergic Diseases Golnik, Slovenia

Anaphylaxis is an acute, life-threatening, systemic, allergic reaction with rapid onset. The activation of mast cells involving IgE and the high-affinity IgE receptor (FcεRI) on these cells is postulated to have a pivotal role in anaphylaxis and to date, no experimental system has directly demonstrated that basophils contribute to IgE-mediated anaphylaxis. Therefore, the exact molecular mechanism as well as the specific role of basophils in human anaphylaxis remains poorly understood. 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, our research has been focus on the determination of the role of basophils in anaphylaxis. Specifically, our aim was to determine whether blood basophils are activated during anaphylaxis and whether anaphylaxis induces basophil migration to the site of inflammation and in that case which chemotactic factors are involved in their migration. Using the global transcriptome profiling of peripheral blood samples during anaphylaxis we aimed to get a more detailed look at the molecular mechanisms during an anaphylactic episode.

We have demonstrated that the absolute number of circulating basophils is significantly decreased during anaphylactic episode, and that decrease was confirmed with the decrease expression of basophil specific genes, specifically FcεRI, carboxypeptidase A3 and L-histidine decarboxylase in whole blood samples. In line with that, CCL2, a major basophil chemotactic factor, significantly increased during anaphylaxis, and there was an inverse correlation between the absolute number of basophils in blood and CCL2 in sera. Importantly the basophils that remained in the circulation exhibited a low level of activation. Transcriptome analysis further revealed that cellular movement, cell-to-cell signalling, interaction and immune cell trafficking are the most important mechanisms taking place during anaphylaxis. Comparative analysis with expression signatures of immune cells showed significant under-expression of basophil and over-expression of activated eosinophil signatures during anaphylactic reaction. Furthermore, measurement of absolute number of circulating basophils, CCL2 and FcεRI expression have the potential to be used as biomarkers to confirm the diagnosis of anaphylaxis, showing better AUC, sensitivity and specificity than serum mast-cell tryptase which is currently the gold standard diagnostic test to confirm anaphylaxis.

In summary our findings suggest that cellular movement and interactions of distinct immune cells are taking an important place during anaphylaxis. We have demonstrated a marked migration of circulating basophils, which correlated with a significant increase in the level of major basophil chemotactic factor CCL2. These data suggest a novel and specific role for basophils in the pathobiology of human anaphylaxis.

CHASING RECYCLED MOLECULES BY MONOCLONAL ANTIBODY-BASED RECYCLING ASSAYS

Gordana Blagojević Zagorac

University of Rijeka Faculty of Medicine, Department of Physiology and Immunology

Membrane proteins and their ligands are continuously internalized and directed to recycling or degradation. Endocytic recycling is a highly regulated, dynamic and complex cellular process and has irreplaceable role in cellular physiology and pathophysiology processes, includin immune response. Studies of endosomal trafficking suggest that membrane proteins use various recycling pathways and much knowledge about recycling route and the regulatory mechanism was generated for transferrin receptor (TfR), a clathrin-dependent cargo protein. In contrast to clathrin-dependent cargo proteins, for many proteins that are endocytosed by the clathrin-independent mechanism, the rate of endocytic recycling and recycling route was not established and remains largely unknown. The best characterized is the recycling route of Major Histocompatibility Class I (MHC-I) proteins. For detection and quantification of recycling, several assays were used. Although antibodies were used in tracing the recycling routes more than a decade, still there were many inconsistencies in using different experimental approaches, resulting with false positive and false negative results.

We performed a systematic study of various protocols known in literature in which antibodies are used as tools to study endosomal recycling. We used TfR recycling, as a paradigm of rapidly endocytosed clathrin-dependent cargo molecule and fully conformed MHC-I proteins as a paradigm for constitutively endocytosed clathrin-independent cargo molecule. We also followed recycling of open MHC-I conformers (peptide-empty MHC-I proteins, eMHC-I) as a control, because they do not recycle from early endosomal recycling circuit by the fast and slow recycling route.

Our study demonstrates that direct and indirect detection of recycled mAb:protein complexes at the cell surface underestimate the recycling pool, especially for clathrin-dependent membrane proteins that are rapidly reinternalized after recycling. Recycling protocols based on the capture of recycled mAb:protein complexes require the use of the Alexa Fluor 488 conjugated secondary antibodies or FITC-conjugated secondary antibodies in combination with inhibitors of endosomal acidification and degradation. Finally, protocols based on the capture of recycled proteins that are labeled with Alexa Fluor 488 conjugated primary antibodies and quenching of fluorescence by the anti-Alexa Fluor 488 displayed the same quantitative assessment of recycling as the antibody-capture protocols.

This work was supported in part by the Croatian Science Foundation (grant IP-2014-9-9564) and by the University of Rijeka (grants 13.06.1.1.4, 13.06.2.1.55, and 13.06.2.1.56).

ROLE OF IL-6 IN CANCER DEVELOPMENT

Janoš Terzić

University or Split School of Medicine

Inflammation is recently being considered as one of the cancer hallmarks and intereleukin 6 (IL-6), one of main proinflamatory cytokines, plays major role in inflammation-related cancer development. We have found that IL-6 has important role in colon cancer development stimulating development of increased number and size of tumors. These effects were mainly mediated by Stat3 transcription factor. On the other hand, IL-6 role in inflammation-influenced bladder cancer is not so well characterized. Using BBN-induced bladder cancer in IL-6 KO and WT mice, we have determined high activation of immune response genes with tumor phenotype being less distinctive between tested groups.

ORAL PRESENTATIONS

IL-33 DRIVES REGULATORY T CELL SUPPRESSION OF SEVERE LIVER DAMAGE UPON MOUSE CYTOMEGALOVIRUS INFECTION

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Regulatory T cells (Tregs) are crucial for immune homeostasis and for dampening immune response to several diseased conditions, including viral infections. Mouse cytomegalovirus (MCMV) is a herpesvirus with pathogenic potential so early immune mechanisms are essential in controlling virus and protecting from virus-induced pathology. Studies on FoxP3+ Tregs have revealed their inhibitory role on the early T cell response to MCMV infection and have suggested Tregs as a target of MCMV's immunoevasion mechanisms. Here we demonstrate that the number and activation status of liver Tregs is strongly induced in mice infected with MCMV. The depletion of Tregs results in an increased virus-specific CD8+ T cell response without alterations in the virus load. Furthermore, depletion of Tregs leads to severe liver damage and adoptive transfer of Tregs rescues mice from T cell mediated hepatitis. Interestingly, liver Tregs constitutively express high amounts of a cellular receptor for IL-33, tissue alarmin strongly upregulated in the liver upon MCMV infection. The accumulation of Tregs in the liver is dependent on IL-33 signalling and mice lacking the IL-33 receptor show a more pronounced liver pathology and higher death rate compared to infected control mice. These results illustrate importance of IL-33 in the accumulation of liver Tregs and their suppression of MCMV-induced immunopathology.

PURIFICATION AND CHARACTERIZATION OF THE MOTOGENIC PROPERTIES OF MIGRATION STIMULATING FACTOR, A GENETICALLY TRUNCATED ONCO-FETAL ISOFORM OF HUMAN FIBRONECTIN 1

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Migration-stimulating factor (MSF) is a porly studiated oncofetal isoform of human fibronectin 1 (FN1) generated from its primary gene transcript by a intron read-through mechanism. Detailed molecular characterization indicates that MSF is a 70 kDa soluble protein identical to the N-terminal portion of full-length FN1 (up to exon III-1a), with the addition of a unique 10 amino acids long peptide in the C-terminus. MSF is mainly produced by epithelial and stromal cells during foetal development and by cancer-associated fibroblasts, but not by adult healthy cells. Available data indicate that it has a potent motogenic activity for fibroblasts, vascular and epithelial cells, and can induce angiogenesis and matrix remodelling, suggesting a possible role in cancer development. We focus our attention on the characterization of reagents to study MSF biology. After immunization with the MSF-specific 10 amino acids long peptide, we selected a monoclonal antibody recognizing specifically human MSF but not human FN1. The antibody was used to purify by immunoaffinity chromatography recombinant human MSF (rhMSF) produced by transfected CHO cells. Initial efforts were aimed at defining the condions ensuring protein stability over the time. Biological activity of purified rhMSF was tested in migration and invasion assays with monocytes and cancer cells, using boyden chamber or transwells respectively. Purified recombinant MSF can promote migration of a different human cancer cell line (PANC-1, MDA-MB231, 8387), confirming the motogenic effect of this protein. In addition purified rhMSF has a chemotactic activity for human monocytes and neutrophils, suggesting that it can promote monocyte/macrophage and neutrophils recruitment into tissues. Further studies are needed to elucidate the engagement of MSF as protumoral molecule.

PERINATAL CYTOMEGALOVIRUS INFECTION DRIVES NK CELL HYPORESPONSIVENESS CHARACTERIZED BY DOWNREGULATION OF T-box TRANSCRIPTION FACTOR

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Human cytomegalovirus (HCMV) is a frequent cause of disease in immunodeficient and immunologically immature hosts such as newborn infants. For that reason, congenital HCMV infection presents a significant health concern since it is frequently manifested with neurodevelopmental sequelae, such as auditory damage and neurodevelopmental disabilities. NK cells have been shown to play an important role in fighting cytomegalovirus (CMV) infection and the adaptive features of NK cells in response to CMV infection are being recently increasingly recognized. However, the extent to which congenital CMV infection affects and shapes NK-cell mediated immunity is largely unknown. To address this issue, we have used mouse CMV (MCMV) infection of newborn mice as a model to investigate the impact of congenital CMV infection on the maturation and functional properties of NK cells. We observed that perinatal MCMV infection leads to persistent alteration of transcriptional activity and strongly affects the maturation and function of NK cells. Surprisingly, NK cell expression of T-box transcription factor Eomes, critical for NK cell development, was dramatically impaired. At the same time the expression of T-bet, another T-box transcription factor, was unaffected. The downregulation of Eomes correlated with major changes in NK cell phenotype, indicating most notably NK cell exhaustion, as well as an impaired NK cell response to different stimuli. To our knowledge this is the first evidence that a viral infection can lead to the perturbation in NK cell expression of the T-box transcription factors. In addition, we have observed an NK cell population with a phenotype characteristic of memory-like NK cells. This population of NK cells persisted for several months in infected mice indicating that congenital CMV infection is a factor that shapes the NK cell response over a long-term.

PROTECTIVE EFFECT OF LIPOPOLYSACCHARIDE-INDUCED INFLAMMATION ON FAS-MEDIATED HEPATOCYTE APOPTOSIS IN MICE

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BACKGROUND: Fas/Fas ligand (FasL) apoptotic pathway is involved in the pathogenesis of various liver diseases. However, the exact effects of acute inflammation on the liver apoptotic processes are still not well elucidated. We investigated the effect of pro-inflammatory mediators on Fas/FasL-mediated hepatocyte apoptosis using a model of lipopolysaccharide (LPS)-induced acute inflammation.

METHODS: Male C57BL/6 mice received intraperitoneal injection of LPS (0.1 μg/g) while the control group of animals received the vehicle (sterile saline). After 2 hours both groups were treated with anti-Fas (JO2) activating antibody (0.25 μg/g, intravenously). Mice were sacrificed after additional 6 hours and plasma (ALT, AST) and liver samples (pathohistology, caspase activity, qPCR) were harvested. In the second set of experiments mice were treated with saline or LPS, non-parenchymal liver cells were harvested and leukocytes populations were determined by flow cytometric analysis. Concentrations of soluble Fas (sFas) in plasma were determined by ELISA. To induce neutrophil depletion mice were intraperitoneally injected with cyclophosphamide (250 mg/kg), four days prior to LPS and anti-Fas application.

RESULTS: Mice pre-treated with LPS were protected from Fas/FasL-mediated hepatocyte apoptosis as evidenced by lower levels of ALT (median (IQR); 82 (32-182) vs. 3709 (1429 – 5922) U/L, p=0.02) and AST (151.5 (96-256) vs. 3137 (1378-5389) U/L, p=0.02) in plasma compared with mice which received saline before anti-Fas antibody. Additionally, LPS pre-treated mice had lower number of apoptotic cells on pathohistological analysis and lower caspase 8 activity than saline pre-treated mice. LPS alone had no effect on aminotransferase levels and caspase 8 activity, while it increased expression of inflammatory mediators TNF-alpha, IL-1 and IL-6 in hematopoietic liver cells, as well as the expression of Fas and antiapoptotic CFLAR and Bcl2l1 in hepatocytes. LPS did not increase the level of sFas in plasma. Flow cytometric analysis of intrahepatic leukocytes showed an increase in neutrophil (7.6 fold), NK cell (1.7 fold) and NKT cell (1.6 fold) population. Accumulation of Fas positive neutrophils in liver following LPS administration was confirmed immunohistochemically. However, cyclophosphamide-induced neutrophil depletion did not abrogate protective effect of LPS.

CONCLUSION: Acute inflammation induced by LPS alleviates Fas/FasL-mediated apoptosis by acting on Fas-apoptotic pathway upstream of caspase 8 activation. We intend to define protective mechanism more precisely by investigating effects of LPS on expression pattern of broader spectrum of pro- and antiapoptotic molecules at various time points following LPS treatment.

THE ROLE OF AUTOPHAGY IN THE EFFECTS OF AMP-KINASE MODULATORS ON ACUTE MYELOID LEUKEMIA CELLS

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Autophagy has been shown to contribute to differentiation of leukemia cells in various experimental settings. Our recent results showed that 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), a compound commonly used as a modulator of AMP-kinase (AMPK), induced differentiation of U937 cells in an AMPK-independent manner. In the present study we tested for the possible role of autophagy in AICAR-mediated effects. The level of LC3B-II was increased after 48 h incubation with AICAR and other differentiation inducers, all-*trans* retinoic acid (ATRA) and phorbol myristate acetate (PMA). No similar effects were observed in cells treated with metformin, an AMPK modulator without differentiative properties. The pretreatment of cells with 3-methyladenine (3-MA) inhibited agonist-mediated increase in the expression of differentiation markers and decreased the number of viable cells. However, although treatment with 3-MA reduced the levels of PtdIns(3)P, the levels of LC3B-II increased after addition of 3-MA. Gene knockdown for Beclin-1 and class III phosphoinositide 3-kinase (PI3KC3) did not abolish the differentiative effects of AICAR, ATRA and PMA. Still, metformin-mediated decrease in cell viability was inhibited in cells with down-regulated Beclin-1.

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FROM SYMPTOM TO GENES: APPLICABILITY OF FUNCTIONAL GENOMIC METHODS IN DISCOVERING THE MECHANISMS OF NEWLY DESCRIBED DISEASE ENTITY

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OBJECTIVE: Clavicular cortical hyperostosis (CCH) is a sterile inflammatory bone disorder of unknown etiology clinically characterized by pain and/or swelling of the clavicle. It has been regarded as a variant of chronic nonbacterial/recurrent multifocal osteomyelitis (CNO/CRMO) but due to lack of other inflammatory sites and recurrence it could also be regarded as a separate disease in the spectrum. Therefore, it is of high importance to elucidate the exact mechanisms responsible for the development and progression of the symptoms. METHODS: Total RNA was isolated from whole blood of 18 new-onset, untreated CCH patients and 8 healthy controls. DNA microarray gene expression was performed in 5 CCH and 4 control patients along with bioinformatical analysis of retrieved data. Carefully selected differentially expressed genes (TRPM2, TRPM3, TRPM7, CASP2, MEFV, STAT3, EIF5A, ERBB2, TLR4, NLRP3, CD24, MYST3) where analyzed by qRT-PCR in all participants of the study. In one patient, the blood cells were processed using a cytosine for a immunofluorescence microscopy with TRPM3 and TRPM7 antibody. RESULTS: Microarray results and bioinformatical analysis revealed 974 differentially expressed genes, while qRT-PCR analysis showed significantly higher expression of TRPM3 and TRPM7, and lower expression of ERBB2. Immunofluorescence microscopy showed high signal of TRPM3 in blood cells of one patient. CONCLUSIONS: Microarray data analysis revealed that majority of differentially expressed genes in CCH patients are involved in various inflammatory processes, while qRT-PCR analysis confirmed statistically significant expression change of 3 genes. Among them, TRPM3 and TRPM7 are members of transient receptor potential (TRP) gene superfamily, which encodes proteins that act as multimodal sensor cation channels for a wide variety of stimuli, one of which is environmental temperature that in the case of CCH could be elicited by overuse of sterno-clavicular joint (SCJ). Upon stimulation, TRP channels transduce electrical and/or Ca2+ signals. Dysfunctions in Ca2+ signaling due to altered TRP channel function can have strong effects on a variety of cellular and systemic processes, including the activation and the regulation of the inflammasomes, which are reported to be involved in CRMO pathogenesis. ERBB2, third gene with significant expression change, belongs to a family of genes that encodes for widely expressed cell surface growth factor receptors. Recently it has been shown that ErbB activation promotes protective cellular outcomes during inflammation, hence lower expression of this gene could cause damage due to inflammation. Finally, the results of transcriptome analysis were confirmed on a protein level with a proof-of-concept experiment which indicated high presence of TRPM3 in blood cells of a patient. Based on the results of these and previous studies, we hypothesize that CCH could be an autoinflammatory disease induced by SCJ overuse, TRP channel overexpression, inflammasome activation and reduced protection during inflammation.

MEMORY CD8 T CELL FORMATION REQUIRES INDUCTION OF BCL-2 BY EOMES IN RESPONSE TO LOW-AFFINITY T CELL RECEPTOR LIGATION

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In response to viral infection, antigen-specific CD8 T cell clones are activated and form effector cells that directly kill infected cells. In addition, memory cells are generated that provide long-term protection against re-infection. The effector cell pool consists of only a few clones of high antigen-specificity. This ensures that only the most efficient cells mediate direct viral clearance. In contrast, the antigen-specific memory cell pool is much more clonally diverse. While reducing its overall affinity, a larger clonal diversity increases the scope of antigens that can be recognized in a secondary infection. Clearly, different mechanisms control selection of clones in the effector and memory cell pools. How these processes are regulated on a molecular level is largely unknown.

Here we investigated how the intensity of T cell receptor triggering translates to the capacity of a CD8 T cell to form memory cells. As a model we used OT-1 transgenic T cells, stimulated with antigens of high- or low affinity. We find that T cells of high affinity generate more effector cells and proliferate more rapidly than low-affinity cells. In contrast, high-affinity cells have reduced memory forming potential compared to cells of sub-optimal affinity, due to a higher level of cell death early during memory cell differentiation. Micro-array screening of memory precurors revealed that the transcription factor Eomes is more highly expressed in cells of low affinity compared to their high-affinity equivalents. In vitro analysis shows that Eomes is induced at low concentrations of antigen, but that its expression is suppressed at higher intensities of T cell receptor triggering by the transcriptional regulator T-Bet. Using cells deficient for Eomes, we show that this molecule is required for induction of the potent pro-survival protein Bcl-2. Low-affinity cells, but not cells of high-affinity are therefore highly sensitive to ABT-199, a specific inhibitor of Bcl-2. In vivo, we demonstrate that Eomes-deficient CD8 T cells of reduced affinity are rapidly lost within the antigen-specific pool upon viral infection.

In summary, we uncover a new molecular mechanism how diversity of the memory CD8 T cell pool is controlled. Our findings may have implications for future CD8 T cell based therapies, as it can be used to increase the number of viral or tumor antigens that are recognized by a single immunization.

WHO WINS THE FIGHT? A GAME OF CAT AND MOUSE BETWEEN Ly49 RECEPTORS AND MCMV ENCODED IMMUNOEVASINS

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The feature of an early murine cytomegalovirus (MCMV) infection is the rapid and effective downregulation of the MHC class I molecules from the surface of infected cells. A virus does it to prevent antigen presentation and CD8 T cell response. This function however, makes infected cells prone to "missing self"-mediated recognition and killing by NK cells. To avoid this, MCMV encodes for m04/gp34, a protein that binds some MHC I molecules, escorts them to the cell surface and engages inhibitory NK Ly49 receptors. Using reporter cells that express single Ly49 receptor, we have observed that WT MCMV infected cells engaged inhibitory Ly49 receptor equally, or even stronger, than uninfected cells despite MHC I downregulation (Babic Cac et al. 2010). Subsequently, we looked for additional viral factor that might affect interaction between inhibitory Ly49 and MHC I-m04 complex. Here we show that this missing viral factor is encoded by the MAT transcript (Most Abundant Transcript), the most highly expressed transcript throughout the infection. With the total length of 1.7 kb, MAT encompasses 2 previously annotated genes, m168-m169, encodes two proteins and contains a binding site for cellular miRNA-27b (Juranic Lisnic et al. 2013, Marcinowski, Tanguy et al. 2012). The deletion of region that encodes for one of MAT protein, 11 kDa MAT uORF, not only abrogated stronger interaction between inhibitory Ly49 receptors and MHC I-m04 complex, but also diminished level of MHC I on the cell surface and affected maturation of MHC I and quality of peptides loaded onto MHC I. We also show that MAT uORF is essential for recognition of MHC I-m04 complex by activating Ly49 receptors (P, L and D2) and thus hypothesize that MAT uORF was originally developed as a missing-self immunoevasin, which drove evolution of activating Ly49 receptors in response to this viral evasion strategy. Moreover, deletion of uORF MAT region of MCMV results in attenuation of the virus *in vivo* in NK and MHC-I-dependant manner, affects NK cells maturation and activation, IFNγ production and strongly induces extramedullar hematopoiesis.

CYTOMEGALOVIRUS INFECTION ENHANCES DEVELOPMENT OF GLUCOSE INTOLERANCE AND INSULIN RESISTANCE IN OBESITY

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Obesity and its complication Diabetes Mellitus type 2 (DM2) represent global health problems. It is well established that low-grade chronic inflammation, which originates in obese visceral adipose tissue (VAT), is an underlying cause of insulin resistance (IR) and DM2. It is characterized by accumulation of Th1-type immune cells in VAT and secretion of pro-inflammatory cytokines. Thus, obese VAT resembles immune response to a viral infection. Although obese people are very often exposed to viral infections, very little is known how a viral infection contributes to the progression of DM2. Cytomegaloviruses are species-specific beta-herpesviruses. Majority of humans are infected with HCMV and after acute infection they establish a life-long latency. Infection of mice with MCMV represents a well-accepted model for HCMV infection.

In this work we investigated how MCMV infection influences the development DM2 in well-established mouse model of diet induced obesity (DIO). The infection was associated with a rapid accumulation of activated NK cells, which was followed by a dramatic increase of M1 macrophages, CD4 and CD8 T cells. MCMV infection itself did not induce GI in mice fed with normal chawing diet. However, it induced fast progression of GI and IR after only 4 - 6 weeks of high fat diet treatment in comparison to the DIO mice. Depletion of NK cells reduced accumulation of M1 macrophages in VAT, suggesting that NK cells were mostly responsible for the induction. Altogether, our results show that MCMV infection can cause aggravation of VAT-inflammation and induce progression of DM2 in obesity.

NK CELLS PERSISTING IN THE BRAIN FOLLOWING MCMV INFECTION INDUCE POLARIZATION OF MICROGLIA TOWARD PROINFLAMMATORY PHENOTYPE AND DELAY IN CEREBELLAR GROWTH VIA INTERFERON γ

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Congenital human cytomegalovirus (HCMV) infection is the most common viral cause of long-term neurodevelopmental sequelae, including mental retardation, microcephaly and sensorineural hearing loss. As HCMV does not cross the species barrier, we employed a mouse model in which newborn mice are infected by intraperitoneal (i.p.) inoculation of mouse cytomegalovirus (MCMV). Following infection the virus disseminates to the central nervous system (CNS), replicates in the brain parenchyma and induces delay in cerebellar growth.

In our model of congenital MCMV infection, the initial neuroimmune responses are dominated by increased cytokine levels of interferon γ (IFNγ) within the brain, polarization of microglia toward proinflammatory phenotype, characterized by upregulation of MHC molecules, and the influx of NK cells, whose appearance coincides with detection of the virus in the brain. The number of NK cells in the CNS peaked at day 8 post infection (p.i.). Phenotypic analysis showed that brain infiltrating NK cells are highly activated and produce IFNγ. In this study we investigated the role of NK cells and IFNγ in polarization of microglia toward proinflammatory phenotype and in altered CNS development following MCMV infection of the developing CNS. In the naive brain the expression of MHC II was minimal. MCMV infection of the brain induced upregulation of MHC II expression on microglia. Depletion of NK cells in MCMV infected mice induced lower expression of MHC II on microglia compared to MCMV infected group of mice. Expression of MHC II was abolished after treatment of MCMV infected animals with anti-IFNγ neutralizing antibody. In adition, treatment of MCMV infected animals with anti-IFNγ neutralizing antibody normalized altered cerebellar development.

These results indicate that IFNγ is a major component of the inflammatory response that is associated with altered neurodevelopment that follows CMV infection and that NK cells that infiltrate the brain represent an effector cell population of IFNγ induced inflammation in this model.

CYTOMEGALOVIRUS VECTOR EXPRESSING RAE-1γ LIGAND SERVES AS A HIGHLY EFFICIENT ANTI-TUMOR CD8 T CELL VACCINE

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The role of CD8 T cells in anti-tumor therapy has been well established. Cytomegalovirus (CMV) is an excellent inducer of effector memory CD8 T cells and therefore represents an attractive candidate for anti-tumor vaccine vector. Here we demonstrate that recombinant murine CMV (MCMV) expressing NKG2D ligand RAE-1γ (RAE-1γMCMV) promoted highly functional CD8 T cell response which provided protection against tumor challenge in both, prophylactic and therapeutic approach. Anti-tumor capacity of RAE-1γMCMV-induced CD8 T cells was retained long-term and protected mice in secondary tumor challenge. Furthermore, RAE-1γMCMV provided efficient anti-tumor protection even in mice immunized as newborns. Altogether, our results demonstrate that CMV expressing RAE-1γ protein represents a great platform for development of CD8 T cell-based anti-tumor vaccine vectors.

URINARY MICRORNAS AS NEW EARLY INDICATORS FOR DISEASES SEVERITY IN HEMORRHAGIC FEVER WITH RENAL SYNDROME

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Objective: Micro RNAs (miRNAs) are a class of small RNAs, 18 – 25 nucleotides in length, that represent a way of posttranscriptional regulation of gene expression by binding to mRNAs and facilitating there inhibition or degradation depending on the degree of similarity. Besides intracellularly they have been detected in several bodily fluids, implicating a possible regulation of selected tissues or organs. Certain miRNAs found in urine can be predictors of disease outcome in various renal pathologies, e.g. glomerulonephritis, IgA or diabetic nephropathy. We measured, for the first time, levels of selected miRNAs in urinary samples of patients with hemorrhagic fever with renal syndrome (HFRS) after Puumala virus infection and compared them to patients with pyelonephritis and healthy controls. Methods: Midstream urinary samples were obtained upon hospitalization and before discharge from 30 patients with HFRS after Puumala virus infection and 15 patients with pyelonephritis. The control group consisted of 15 sex and age matched individuals. Urinary miRNA and a spike-in control RNA were isolated and transcribed into cDNA. A custom real-time PCR Array was designed for the detection of seven selected miRNAs (miR-21-5p, miR-24-3p, miR-27a-3p, miR-127-3p, miR-146a-5p, miR-155-5p, let-7e-5p). Laboratory and clinical parameters were correlated by descriptive statistics. Differences between two independent groups were calculated using non-parametric statistics, e.g. Mann-Whitney U test. Differences between more than two groups of numeric variables were determined through Kruskal-Wallis and ANOVA & Median test. The calculated correlations were evaluated with the Spearman rank correlation. Results: Except miR-146a-5p and miR-155-5p all have been readily detected in patients urine albeit low concentrations. In urinary samples of HFRS patients and patients with acute pyelonephritis, miR-21 and miR-27a were more abundant, while let-7e could be HFRS specific. In mild patients with HFRS only miR-21 and let-7e were deregulated, whereas in severe patients miR-24, miR-27a, and miR-127 were also altered. Conclusions: Here we show for the first time a distinct profile of miRNA abundance in urine of HFRS patients and patients with acute pyelonephritis, which could serve as HFRS biomarkers as they correlate significantly with urea and creatinine levels, which in turn are hallmarks of HFRS progression and severity.

NATIVE ELUTION IN IMMUNOAFFINITY CHROMATOGRAPHY OF VIRUSES – A STEP TOWARD HIGH-PURITY VIRUS PARTICLE PURIFICATION

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Whole viral particles are the active principles in prophylactic (attenuated viruses) and therapeutic vaccines (viral vectors). The manufacturing process of viral particles consists of upstream (USP, virus *in vivo* production in bioreactors) and downstream (DSP, virus purification) part. The immunogenicity and stability of viruses throughout the entire production chain should be maintained. The main aim of DSP is to eliminate contaminants, either process related or product related (host cell proteins, DNA, free proteins, aggregated and empty capsids, host cell exosomes). Downstream processing of viruses has currently been a bottleneck of virus manufacturing, and accounts for up to 70% of overall production costs.

Immunoaffinity chromatography is one of the most powerful techniques in the protein purification. High specificity and high affinity of antigen-antibody interaction enables high purification (up to 1000x) and concentration in a single step. However, the drawback of immunoaffinity is that the high affinity interaction between antigen and antibody can be disrupted only under harsh elution conditions that inevitably disturb also intramolecular non-covalent interaction, disordering the protein conformation, and consequently function. Particularly viruses are especially sensitive to elution condition effective in immunoaffinity chromatography, so the immunoaffinity chromatography has not been used in purification of viable viruses.

Here we describe the novel principle of native elution, *i.e.* effective elution of viruses under native, physiological conditions that maintains virus stability and infectivity. It is based on elution of antigen in immunoaffinity chromatography by different amino acid solutions of high molarity, under physiological pH (7.2-7.4). The mechanism of antigen (virus) desorption from antibody is competition. Using attenuated mumps virus strain, as a model virus, we were able to elute 49±16.5% (n=10) infective virus particles using 0.75 M Arg/0.75 M imidazole as eluting agent and 68±13.5% (n=12) infective virus particles using 0.75 M Arg/0.75 M Ser. Moreover, we were able to demonstrate that native elution was effective in separation of infective from non-infective particles.

Since amino acids have already been experimentally demonstrated to have virus stabilizing features, a combination of high specificity exhibited by immunoaffinity chromatography and efficient elution with pH neutral, stabilizing solution opens new possibilities for commercial use.

Data presented are subject matter of Croatian pending patent application No. P20160086A filed on January 27, 2016, and have been published in Brgles et al. J Chrom A 2016; 1447:107-114.

DISRUPTION OF PROTEASOMAL FUNCTION, ENDOSOMAL ACIDIFICATION AND ACTIN INTEGRATION INFLUENCE IE1 EXPRESSION IN MCMV INFECTED CELLS

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INTRODUCTION: Mouse cytomegalovirus (MCMV) is a member of *Herepesviridiae* family. It is a large DNA virus with highly developed immunoevasive strategy. Upon infecting the host cell it alters its functions and reorganizes its endosomal system. There is evidence suggesting that *in vitro* infection with several other members of the *Herpesviridiae* family (i.e. Kaposhi's sarcoma virus, Herpes simplex virus 1) can be prevented if the targeted cells are treated with chemical inhibitors of proteasomal acitivity.

OBJECTIVES: During this resarch, we wanted to investigate if the level of infection with MCMV could be connected with cellular proteasomal activity levels and with polymerization of actin fibers. Also, we compared the distribution patterns of fully conformed K^d molecules in infected cells pretreated with the apropriate inhibitors.

MATHERIALS AND METHODS: Murine embryonic fibroblasts (MEFs) and Balb 3T3 fibroblasts were infected for 2, 4, 6, or 8 hours with recombinant murine cytomegalovirus Δm138-MCMV (ΔMC95.15) that has deleted FcR. In this research, we treated the cells with proteasomal inhibitor MG132, selective inhibitor of the vacuolar ATPase proton pump Concanamycin A (ConA) and one compound preventing the polymerization of the actin fibers: Latrunculin A (LatA). The expression of IE1 protein was followed by immunofluorescent microscopy and with western blot.

RESULTS: The cells treated with MG132 show reduced levels of IE1 expression regardless of the duration of the treatment. Lower PFU rates in the infection process keep the IE1 expression levels permenantly low, but when the PFU level increases above 2 PFU/cell, the inhibitor influence starts to diminish. ConA and LatA treatments show time-dependent pretreatment negative effect on the IE1 expression.

Fully conformed K^d molecules change their distribution throughout the cells, when MG132 is present. The predominant phenotype of an accumulted "hat" sitting on the cell nucleus that can be found in infected cells nearly vanishes when the MG132 is presented to the cells.

CONCLUSION: Infection rates of MEF and Balb3T3 fibroblasts with the MCMV can be influenced by various chemical inhibitors of different cellular processes. MG132 proves to be highly potent inhibitor of early stage of infection regardless of the duration of the treatment. Prolonged treatment with the ConA and LatA the early infection of the cells with MCMV can be prevented.

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POSTER PRESENTATIONS

CD32+ AND CD86+ B CELLS ARE ASSOCIATED WITH DISEASE ACTIVITY AND INCREASED LEVELS OF PROINFLAMMATORY CYTOKINES IN PATIENTS WITH RHEUMATOID AND PSORIATIC ARTHRITIS

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Background: Autoimmunity is a major driving force in pathogenesis of chronic rheumatic diseases, including rheumatoid arthritis (RA) and psoriatic arthritis (PSA). Although they differ in their clinical features, both are marked by persistent inflammation and osteoresorption underpinned by aberrant lymphocyte populations and disturbed cytokine network.

Aim: Various subpopulations of both T and B cells have been implied in RA and PSA, but their relevance to disease onset and progression remains unclear. Aim of our study was to define association of T, B and NK cell subpopulations with cytokine levels and clinical parameters in RA and PSA patients.

Methods: Mononuclear cells were isolated from peripheral blood of healthy controls (n=35), RA (n=36) and PSA (n=13) patients, after obtaining Ethical approval and informed consent. Flow cytometry was used to discriminate between T cell (CD3+) subpopulations: Th1/2 (CD4+CCR6-), Th17 (CD4+CCR4+CCR6+), Tfh (CD4+CXCR5+), Tc (CD8+) and memory Tc (CD8+CCR4+); B cell (CD19+) subpopulations: naïve (IgD+CD27-), unswitched memory (IgD+CD27+), class-switched memory (IgD-CD27+), double-negative memory (IgD-CD27-) and plasmablasts (IgD+CD27hiCD38+); and NK cells (CD3-CD19-CD56+). Markers of lymphocyte maturation (CD32), activation (CD86, IL21R, CD25) and migration (CD11b) were analyzed. Frequencies of lymphocyte subpopulations were correlated with DAS28 (for RA and PSA), rheumatoid factor (RF) and anti–citrullinated protein antibody (ACPA) levels (for RA). Serum levels of various cytokines (TNF, IL4, IL6, IL10, IL17, CCL2, CCL3, CCL4, CCL5, CXCL9, CXCL10) were measured by flow cytometry bead based assay.

Results: Several subpopulations were found to be significantly expanded: CD32+ B cells - naïve (Ctrl 1.8%, RA 5.8%, PSA 6.0%) and memory class-switched (Ctrl 1.8%, RA 5.3%, PSA 3.8%), unswitched (Ctrl 4.0%, RA 16.8%, PSA 13.7%), double-negative (Ctrl 7.1%, RA 16.3%, PSA 19.1%); memory Tc (Ctrl 4.8%, RA 6.8%, PSA 9.1%). Significant correlations between lymphocyte subpopulations and clinical parameters included: positive association of CD86+ unswitched memory B cells with DAS28 (both RA and PSA, ρ=0.40) and RF levels (ρ=0.55); CD86+ naïve B cells with ACPA (ρ=0.55) and RF (ρ=0.73); CD32+ B cells with ACPA, both naïve (ρ=0.581) and memory (switched ρ=0.55, unswitched ρ=0.50, negative ρ=0.55); CD32+ unswitched memory B cells and memory T cells with RF (ρ=0.58, 0.60). Correlation of lymphocyte subpopulations with cytokine levels showed association of CD32+ naïve, and class-switched and unswitched memory B cells with TNF (ρ=0.50, 0.47, 0.55); CD32+ double-negative memory B cells with CXCL9 and CXCL10 (ρ=0.54, 0.48).

Conclusion: Our results indicate novel B cell subpopulations induced in both RA and PSA. CD32+ and CD86+ B cells may be of particular interest as possible therapeutic targets, since their frequency is associated with disease activity and increased levels of proinflammatory and proresorptive cytokines.

POTENTIAL ALS MOUSE MODEL OF OPTINEURIN INSUFFICIENCY SHOWS DECREASED TBK1 ACTIVATION IN MICROGLIA AND IMPAIRED AUTOPHAGY IN NEURONS

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ALS is a rapidly fatal neurodegenerative disease characterized by progressive loss of motor neurons. Although ALS is a complex disease, which can be caused by mutations in more than 20 different genes, neuroinflammation is one of its earliest and invariable hallmarks. Mutations in the ubiquitin-binding scaffold protein optineurin have recently been described in ALS patients. Unlike many proteins known to cause ALS by their toxic and/or prion-like properties, optineurin is largely thought to cause disease by loss-of-function, arguing for its protective role. However, although optineurin has been proposed to act as an adaptor protein in a variety of cellular processes including signalling, apoptosis, necroptosis, autophagy, and vesicle trafficking, its direct link to ALS pathogenesis remains unclear. To address this, we have performed analysis of microglia and neurons from optineurin insufficiency mouse model, in which C-terminal ubiquitin-binding region was deleted (Optn470T), thus mimicking some of mutations found in ALS patients. Here, we report that optineurin is dispensable for NF-□B activation in primary microglia upon Toll-like receptor (TLR) stimulation measured by p65 phosphorylation and IκB degradation. However, TLR-stimulated Optn470T microglia had impaired Tank-binding kinase 1 (TBK1) activation, interferon regulatory factor 3 (IRF3) phosphorylation and subsequently decreased IFN-□ production, indicating that optineurin and/or its C-terminus is important for positive regulation of type I IFN production. Although TLR stimulation can also induce autophagy in myeloid cells, our analysis revealed that optineurin is not important for autophagy in microglia. Contrary to this, basal and starvation induced autophagy flux in Optn470T cortical neurons was diminished, as measured by the lack of LC3 and p62 accumulation upon blockage of lysosomal degradation. Furthermore, Optn470T mice showed defects in motor coordination late in life and reduced life span compared to their WT littermates. Thus our results suggest that optineurin exerts different protective functions in different cell types i. e. that it regulates autophagy in neurons and anti-inflammatory cytokine secretion in microglia. Further analysis of the cross-talk between these processes in co-cultures and pathohistological analysis of aged Optn470T mice brains and spinal cords will help us determine if these are physiologically relevant mechanisms for maintaining neuronal homeostasis.

INTENSIVE SPLICING IN MCMV TRANSCRIPTOME AND INTRIGUING FUNCTION OF A NOVEL MCMV PROTEIN ENCODED BY THE M116 REGION

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New high-throughput sequencing technologies have enabled us to gain understanding of the mechanisms that cytomegaloviruses employ to invade the host, manipulate its immune system and cause disease on a much deeper level than was previously possible. Using next gen sequencing, we and others have demonstrated that the transcriptome of the murine cytomegalovirus (MCMV) is far more complex and has the potential to encode greater number of proteins and RNA molecules than has previously been anticipated. In addition, our analysis identified several novel spliced CMV genes with completely unknown functions, such as MAT, M116 and m119, which dominate the MCMV transcriptome. To analyze splicing within the MCMV transcriptome further, we have performed a deeper temporal analysis of the MCMV and host transcriptomes during infection using strand-specific RNASeq with 72-bp read length and several splice-aware short read mappers. All mappers identified several hundreds of potentially novel splice-sites in the MCMV transcriptome, of which >70 with highest splice-junction coverage have so far been confirmed by PCR.

In addition to global analysis of splicing, we have also performed molecular, in vitro and in vivo characterization of a novel spliced MCMV transcript and its product - M116. M116 locus is transcribed into two 5' co-terminal transcripts and gives rise to at least one protein. Virus lacking M116 protein is attenuated in macrophages and dendritic cells, but has normal growth curve in murine embryonic fibroblasts. Macrophages have long been suspected to play a role as a viral reservoir during latency, as well as Trojan horses that help disseminate the virus through the body. Thus, a virus with diminished ability to infect macrophages is a useful tool in studying these important questions. Interestingly, our preliminary data does not show major differences in virus dissemination or titers between wild type and virus lacking M116.

CONGENITAL CYTOMEGALOVIRUS INFECTION INDUCES BRAIN TISSUE-RESIDENT MEMORY CD8$^+$ T CELLS BY RECRUITING PERIPHERAL IMMUNE CELLS

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The brain is an immune-privileged organ and it is not routinely surveyed by lymphocytes. Viral infection of the brain results in infiltration and long-term retention of virus-specific CD8$^+$ T cells. It has been recently shown that brain tissue-resident memory CD8$^+$ T cells (bTRM) persist at sites of prior infection and enhance pathogen clearance. Their persistence in organs is mediated by specific adhesion molecules, such as CD103, and expression of CD69 which antagonizes their tissue egress. In order to investigate the role of bTRM in CMV infection, we took advantage of mouse model of congenital CMV infection using intraperitoneal inoculation of mouse cytomegalovirus (MCMV) in newborn mice. Upon infection we observed high frequency of immune cells in the brain. CD8$^+$ T cells were the most numerous and peaked on day 21 post infection. Phenotypic analysis showed that MCMV-specific CD8$^+$ T cells are highly activated early post infection and display tissue resident memory phenotype during latency. The majority of bTRM expressed CD69 and more than half of these cells expressed CD103. Both CD103$^+$ and CD103$^-$ CD69$^+$ bTRM subsets persisted in stable relative proportions during latency. Furthermore, by using an adoptive transfer of MCMV specific MHC class I restricted CD8$^+$ T cells, we demonstrated that upon MCMV infection CD8$^+$ T cells migrate from the periphery to the brain and become bTRM. Importantly, transferred MCMV specific CD8$^+$ T cells reduced viral load in organs and brain pathology.

Altogether, we show that in congenitally CMV-infected mice, CD8$^+$ T cells migrate from the periphery to the brain and become bTRM where they persist over time. Which subset of tissue resident memory cells provides virus control is subject of ongoing studies.

CHEMOTACTIC SIGNALING CONTRIBUTES TO OSTEOCLAST PROGENITOR HOMING TOWARD INFLAMED JOINTS IN COLLAGEN INDUCED ARTHRITIS

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Introduction. Collagen induced arthritis (CIA) is a mouse model of rheumatoid arthritis (RA), characterized by osteoclastic bone destruction. Osteoclasts differentiate from myeloid progenitors normally presented within bone marrow and circulating monocyte pool.

Objectives. Our aim was to identify chemotactic signals important for homing of osteoclast progenitors (OCPs) to the sites of osteitis, the inflammatory infiltrate in bone marrow, in CIA. Therefore, we analyzed chemokine receptor profile of OCPs in circulation, affected joints and periarticular bone marrow, chemokine expression and OCP migration potential.

Methods. After receiving Ethical approval, C57BL/6 mice were immunized with chicken type II collagen in complete Freund's adjuvant. CIA development was evaluated by clinical scoring and anti-collagen antibody detection. Hind paw joints were assessed by micro CT, histology and histomorphometry. Peripheral blood (PBL), bone marrow of distal tibia (periarticular BM) and collagenase-digested tarsometatarseal joints were analyzed by flow-cytometry for the expression of hematopoietic markers and chemokine receptors. Serum chemokines were measured by ELISA and expression was detected by qPCR. For *in vitro* migration assay, M-CSF and RANKL stimulated PBL cells were seeded into transwell inserts and analyzed for the migration potential toward CCL2 gradient. For migration tracking, labeled PBL cells were transferred to recipient CIA mice and assessed by fluorescent microscopy and flow cytometry.

Results. Approximately 60% of immunized mice developed CIA. Osteitis was present at affected joints, with increased number of mature osteoclasts (1.4±1.1 in control vs 5.4±4.7 in CIA) and significant subchondral bone loss (BV/TV 46.8±13.4% in control vs 32.1±8.6% in CIA). Flow-cytometry analysis showed expansion of lymphoid-negative CD11b+CD115+ OCP subset in CIA: in PBL (4.6±0.7% in control vs 8.8±3.3% in CIA) and periarticular BM (9.6±1.1% in control vs 21.9±3.4% in CIA). OCP subsets of both groups equally expressed CCR2 (35±9% in periarticular BM, 28±6% in PBL), but CCL2 serum level was significantly increased in CIA group (115±77 pg/mL in control vs 408±102 pg/mL in CIA). PBL cells from CIA mice demonstrated significantly enhanced migration toward CCL2 chemotactic gradient (11.5 (IQR 11-13.25) in control vs 20.5 (IQR 20-25) in CIA). After *in vivo* transfer to mice with CIA, fluorescently-labeled PBL cells of CIA mice were efficiently attracted to tarsometatarseal joints, which showed enhanced gene expression of CCL2 compared to controls.

Conclusions. OCP populations are highly induced in CIA, with substantial expression of CCR2, possibly causing their increased migration and homing to bone surfaces of the inflamed joints. Therapeutic blocking of chemokine signaling may therefore be a promising approach to antagonize enhanced osteoresorption in RA.

CYTOMEGALOVIRAL PROTEIN m154 REDUCES PVR EXPRESSION IN INFECTED CELLS

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Cytomegaloviruses (CMVs) are species-specific herpesviruses causing severe disease in immunocompromised and immunologically immature hosts. CMVs are equipped with the capacity to encode multiple products committed to alter components of the innate and adaptive immunity. They are well known for their regulation of the expression of cellular ligands of different immune receptors in order to avoid immune surveillance. Human CMV (HCMV) blocks the surface expression of CD155 (PVR) molecule, which serves as a ligand for activating receptor CD226 (DNAM-1), conserved between mice and humans and expressed on NK cells and T cells. On the other hand, PVR also binds the inhibitory receptor TIGIT and CD96 (TACTILE), a receptor exhibiting both inhibitory and activating roles. Due to the species-specific nature of HCMV replication, infection with murine CMV (MCMV) has proven to be an invaluable system widely used to unveil new immunomodulatory molecules and to explore the processes underlying CMV infection. We have observed that, similarly to HCMV, MCMV dampens the expression of PVR on the surface of infected cells. We previously reported that MCMV-encoded glycoprotein, m20.1, retains PVR in the endoplasmic reticulum and promotes its proteolytic degradation, thus interfering with PVR-DNAM-1 activating pathway of NK cells, essential in the early control of CMV. By screening a panel of MCMV deletion mutants, we identified another MCMV product, m154, as a second regulator of PVR surface expression. The deletion of either m154 or m20 or both resulted in the rescuing of PVR on the cell surface. Using a viral mutant lacking m20.1, we observed cytoplasmic accumulation of PVR, which colocalized with adaptor protein-1 (AP-1) complex, responsible for the transport of lysosomal hydrolases. This result is suggestive of inhibitory role of m154 in the PVR maturation. Despite the surface downregulation of PVR, a wild type MCMV infection significantly upregulated PVR gene transcription, and the effect was more pronounced when using viral mutants lacking either m154 or m20.1 or both regulators. Hence, we speculate that m154 could possibly have a dual role in the regulation of PVR, both at the transcriptional and protein level. Our ongoing study is aimed to further decipher the MCMV mechanisms employed in the PVR modulation.

AUTOINFLAMMATORY DISORDERS PRESENTING AS CHRONIC URTICARIA

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Urticaria is one of the most common disorders in allergy and clinical immunology daily practice. The most common form of chronic urticaria is spontaneous urticaria mediated by persistant mast cells activation and release of histamine. Autoinflammatory diseases are rare systemic disorders caused by increased IL-1 secretion which can also present with chronic non-pruritic urticarial rashes. These patients present with a variety of systemic symptoms and are not responsive to antihistamines.Two chronic urticaria patients with different autoinflamamtory diseases are presented.

Muckle-Wells syndrome (MWS) is a hereditary autoinflammatory disorder characterised by episodes of fever, urticarial rash, musculoskeletal symptoms and progressive sensorineural hearing loss. Mutations in the NLRP3 gene responsible for overproduction of IL-1β are pathognomonic although not always detected.

We report a 58-year-old man with a 9 year history of chronic nonpruritic urticaria, ankle pain, and bilateral hearing loss. Over the years he was treated with various antihistamines, montelukast, glucocorticoids, sulphasalazine and methotrexate which all proved inefficient.

Laboratory tests showed ESR 70- 90 mm/h, CRP 80-145 g/L, mild leukocytosis with neutrophilia, normocytic anemia (hgb 105 g/L) and polyclonal hypergammaglobulinemia. ANA, ENA-6, antidsDNA and ANCA were negative, C3 and C4 levels were normal. Skin biopsy showed no vasculitis. Genetic analysis of NLRP3 gene was performed but revealed no typical mutations.

Since clinical features strongly suggested MWS anakinra (IL-1 receptor blocker) 100 mg SC/ day was introduced. The patient responded immediately with complete resolution of urticaria and normalisation of serum inflammatory markers.

Schnitzler syndrome is a rare acquired auto-inflammatory disorder. Diagnostic criteria include urticarial rash and monoclonal IgM serum immunoglobulin.The exact pathogenesis is yet unknown but it is assumed to be IL-1 mediated similar to hereditary syndromes.

We report a 50-year –old female patient with a 8-year history of chronic urticaria, intermittent fever, bone pain, axillary limfadenopathy and hepatosplenomegaly. Laboratory test showed persistantly elevated ESR and CRP and monoclonal IgM kappa. Immunological parameters were all negative. Skin biopsy showed neutrophilic urticaria. She was treated with antihistamines and various immunosupressive drugs over the years which all proved ineffective. Anakinra 100 mg SC/ day induced rapid and complete remission.

It is important to consider autoinflammatory disorders in differential diagnosis of chronic urticaria since targeted therapies with IL-1 blockers are very efficient. When untreated chronic inflammation may lead to amyloidosis and irreversible organ damage in these patients.

ALTERATIONS IN THE SYNOVIAL CELLULAR COMPOSITION ASSOCIATED WITH OSTEORESORPTION IN ANTIGEN-INDUCED ARTHRITIS

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Introduction The inflammatory arthritides are chronic joint diseases characterised by synovial infammation and thickening. Most common is rheumatoid arthritis, often accompanied by structural bone damage, in contrast to arthritis associated to systemic lupus erythematosus (SLE), which rarely affects bone. Murine phenotype resembling human SLE may be induced by inactivation of Fas/Fas receptor pathway. Mice with a non-functional Fas gene develop ameliorated form of experimentally induced arthritis, with less severe joint damage and bone destruction. However, the exact cellular interactions and signalling pathways mediating this effect are not fully understood. In present investigation we aimed to compare the cellular composition of synovial compartment in resorptive vs. non-resorptive forms of antigen-induced arthritis (AIA), and to assess the association of altered populations with bone resorption.

Methods Wild-type (WT) and Fas-deficient (Fas -/-) mice were immunized with methylated(m)BSA in complete Freund's adjuvant, followed by intra-articular injection of mBSA. Control mice (ctrl) were intra-articularly injected with PBS. Five weeks post-immunization, arthritis was assessed by histology and micro-CT. After collagenase digestion and labelling, the cellular phenotypes were determined by flow cytometry for the following markers: CD3, CD4, CD8, CD11b, CD29, CD31, CD44, CD45, CD51, CD90.1, CD105, CD140b, CD166, CD200, B220, Gr-1, Sca-1, and TER119.

Results Histologically assessed, arthritis was more severe in the AIA group of WT mice than in the Fas -/- AIA group. Micro-CT analysis revealed metaphyseal trabecular bone loss not only as a result of arthritis, but also as a consequence of systemic immunization in both WT and Fas -/- mice. Epiphyseal trabecular bone from Fas -/- mice was not affected by immunization or arthritis induction. Myeloid (CD11b+/Gr-1+) and macrophage (F4/80+) subpopulations accumulated in the synovial compartment of both WT-ctrl and WT-AIA mice, in comparison to non-immunized (NI) mice, while this up-regulation was absent in Fas-/- mice. Proportions of myeloid cells were negatively associated with the femoral epiphyseal trabecular bone volume (BV/TV, $p<0.05$). Populations containing bone and cartilage progenitors were downregulated in the synovial compartment of WT-AIA mice. A positive association with femoral epiphyseal BV/TV was established in particular for proportions of CD140b+ cells whereas the CD44+ population was negatively associated with femoral epiphyseal BV/TV. Skeletal progenitor population (lin-CD51+CD200+) and bone/cartilage stromal progenitor populations (lin-CD51+CD200-CD105+) were reduced (by absolute numbers) and positively associated with femoral epiphyseal BV/TV.

Conclusions Absence of Fas signalling prevents periarticular bone destruction in a murine model of AIA. Non-destructive arthritis in Fas -/- mice is characterised by decreased proportions of synovial myeloid cells and macrophages, which are strongly negatively associated with bone volume. Mesenchymal cells, containing bone and cartilage progenitors, were more abundant in the joints of Fas -/- mice with non-destructive arthritis, associated with higher bone volume.

KINETICS OF SELECTED CYTOKINES AND CHEMOKINES IN PATIENTS WITH PUUMALA VIRUS INFECTION

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Objective: Hemorrhagic fever with renal syndrome (HFRS) is a viral disease caused by hantaviruses, however, it is primarily considered as an immune-mediated disease. Monocytes/macrophages (mo/ma) are important immune cells and are considered one of the target cells for hantaviruses. The aim of our study was to analyze the mo/ma related cytokines/chemokines profile in sera of HFRS patients infected with Puumala virus (PUUV) according to the severity of their clinical picture and in correlation with some laboratory parameters.

Methods: The study included 34 HFRS patients with a serologically confirmed PUUV diagnosis, hospitalized in 2014 in Zagreb, Croatia. Patients' serum samples were taken in two acute time points – upon arrival at the hospital and before discharge. Luminex technology of (multiplexed) immunoassay with magnetic beads was used for 16 analytes, namely: IL-1β, IL-1RA, IL-12(p70), IL-15, IL-18, IL-23, IL-27, MIF, CCL2, CCL3, CCL4, CCL5, CCL7, CCL20, CXCL12a, TGF-β1.

Results: The results showed, suppression of the early acute immune response to PUUV - some pro-inflammatory cytokines were down-regulated or absent. CCL4, IL-1RA, IL-18 and MIF were elevated in both phases compared to the controls, while CCL5 was downregulated in the first and upregulated in the second phase. In opposite, low levels of IL-15 were recorded in the first serum and not in the second serum sample. Correlations with some laboratory parameters revealed strong positive correlations of CCL5 and TGF-β1 with platelets in early acute mild form and positive CCL5 and negative CCL2 correlations with platelets for late acute moderate HFRS form.

Conclusions: Some differences in the levels of monocytes/macrophages related cytokines/chemokines in HFRS patients were detected in patients with mild in comparation to the patients with moderate clinical picture and correlation with several clinical laboratory parameters were observed as well.

THE ROLE OF INNATE IMMUNE CELLS IN DEVELOPMENT OF NAFLD

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With an increasingly obese population non-alcoholic fatty liver disease (NAFLD) has become the leading cause of chronic liver disease in western countries. It is an important cause of morbidity and mortality and is considered as hepatic manifestation of metabolic syndrome. NAFLD represents a wide spectrum of liver disease from simple steatosis, to steatohepatitis, cirrhosis and end-stage liver failure without alcohol consumption. Even though NAFLD has a relatively benign prognosis, somewhere around 20% of patients with fatty liver eventually develop non-alcoholic steatohepatitis (NASH).

In our research, we used murine amilyn diet model (AMLN) that induced all stages of NAFLD within 16 weeks after the start of diet and we analysed changes in cell populations within that time period. In first 4 weeks after the start of AMLN diet we could see that hepatocytes were starting to accumulate fat. This accumulation was followed by an increase in cNK cells and γδ T cells. Since both of these cell populations are a part of innate immunity we believe that they might be the first cells that sense obesity-induced cellular stress in liver.

After 8 weeks of AMLN diet we could already see well established steatosis with some minor inflammation that was mainly marked with an increase of $Gr\text{-}1^+$ activated macrophages. On week 12 liver inflammation became severe and was followed by further increase in $Gr\text{-}1^+$ macrophages, $CD8^+$ T cells and Tregs. Week 16 was marked by a decrease in inflammation but the start of fibrosis.

VIPERA AMMODYTES BITES TREATED WITH ANTIVENOM VIPERATAB®: A CASE SERIES AND PHARMACOKINETIC EVALUATION

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In the southeastern parts of Europe *Vipera a. ammodytes* and *Vipera berus* are the only medically important poisonous snakes. Differentiation of their bites based on clinical presentation is very difficult and unreliable. In the past this was not a concern, since snakebites were successfully treated with Viperfav™ (Aventis Pasteur, France) or European viper venom antiserum (Zagreb antivenom) (Institute of Immunology, Croatia) as formulations containing equine $F(ab')_2$ fragments that are either specific for both venoms, either clinically proved to be safe and effective for the treatment of *V. a. ammodytes* and *V. berus* envenomings. However, due to current shortage in Viperfav™ and Zagreb antivenom availability, *V. a. ammodytes* and *V. berus* bites have recently been treated with ViperaTAb® (MicroPharm Limited, United Kingdom) composed of ovine Fab fragments as active principle against the venom of *V. berus* only. Its therapeutical convenience for use against *V. a. ammodytes* venom-induced toxicity in human has not been described yet, although neutralisation efficacy has been proved preclinically. In view of this for the first time we present cases of several *V. a. ammodytes* snakebites treated with ViperaTAb® whose pharmacokinetics has been measured and correlated with clinical picture.

CORRELATION OR RECIPROCITY OF NOTCH AND AIOLOS IN LEUKEMIA

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Notch signaling pathway is highly conserved during evolution and critical for cell development. Leukemia cells have the associating characteristic of hematopoietic cells being blocked in the progression of differentiation to their mature form. It has been shown that Notch receptors and ligands in the immune system affect hematopoietic stem cells as well lymphocyte precursors. Notch guides lineage specification, cell progression, commitment and survival and deregulation of this pathway has been shown to be involved in either the occurrence or in the progression of T-cell acute lymphoblastic leukemia, as historically known, but also in other types of leukemia of B cell origin like B-cell chronic lymphocytic leukemia (B CLL). Its role has also been studied in myelopoiesis and myeloid leukemia.

In addition to Notch, the Ikaros family of zinc - finger proteins, Ikaros, Aiolos and Helios are crucial for the development of lymphocytes. By acting as chromatin remodeling transcription factors they influence gene expression and guide the differentiation pathways of lymphocytes. Another layer of complexity to the story of differentiation is added when we take into account the various isoforms of proteins in the Ikaros family and the homo- and heterodimers the proteins in the pathway form.

We and others have previously shown an elevated expression of Aiolos in CLL patients. The aim of the present study is to compare the mRNA and protein expression of Notch, Ikaros family members, especially Aiolos, and their downstream regulators to better understand their implication in leukemia. Considering the importance of the Notch pathway and the proteins involved, elucidating sequential steps from stem cells to mature immunocompetent cells will pave the way for better and more efficient treatments, as well as help us understand the regular task of these proteins in lymphocyte development.

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