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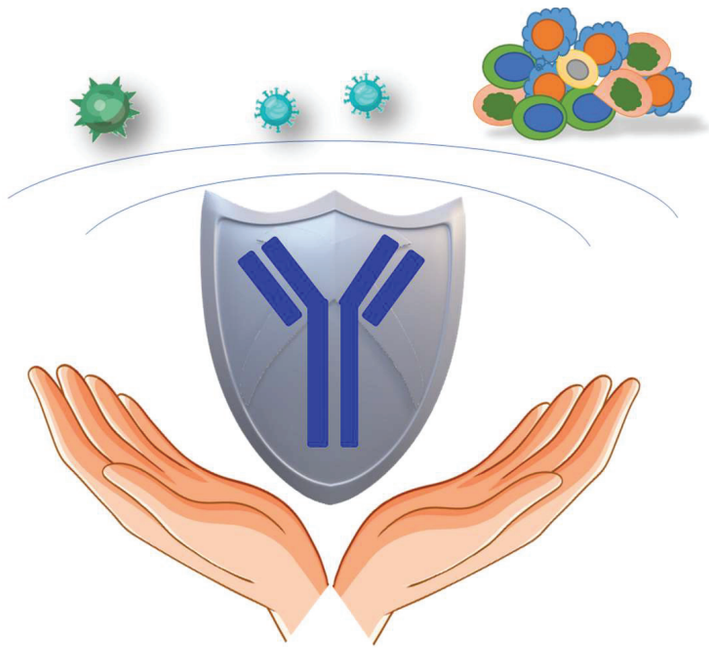
The Annual Meeting of the Croatian Immunological Society 2024

11TH to 13TH October, 2024

Hotel Panonija, Sisak



SMN DESTINACIJA SVAGDJE

U LIJEČENJU SPINALNE MIŠIĆNE ATROFIJE (SMA),

Što saznajemo o ulozi SMN proteina u središnjemu živčanom sustavu i perifernim tkivima?

SMA = spinalna mišićna atrofija; SŽS = središnji živčani sustav; SMN = neuralni čimbenik rasta (od engl. survival motor neuron)

Nedostatak SMN proteina može imati učinak na više organskih sustava nego što se to prije mislilo[1]

Ljudi sa SMA-om imaju nedostatak SMN proteina u SŽS-u i u perifernim tkivima[2,3]

Istraživanja provedena na životinjskim modelima upućuju na to da nedostatak SMN proteina može izravno utjecati i na stanice u perifernim tkivima[4-6]

Referencije: 1. Park, G. H., Maeno-Hikichi, Y., Awano. T., Landmesser, L. T., Monani, U. R. Reduced survival of motor neuron (SMN) protein in motor neuronal progenitors functions cell autonomously to cause spinal muscular atrophy in model mice expressing the human centromeric (SMN2) gene. J. Neurosci. 2010: 30(36):12005-12019. 2. Wadman ,R. I., Stam, M., Jansen, M. D. et al. A comparative study of SMN protein and mRNA in blood and fibroblasts in patients with spinal muscular atrophy and healthy controls. PLoS One, 2016:11(11):e0167087. 3. Coovert D. D., Le. T. T., McAndrew ,P. E. et al. The survival motor neuron protein in spinal muscular atrophy. Hum Mol Genet. 1997: 6(8):1205–1214. 4. Hamilton, G., Gillingwater, T. H. Spinal muscular atrophy: going beyond the motor neuron. Trends Mol Med. 2013: 19(1):40–50. 5. Martinez T. L., Kong, L., Wang, X. et al. Survival motor neuron protein in motor neurons determines synaptic integrity in spinal muscular atrophy. J. Neurosci. 2012: 32(25):8703–8715. 6. Bricceno, K. ., Martinez, T., Leikina, E. et al. Survival motor neuron protein deficiency impairs myotube formation by altering myogenic gene expression and focal adhesion dynamics. Hum Mol Genet. 2014: 23(18):4745–4757.

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Welcome letter

Dear Friends and Colleagues,

We are pleased to extend our sincere welcome to the Annual meeting of the Croatian Immunological Society (HID) with international participation for 2024.

This year it will be held from 11th to 13th October 2024 at the Panonija Hotel in Sisak, known as a city on the three rivers Sava, Odra, and Kupa. During the meeting of the Croatian Immunological Society, key researchers, post-doctoral fellows, and PhD students will get together to share and discuss the most recent advances in the fields of immunology. The Society board (Malo vijeće), with the help of our members, continuously strives to provide exceptional and cutting-edge sessions focused on research and education in immunology. The topics of discussion will span over a wide spectrum of research fields, including organogenesis, lymphocyte selection and differentiation, development of immune and stromal cells, tumor immunology, self-tolerance, immune responses, autoimmunity, allergy, transplantation, inflammatory diseases, immunity to microbes, immune regulation, cell metabolism, T-cell-based immunotherapy, aging and beyond. Since HID is an integral part of the European Federation of Immunological Societies (EFIS), which is a network of immunological societies across Europe, we actively participated in a number of EFIS activities, including EFIS Young Immunologist Task Force (yEFIS) webinars, EFIS Gender and Diversity Task Force discussions and 7th European Congress of Immunology in 2024, at which Croatian immunologist Prof Bojan Polić started his EFIS presidentship.

We wish to thank our international guests, invited speakers, our members, and all participants for their contribution to this event, opening the possibilities for new connections and collaborations. We are also grateful to our sponsors for their continued support. Our meeting is an excellent opportunity particularly for young scientists to present their work and engage in discussions with the leading experts in the realm of immunology. With our joined effort, we would generate an inspiring and intellectually very rewarding meeting!

We look forward to your participation and wish you a pleasant stay in Sisak.

Lidija Milković and Mariastefania Antica

THE ANNUAL MEETING OF THE CROATIAN IMMUNOLOGICAL SOCIETY 2024

Hotel Panonija, Sisak, 11. - 13.10.2024.

ORGANIZED BY

CROATIAN IMMUNOLOGICAL SOCIETY

Bijenička cesta 54, Ruđer Bošković Institute, Zagreb

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PROGRAM

Friday, October 11th, 2024.

14:00-24:00 Hotel check-in

14:30-15:30 Registration

15:30-15:40 Opening ceremony

MARIASTEFANIA ANTICA, President of the Croatian Immunological Society

BOJAN POLIĆ, EFIS President-elect

CHAIRS: Stipan Jonjić & Alenka Gagro

15:40-16:20 Invited lecture: **BERISLAV BOŠNJAK,** Hannover Medical School, Institute of Immunology, Hannover, Germany

Genomic, phenotypic, and functional analysis of cytomegalovirus-specific T cells

16:20-17:20 Selected oral presentations – **Session 1**

16:20 **Lydia Gaćina,** Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

The role of NKG2D ligand H60a in the immune surveillance of murine cytomegalovirus infection

16:40 **Lidija Cvetko Krajinović,** Research Department, University Hospital for Infectious Diseases "Dr. Fran Mihaljević", Zagreb, Croatia

Differential monocyte activation by SARS-CoV-2 variants: Implications for early immune response

17:00 **Sara Aničić**, University of Zagreb School of Medicine, Zagreb, Croatia

Effect of Notch signaling on human trilineage monocyte progenitors in patients with rheumatoid arthritis

17:20-18:00 Invited lecture: **TOMISLAV KELAVA,** Department of Physiology and Immunology, School of Medicine, Zagreb, Croatia

Targeting Notch signaling pathway in liver fibrosis - possibilities and limitations

Saturday, October 12th 2024

CHAIRS: Vanda Juranić Lisnić & Asja Stipić Marković

9:00-9:40 Invited lecture: **MARIA PIA FELLI,** Department of Experimental Medicine, Faculty of Medicine and Surgery, Sapienza University of Rome, Roma, Italy

Notch-ed up T-cell acute lymphoblastic leukemia development and progression

9:40-10:40 Selected oral presentations – **Session 2**

9:40 **Dora Višnjić,** University of Zagreb School of Medicine, Zagreb, Croatia

The Role of Ribonucleotide Reductase Activity in Monocytic Differentiation Induced by Pyrimidine Synthesis Inhibitors

10:00 **Sanja Mikašinović,** Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia

IGF-1 signaling enhances CD8 memory cell formation

10:20 **Sara Priselac,** Laboratory for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia

Midline 1 promotes bone and cartilage damage during antigen-induced arthritis

10:40-11:20 **Coffee break sponsored by GOREA PLUS**

CHAIRS: Danka Grčević & Bojan Polić

11:20-12:00 Invited lecture: **BENCE RETHI,** Department of Medicine, Karolinska Institutet, Stockholm, Sweden

Early pathogenesis in rheumatoid arthritis - understanding and targeting key pathways in disease development

12:00-13:00 Selected oral presentations – **Session 3** - **Bright Spark**

12:00 **Maša Filipović,** Department of Medicine, Solna, and Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden

Role of the chemokine CCL22 in rheumatoid arthritis development

12:20 **Maja Jirouš Drulak,** Dept. of Medical Chemistry, Biochemistry and Clinical Chemistry, Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Croatia

The distribution, transcriptome, and TCR profiles of peripheral γδ T cells in psoriasis vulgaris

12:40 **Dubravka Karner,** Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia

Cellular prion protein alters viral control and enhances pathology after perinatal cytomegalovirus infection

13:00-13:40 Invited lecture: **SANJA NOVAK,** Center for Regenerative Medicine and Skeletal Development, School of Dental Medicine, UConn Health, Farmington, Connecticut, USA

Notch signaling regulation of skeletal repair

13:40-15:20 Lunch break

15:20-16:20 **GENERAL ASSEMBLY OF THE CROATIAN IMMUNOLOGICAL SOCIETY**

CHAIRS: Dora Višnjić & Alemka Markotić

16:20-17:00 Invited lecture: **ILIJA BRIZIĆ**, Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia

Cytomegalovirus infection and inflammation in developing brain

17:00-19:00 **POSTER SESSION –** *CHAIRS: Beata Halassy & Alan Šućur*

19:00 Dinner

Sunday, October 13th 2024

CHAIRS: Ines Mrakovčić-Šutić & Felix Wensveen

9:00-9:40 Invited lecture: **IGOR AURER,** Department of Hematology, University Hospital Centre Zagreb, Croatia

CAR-T cells - a new generation of adoptive cellular immunotherapy

9:40-11:00 Selected oral presentations – **Session 4**

9:40 **Marina Babić Čač,** Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

Immune cell crosstalk during neuroinflammation - role for cellular stress sensor NKG2D

10:00 **Maja Cokarić Brdovčak,** Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

Immunization against SARS-CoV-2 using cytomegalovirus as a vaccine vector

10:20 **Beata Halassy**, Centre for Research and Knowledge Transfer in Biotechnology, University of Zagreb, Zagreb, Croatia

Is there a need to develop new procedures for the preparation of high-quality human immunoglobulin G, in addition to the registered ones?

10:40 **Karlo Mladenić**, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

The IL-17A and IFN-γ dichotomy in MASLD compromises the immune response to mCMV infection

11:00-11:30 Coffee break

CHAIRS: Stana Tokić & Alan Šućur

11:30-12:10 Invited lecture: **MARKO ŠESTAN,** Department of Histology and Embryology, Faculty of Medicine University of Rijeka, Croatia

Immune-mediated control of glucose metabolism in health and disease

12:10-13:00 Award ceremony and closing remarks

Invited Lecture

Genomic, phenotypic, and functional analysis of cytomegalovirus-specific T cells

Berislav Bošnjak[1,2]

[1]Institute of Immunology, Hannover Medical School, Hannover, Germany
[2]Cluster of Excellence RESIST (EXC 2155), Hannover Medical School, Hannover, Germany

Acquired immunity provides long-lasting protection from pathogens that breach innate immune defenses. Key players in the adaptive immune response against viruses are cytotoxic CD8 T-cells (CTLs). Activated CTLs eliminate target cells through the production of the effector molecules granzyme-B, perforin, interferon-γ (IFNγ), and tumor necrosis factor-α (TNFα). After pathogen elimination, some CD8 T cells will become memory cells and provide a rapid and effective recall response after the pathogen re-encounter. Antigen-specificity of CTLs provides an elegant way to fight tumors and viral infections through adoptive T-cell therapy (ATCT). Immunosuppressed persons and transplant patients are endangered by human cytomegalovirus (HCMV). HCMV establishes a life-long latent infection requiring constant immune system surveillance. I will discuss our recent insights on the function of cytomegalovirus-specific CTLs obtained from a mouse model of cytomegalovirus infection and ex vivo analyses of HCMV-specific CTLs from healthy donors. These findings are crucial for selecting T cell donors for successful ATCT against HCMV infections.

Invited Lecture

Targeting Notch signaling pathway in liver fibrosis - possibilities and limitations

Tomislav Kelava

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

In chronic liver injury, immune response is activated leading to activation of hepatic stellate cells, their differentiation to collagen secreting myofibroblast and fibrosis development. Recent research suggest a possible role of Notch signaling pathway in fibrogenesis, but exact cellular and molecular mechanisms are still not defined. We modulated Notch signaling in myofibroblasts using the model of inducible activation or inhibition of Notch signaling in murine models of liver fibrosis. Our results confirm that Notch signaling pathway is activated in both CCL4 and DDC model of liver fibrosis and that αSMA positive myofibroblasts are of activated hepatic stellate cells origin. However, neither the inhibition of canonical Notch signaling (in tamoxifen treated aSMACreER/RBP-Jfl/fl mice) nor its overactivation (in tamoxifen treated aSMACreER/NICD1 mice) changed the degree of liver fibrosis in comparison to the control groups in either of the investigated models. Furthermore, after the withdrawal of the fibrogenic treatment the degree of resolution of fibrosis was similar between the animals with Notch overactivation and controls. In addition to genetic manipulation, we investigated the effect of antibodies against NOTCH1 and NOTCH2 on the development of liver fibrosis, the antibodies had effects on thymus and spleen respectively, but failed to ameliorate liver fibrosis. Our data demonstrate that modulation of Notch activity in activated HSC is not sufficient to change the outcome of liver fibrosis. The results obtained with inhibitory antibodies further demonstrate limitations of targeting Notch 1 and 2 receptors as a promising antifibrotic therapy. Notch pathway remains a potential target for the treatment of liver fibrosis, but finding the appropriate application regimen continues to be a research challenge.

Invited Lecture

Notch-ed up T-cell acute lymphoblastic leukemia development and progression

Maria Pia Felli

Department of Experimental Medicine, Faculty of Medicine and Surgery, Sapienza University of Rome, Roma, Italy

Malignant transformation of T-cell progenitors causes T-cell acute lymphoblastic leukemia (T-ALL), an aggressive childhood lymphoproliferative disorder. Activating mutations of Notch receptors, Notch1 and Notch3, have been detected in T-ALL patients. By characterizing the Notch pathway, ligand and receptors, in the thymocyte differentiation, we generated a transgenic mouse model with the LCK-driven expression of the intracellular (IC) active form of Notch3 (N3-ICtg). Our studies deeply characterized hyperactive Notch3-triggered pathways, PKCtheta- and NF-kB-depedent, as important cues in T-ALL. In addition, we have shown that Notch3 enhances CXCR4-dependent migration of immature thymocytes, $CD4^{+}CD8^{+}$ (DP) cells, notably $Notch3^{high}CXCR4^{high}$, that prematurely egress from the thymus and early infiltrate the bone marrow (BM) and spleen of N3-ICtg mice. More recently, we demonstrated that Notch3-regulated microRNAs are involved in the thymocyte dynamics by expanding immature $CD4^{-}CD8^{-}$ (DN) $CD3\varepsilon^{high}$ $CXCR4^{-/low}$ cells, which dominate N3-ICtg thymus-resident DN subset in T-ALL progression. MicroRNAs might be of significance in T-ALL pathobiology, however, whether required for leukemia maintenance is not fully understood. The selection of specific DN subsets demonstrates the inverse correlation between CXCR4 expression and a panel of Notch3-deregulated microRNAs that cooperatively impinge on thymocyte differentiation with accumulation of $DNCD3\varepsilon^{+/high}CXCR4^{-/low}$ cells. These data point out that deregulation of Notch3 in T-ALL, besides its role in sustaining the early dissemination of abnormal DP T cells, as we previously demonstrated, could select specific DN immature T-cells within the thymus, thus impeding T-cell development, to facilitate T-ALL progression inside the BM.

Invited Lecture

Early pathogenesis in rheumatoid arthritis - understanding and targeting key pathways in disease development

Bence Réthi

Department of Medicine, Solna, Karolinska Institutet & Center for Molecular Medicine, Stockholm, Sweden

The majority of rheumatoid arthritis (RA) cases are characterized by circulating autoantibodies against autoantigens subjected to different types of post-translational modifications, such as citrullination, carbamylation or malondialdehyde/acetaldehyde (MAA) modification. Autoantibodies, and particularly anti-citrullinated protein antibodies (ACPAs) predate RA onset often by several years and indicate a high risk for later disease development.

Why studying early pathogenesis in RA? Although circulating ACPAs could help us to identify individuals at risk of eventual RA development, we can't predict an imminent disease onset and we lack preventive therapies.

To understand early triggers that lead to RA we follow two different strategies. First, we study immunological mechanisms in a group of individuals at risk of RA, people with joint pain and circulating ACPAs but no systemic inflammation, focusing particularly on mediators that could be linked to arthritis onset. Moreover, we transfer autoantibodies from patients to mice, or to cell cultures, and analyze the potential pathogenicity of these.

Importantly, by transfering patient-derived ACPAs to mice we could recapitulate the earliest symptoms preceding RA onset, namely joint pain, microscopical bone erosions and tenosynovitis. Anti-MAA antibodies could contribute to inflammation and bone loss. Nevertheless, ACPAs inhibited joint inflammation in animal models, suggesting that these antibodies may not be responsible for arthritis. In addition to autoantibodies, our research indicated a potential role for the cytokines IL-6, IL-15 and CCL22 in very early stages of RA development.

In summary, we described the roles of certain immune mediators in early disease progression and we developed an animal model for pre-RA phases.

Invited Lecture

Notch signaling regulation of skeletal repair

Sanja Novak[1], Hitoshi Tanigawa[1], Vijender Singh[2], Ivo Kalajzic[1]

[1]Center for Regenerative Medicine and Skeletal Development, School of Dental Medicine, UConn Health, Farmington, Connecticut, USA
[2]Institute for Systems Genomics, Computational Biology Core, UConn, Storrs, Connecticut, USA.

Bone has great regenerative capacity. Upon fracture there is an influx of hematopoietic cells, followed by expansion of progenitors and their commitment to mature lineages. Notch signaling has a role in maintaining progenitor pool and cell differentiation, but mechanisms controlling healing are unclear.

We performed scRNA-seq from sorted mesenchymal (MSCs) and hematopoietic cells of periosteum 3 days after the fracture (dpf). Mice with Notch1 overexpression in MSCs (αSMACre$^+$/NICD1) and Cre- mice were used. scRNA-seq analysis of periosteum identified EC, satellite and muscle clusters and trajectories from MSCs into chondrocytes and osteoblasts. NICD1 overexpression in the αSMA expressing cells led to significant increase of *Alpl* and *Ibsp* expression in MSCs, while causing decrease in proinflammatory signals (*Tnf* and *Il6*) in CD45$^+$ cells upon fracture compared to control.

To evaluate the effects of Notch ligand Dll4 in fracture healing, we used Cdh5CreERT2 and induced deletion of Dll4 in endothelial cells (EC). Cre activity was induced by injection of tamoxifen on 0/2/4 dpf and healing evaluated by qPCR, histology and μCT. Deletion of Dll4 in EC led to decreased number of proliferating cells within the periosteal callus (4 dpf). Cre$^+$ mice have smaller callus, less cartilage and decreased Sca1 cells, at 14 dpf decreased callus bone mass.

Induced Notch1 activation in MSCs led to increased expression of osteogenic genes within CD45$^-$ and decreased proinflammatory genes within CD45$^+$ cells. Deletion of Dll4 in EC has a negative effect on the early fracture healing. Notch signaling is important in early fracture healing and in osteoprogenitor differentiation.

Invited Lecture

Cytomegalovirus infection and inflammation in developing brain

Ilija Brizić

Center for Proteomics, University of Rijeka, Faculty of Medicine, Rijeka

Human cytomegalovirus (HCMV) is a highly prevalent herpesvirus that can cause severe disease in immunocompromised individuals and immunologically immature fetuses and newborns. Congenital human cytomegalovirus (cHCMV) infection of the brain is associated with a wide range of neurodevelopmental and cognitive sequelae. We are using infection of newborn mice with mouse cytomegalovirus (MCMV) as a reliable model that recapitulates many aspects of cHCMV infection, including virus dissemination to the central nervous system (CNS), altered neurodevelopment, and sensorineural hearing loss. The inflammatory response in the brain is required to control the infection. However, host inflammatory factors are also critical drivers of neurodevelopmental delay. Furthermore, MCMV establishes latency in the brain, causing lifelong microglia priming and retention of MCMV-specific T cells in the brain tissue. In this presentation, our recent studies on cytomegalovirus infection in the brain, local immune response to infection, and mechanisms leading to CNS sequelae will be discussed.

Invited Lecture

CAR-T cells - a new generation of adoptive cellular immunotherapy

Igor Aurer

University Hospital Centre Zagreb and Medical School, University of Zagreb, Zagreb, Croatia

The immunological system has evolved over millions of years, mainly as a defence against infections, but immunological mechanisms can be used to fight cancer. A very effective method of cellular immunotherapy is allogeneic hematopoietic stem cell (aka "bone marrow") transplantation. Still, despite many advances, many unmet needs remain in the fight against cancer.

In 1993 Dr. Zelig Eshhar from the Weizmann Institute in Israel described a method for *ex vivo* modifications of endogenous T-cell by inserting a monoclonal antibody derived receptor, making them reactive against a tumor antigen. These cells are called CAR-T cells, an abbreviation from „chimeric modified antigen recognizing T-cells" or „chimeric antigen receptor T-cells". It took many years to develop CAR-T cells into a viable therapeutic option, but nowadays they have become a very important treatment modality for B-lymphoid malignancies.

All CAR-T cells used in humans are autologous T-cells collected from the patient into which the chimeric antigen receptor (CAR) is inserted using a proliferation-defective lentiviral vector. Second generation CARs, which are used nowadays, consist of an extracellular MHC-unrestricted antigen binding domaine, a costimulatory domaine (41-BB or CD28) and a CD3-zeta derived signaling domaine important for T-cell activation and persistence.

CAR-T cells have unusual toxicities, mainly the cytokine release syndrome (CRS) and immune cell associated neurological syndrome (ICANS). CRS is a hyperinflammatory reaction mediated by IL6 and treated with tocilizumab and steroids. ICANS is probably caused by direct CAR-T infiltration of the CNS and treated with steroids. If these first line treatment options fail, anakinra, an IL1 antagonist, is used.

CAR-T cells, used nowadays in routine clinical practice, are directed against CD19 for treatment of relapsed/refractory B-large cell, mantle-cell and follicular lymphoma and B-cell acute lymphoblastic leukemia or against BCMA for treatment of relapsed/refractory multiple myeloma. Experimental approaches include targeting other antigens (for treatment of other tumor types), targeting double or triple antigen combinations, use of CAR-T cells in earlier treatment lines or in autoimmune diseases and local production of CAR-T cells.

Invited Lecture

Immune-mediated control of glucose metabolism in health and disease

Marko Šestan

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

The immune and endocrine systems play crucial roles in the body. The immune system defends against lethal pathogens, while the endocrine system maintains proper metabolic function in peripheral organs by regulating systemic metabolic homeostasis. Traditionally, these systems were thought to operate independently, with the immune system using cytokines and immune receptors, and the endocrine system using hormones to regulate metabolism. However, our recent findings reveal a close interaction between the immune and endocrine systems, particularly in the regulation of glucose metabolism during both homeostasis and viral infections. In summary, new findings on immune-induced changes in systemic metabolism during homeostasis and following viral infections, with a focus on glucose metabolism regulation, will be discussed.

Selected oral presentations – SESSION 1

S1-O1 ***The role of NKG2D ligand H60a in the immune surveillance of murine cytomegalovirus infection***

Lydia Gaćina[1], Irena Slavuljica[2,3], Maja Cokarić Brdovčak[4], Jelena Materljan[1,4], Stipan Jonjić[4], Astrid Krmpotić[1]

[1]Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[2]Department of Infectious Diseases, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[3]Clinical Hospital Center Rijeka, Rijeka, Croatia
[4]Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

NKG2D is a strong activating receptor expressed by immune cells, whose importance in the immunosurveillance of cytomegalovirus (CMV) infection is illustrated by the fact that CMVs encode genes involved in the immune evasion of NKG2D-mediated immune control. CMV infection induces an inflationary $CD8^+$ T cell response of highly functional CMV-specific CD8 T cells. We have previously shown that murine CMVs (MCMV) expressing RAE-1γ or MULT-1, two of the cellular ligands for the NKG2D receptor, although highly attenuated *in vivo,* induce a strong $CD8^+$ T cell response.

Here, we demonstrate that a recombinant MCMV expressing the third NKG2D ligand, H60a, (H60aMCMV) inserted in the place of its viral inhibitor m155, is dramatically attenuated *in vivo* in an NKG2D- and NK cell-dependent manner. NK cell response in mice infected with H60aMCMV shows features of a stronger early activation which wanes by the second day, compared to the wild type MCMV (WT MCMV). Despite efficient control by the host innate immunity, H60aMCMV induces a potent and long-lasting virus specific $CD8^+$ T cell response. Moreover, H60aMCMV is attenuated in immunologically immature newborn mice. H60aMCMV infection is associated with lower viral titers and fewer pathoanatomical lesions in the central nervous system of newborn mice as compared to WT MCMV infection. Furthermore, H60aMCMV infection induces the production of anti-viral antibodies which protect the offspring of H60aMCMV vaccinated mothers from challenge MCMV infection. Altogether, our study further supports the concept of CMV expressing NKG2D ligand as a promising model for a CMV vaccine or CMV-based vaccine vector.

S1-O2 ***Differential monocyte activation by SARS-CoV-2 variants: Implications for early immune response***

Mihaela Kordun[1], Željka Mačak Šafranko[1], Kristijan Bodulić[1], Alemka Markotić[1,2,3], Lidija Cvetko Krajinović[1]

[1]Research Department, University Hospital for Infectious Diseases "Dr. Fran Mihaljević", Zagreb, Croatia
[2] School of Medicine, Catholic University of Croatia, Zagreb, Croatia
[3]Faculty of Medicine, University of Rijeka, Rijeka, Croatia

Monocytes are essential during the early immune response against SARS-CoV-2. Except being precursor cells for macrophages or dendritic cells, they perform effector functions like early detection of the virus, pathogen uptake and cytokine production as well. The rapid evolution of SARS-CoV-2 has led to a tremendous genomic diversity of the virus, manifesting in the appearance of viral variants, which increase the efficiency of virus transmission, cellular tropism or pathogenicity of the virus, and escape recognition by the immune system. In this study, we wanted to elucidate the susceptibility and permissiveness of monocytes to genomically different lineages of SARS-CoV-2 and further investigate the adaptation of monocytes to the viral genomic microevolution by examining transcriptional reprogramming of innate immune response. Primary human monocytes infected with four SARS-CoV-2 variants of concern (VoCs): Alpha (lineage B.1.1.7), Beta (B.1.351), Delta (B.1.617) and Omicron (BQ.1.1), and SARS-CoV-2 strain ZG/297 (lineage B.1.1.1) were susceptible but non-permissive for SARS-CoV-2 as indicated in the early phase of infection (6 h.p.i.) by low viral titers, decrease in viral N and E gene copies throughout the cultivation period and absence of virus cytotoxicity, with the Delta variant showing higher replicative potential compare to other VOCs. Transcriptomic profiling showed early activation (6 h.p.i.) of monocyte innate response and the establishment of the antiviral state to Omicron and Delta variants while milder and late activation (48 h.p.i.) to Alpha variant and original ZG/297 isolate suggest possible subversion of host response by those viruses.

S1-O3 ***Effect of Notch signaling on human trilineage monocyte progenitors in patients with rheumatoid arthritis***

Sara Aničić[1,2], Marta Radošević[2], Maša Filipović[1,2], Darja Flegar[1,2], Pavao Planinić[4], Ivo Krešić[4], Tomislav Kelava[1,2], Nataša Kovačić[2,3], Alan Šućur[1,2], Danka Grčević[1,2]

[1]Dept. of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia
[2]Laboratory for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia
[3]Dept. of Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia
[4]Dept. of Physiology, University of Mostar School of Medicine, Mostar, Bosnia and Herzegovina

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by sustained inflammation and enhanced bone destruction. Monocyte progenitors play a pivotal role in pathogenesis of RA due to their inflammation-induced activation and oligopotent differentiation into macrophages, dendritic cells (DCs) and osteoclasts. Since Notch pathway is known to direct hematopoietic lineage commitment, our aim was to determine the effect of Notch modulation on monocyte progenitors' differentiation under inflammatory conditions. Mononuclear cells from peripheral blood and periarticular tissue were collected from RA patients and controls. Classical monocytes were sorted and phenotyped for activation markers and Notch receptors. Notch receptors and ligands expression on periarticular tissue was analyzed using immunohistochemistry. Macrophages, DCs and osteoclasts were differentiated in vitro from common monocyte progenitors under stimulation by immobilized Notch ligands.

We showed that trilineage monocyte progenitors within the classical monocyte subset (CD45+CD15-CD3-CD19-CD56-CD11b+CD16-CD14++CCR2+) can yield functional macrophages, DCs and osteoclasts. Substantial proportion of monocyte progenitors expresses Notch receptors, with a decrease of Notch 1 and 2 expression in peripheral blood and periarticular monocyte progenitors of RA compared to controls. Negative association was found between Notch 1 and 2 expression with RA activity score DAS28. In vitro stimulation of osteoclastogenic cultures by Notch ligands (JAG1, DLL1) inhibited differentiation gene expression and osteoclast formation. Macrophage differentiation was also functionally regulated by Notch, with DLL1 suppressing phagocytosis but enhancing antigen-presenting potential.

We demonstrated that Notch signaling hinders monocyte progenitors' differentiation into macrophages and osteoclasts, while also impairing their function.

The work was supported by Croatian Science Foundation projects IP-2020-02-2431, DOK-2021-02-6365, DOK-NPOO-2023-10-4220.

S2-O1 ***The role of ribonucleotide reductase activity in monocytic differentiation induced by pyrimidine synthesis inhibitors***

Tomislav Smoljo[1,2], Barbara Tomić[1,2], Hrvoje Lalić[1,2], Vilma Dembitz[1,2], Dora Višnjić[1,2]

[1]Department of Physiology, University of Zagreb School of Medicine, Zagreb, Croatia
[2]Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia

Our previous study demonstrated that 5-aminoimidazole-4-carboxamide ribonucleoside (AICAr) promotes leukemia differentiation by inhibiting pyrimidine synthesis at the UMP synthase step, downstream of dihydroorotate dehydrogenase (DHODH). Both AICAr and brequinar, a DHODH inhibitor, induce differentiation through the activation of checkpoint kinase 1 (Chk1), a mechanism also observed in low-dose cytarabine (LDAC)-induced differentiation. However, it remains unclear whether nucleotide triphosphates (NTPs) or deoxynucleotide triphosphates (dNTPs) play a critical role in the DNA damage response during this process. This study aims to investigate the role of ribonucleotide reductase (RNR), the key enzyme responsible for dNTP synthesis, in the differentiation of monocytic cell lines.

The addition of nucleosides abolished the effects of AICAr and brequinar but had no effect on differentiation induced by LDAC. The effects of all three agents were abolished when cells were incubated in αMEM, which contains high levels of both ribonucleosides and deoxyribonucleosides, unlike RPMI. When THP1 and U937 cells were treated with differentiation inducers in RPMI, Western blot analysis revealed elevated levels of both total RNR subunit M2 (RRM2) and Thr33-phosphorylated RRM2; this increase was significantly reduced in αMEM. Pretreatment with COH29, a pharmacological inhibitor of RNR, prevented differentiation in cells treated with AICAr, brequinar, and LDAC. Inhibition of Wee1 kinase by MK1776 resulted in decreased RRM2 levels and significantly reduced the differentiation effects of all tested agents. Downregulation of Wee1 decreased cell viability and reduced differentiation.

In summary, our findings suggest that ribonucleotide reductase (RNR) plays a key role in mediating monocytic differentiation induced by pyrimidine synthesis inhibitors.

S2-O2 ***IGF-1 signaling enhances CD8 memory cell formation***

Sanja Mikašinović[1], Marko Šestan[1], Bojan Polić[1], Felix M. Wensveen[1]

[1]Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia

The immune and endocrine systems are intricately intertwined. In response to viral infection, the immune system triggers metabolic changes that manifest as "sickness behavior" through the modulation of endocrine signals. These adjustments ensure that the immune system combats infections more effectively. On the other hand, endocrine hormones exert a strong influence on immune cells, ensuring that their consumption adapts to systemic availability. However, many immunomodulatory effects of hormones remain insufficiently explored.

Insulin and insulin-like growth factor 1 (IGF-1) are two hormones that are cross-reactive for each other's receptor. Previously, we demonstrated that insulin stimulation enhances cytokine production by CD8 T-cells, but found that this also occurred in cells deficient for the insulin receptor. We therefore hypothesized that IGF-1 may also modulate CD8 T cell responses. Here, we show that IGF-1 promotes the formation and function of CD8 memory cells. In vitro IGF-1 stimulation increased IFNγ production by memory, but not effector CD8 T cells. Metabolic profiling of these cells using SCENITH and Seahorse revealed that IGF-1 promotes glycolytic metabolism, thus increasing overall ATP production and potentiating functionality. In vivo, IGF-receptor deficient T cells showed reduced capacity to form memory cells, whereas their functionality and recall capacity were not notably impacted.

Our results indicate that IGF-1 signaling plays a crucial role in the generation of CD8 memory cells, both in vitro and in vivo. Since IGF1 levels are reduced in people with diabetes, our findings may explain T-cell dysfunction in these patients.

S2-O3 ***Midline 1 promotes bone and cartilage damage during antigen-induced arthritis***

Sara Priselac[1], Tomislav Balen[1,2], Nina Lukač[1], Darja Flegar[1,3], Pavao Planinić[4], Sara Aničić[1,3], Ivo Krešić[4], Tomislav Kelava[1,3], Alan Šućur[1,3], Vedran Katavić[1,2], Danka Grčević[1,3], Nataša Kovačić[1,2]

[1]Laboratory for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia
[2]Department of Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia
[3]Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia
[4]Department of Physiology, School of Medicine, University of Mostar, 88000 Mostar, Bosnia and Herzegovina.

Rheumatoid arthritis (RA) is an autoimmune disease, characterized by bone destruction and joint inflammation, regulated by immune mediators including the Fas/Fas ligand system. Our group has previously documented the protective role of *Fas* deletion (*Fas* –/–) in a murine model of antigen-induced arthritis (AIA). One of the genes downregulated in *Fas* –/– mice is *Midline 1* (*Mid 1*). We examined if the course of AIA would be less severe in mice with inactivated *Mid 1* (*Mid 1* –/–).

Arthritis was induced by immunization with methylated bovine serum albumin (mBSA) and subsequent intra-articular (i.a.) injection of mBSA. Wild-type (C57BL/6J, wt) and *Mid 1* –/– mice were assigned to non-immunized control (NI) and AIA groups and sacrificed on day 10 after i.a. injection. Arthritis was assessed by visual score, histology, micro-CT and flow cytometry.

Mid 1 –/– AIA mice developed arthritis, but joint swelling and visual score were less severe in comparison to wt AIA group. Myeloid cell accumulation was also present in *Mid1* –/– AIA. However in comparison to the wt AIA group, *Mid 1* –/– AIA mice had lower average histology scores, and reduced cartilage and bone destruction. Furthermore, a significant reduction in trabecular number and increase in trabecular separation were observed in wt mice with AIA, while the number and separation of distal epiphyseal trabeculae in *Mid1 –/–* mice with AIA were similar to corresponding controls.

Taken together, these results point to a partially protective effect of *Mid1* inactivation on cartilage and bone damage in inflammatory arthritis.

S3-BS1 ***Role of the chemokine CCL22 in rheumatoid arthritis development***

Maša Filipović[1], Marcelo Afonso[1], Alexandra Argyriou[1], Alexandra Cîrciumaru[1,6], Mikael Ringh[2], Konstantin Carlberg[1,3], Vijay Joshua[1], Szu-Ying Chen[1], Marianne Engström[1], Heidi Wähämaa[1], Tomas J. Ekström[4], Marc H Wadsworth II[5], Ravi Kumaar[1], Aaron Winkler[5], Vivianne Malmström[1], Anca I. Catrina[1], Karine Chemin[1], Aase Hensvold[1,6], Bence Réthi[1]

[1]Department of Medicine, Solna, and Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden; [2]Department of Clinical Neuroscience, and Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden; [3]Department of Gene Technology, Royal Institute of Technology, Science for Life Laboratory, Stockholm, Sweden; [4]Department of Molecular Medicine and Surgery, and Center for Molecular Medicine, Karolinska Institutet, Sweden; [5]Inflammation & Immunology Research Unit, Pfizer Inc., Cambridge, MA, 02139, USA; [6]Center for Rheumatology, Academic Specialist Center, Stockholm Health Services, Stockholm, Sweden

Joint pain in rheumatoid arthritis (RA) patients usually precedes arthritis onset. The analysis of various pain-associated immune mediators in serum of individuals at risk of RA and in early untreated RA revealed elevated CCL22 concentrations in both cohorts, compared to healthy individuals. Therefore, we analysed potential sources and effects of CCL22 in RA.

Smoking affected CCL22, as higher CCL22 levels were found in smokers in the RA cohort, and we detected a significant demethylation of the CCL22 gene in bronchoalveolar lavage cells of healthy smokers. Spatial tissue transcriptomics suggested CCL22 expression in the inflamed joints, and CCL22 levels in synovial fluid (SF) correlated with M-CSF and GM-CSF levels. Both cytokines induced CCL22 release in cultured macrophages. Single-cell RNAseq and flow cytometry identified peripheral blood and SF dendritic cells (DCs) as the most prominent producers of CCL22. The receptor of CCL22, CCR4, was expressed in various CD4+ and CD8+ T cell populations, including regulatory but also naïve, central memory, and subsets of activated and effector T cells. In vitro, CCR4 inhibitor C-021 impaired IL-12 production in DCs, increased IL-10 production in macrophages and reduced osteoclast differentiation. Importantly, arthritis development was reduced in mice treated with C-021.

The increase of CCL22 concentrations prior and after RA onset and the decreased joint inflammation in mice treated with CCR4 inhibitor suggest important roles for this chemokine in arthritis development. DC-derived CCL22 might help orchestrating DC-T cell interactions and CCL22 could contribute to macrophage and DC activation as well as the development of osteoclasts.

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S3-BS2 ***The distribution, transcriptome, and TCR profiles of peripheral γδ T cells in psoriasis vulgaris***

Maja Jirouš Drulak[1], Vera Plužarić[2,3], Marija Šola[3], Martina Mihalj[3,4], Peter Balogh[5], Mario Štefanić[6], Stana Tokić[2]

[1]Dept. of Medical Chemistry, Biochemistry and Clinical Chemistry, Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia; [2]Dept. of Laboratory Medicine and Pharmacy, Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia; [3]Dept. of Dermatology and Venerology, University Hospital Osijek, Osijek, Croatia
[4]Dept. of Physiology and Immunology, Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia; [5]Dept. of Immunology and Biotechnology, Faculty of Medicine, University of Pécs, Pécs, Hungary; [6]Dept. of Nuclear Medicine and Oncology, Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

Psoriasis vulgaris is a chronic autoinflammatory skin disease with systemic manifestations. Despite well acknowledged role of T cells in PV pathogenesis, the precise mechanisms driving skin and systemic inflammation remain unclear. Emerging evidence suggests that unconventional γδ T cells may contribute to PV pathogenesis, yet their specific functions and contributions to disease are not well understood.

To address this, we employed immunophenotyping, TCR sequencing, and targeted transcriptome RNA-sequencing of peripheral γδ T lymphocytes.

Immunophenotyping showed no major alterations in the overall γδ T cell compartment, though a significant reduction in Vδ2+ cells within the CD3+ T cell population was observed in male PV patients. Transcriptomic analysis revealed 36 differentially expressed genes in γδ T lymphocytes from PV patients. Upregulated genes were associated with immune signaling and activation (ZAP70, CD3D/E/G, IL2RB, CD247), Th1/Th2 regulation (KLF2, TBX21, STAT6), and cytotoxicity (PRF1, GZMA, NKG7, SRGN). Additionally, genes associated with chemokine signaling (CXCR4), cell-cell adhesion (ITGAL, SELL, CD47), and interferon responses (IRF1, ISG20, IFITM1), were elevated, while antiviral defense (MX1, OAS3, IFI44L) and metabolic regulation (MTOR) genes were downregulated. TCR profiling revealed a significant age-related decline in TRG and TRD clonotype diversity in PV patients, a pattern absent in age-matched healthy controls. This reduction was driven by the loss of rare clonotypes and an increased frequency of hyperexpanded clonotypes (> 5% of the repertoire). Moreover, disease severity was associated with increased TRG clonotype expansion and reduced TRG/TRD diversity.

These findings underscore the complex interplay between sex, age, and disease severity in shaping TCR dynamics, gene expression, and γδ T cell distribution in psoriasis, highlighting the need for further research.

S3-BS3 ***Cellular prion protein alters viral control and enhances pathology after perinatal cytomegalovirus infection***

Dubravka Karner[1], Daria Kvestak[1], Paola Kucan Brlic[1], Maja Cokaric Brdovcak[1], Berislav Lisnic[1], Ilija Brizic[1], Vanda Juranic Lisnic[1], Mijo Golemac[2], Jelena Tomac[2], Astrid Krmpotic[2], Esma Karkeni[3], Valentina Libri[3], Sebastien Mella[3], Giuseppe Legname[4], Hermann C. Altmeppen[5], Milena Hasan[3], Stipan Jonjic[1], Tihana Lenac Rovis[1]

[1]Center for Proteomics; Faculty of Medicine; University of Rijeka; Rijeka, 51000; Croatia; [2]Department of Histology and Embryology; Faculty of Medicine, University of Rijeka; Rijeka, 51000; Croatia; [3]Cytometry and Biomarkers Unit of Technology and Service (CB TechS); Institut Pasteur, Université Paris Cité; Paris 75015; France; [4]Prion Biology Laboratory; Department of Neuroscience, Scuola Internazionale Superiore di Studi Avanzati (SISSA); Trieste 34136; Italy; [5]Institute of Neuropathology; University Medical Center Hamburg-Eppendorf (UKE); Hamburg 20246; Germany.

Human cytomegalovirus (CMV) is the most common congenital viral infection and frequently leads to lasting brain damage in newborns, primarily due to inflammatory responses, underscoring the need for effective therapeutic strategies. Considering the complexity of damage involving both viral infection of brain tissue and pathology generated by immune responses, our investigation focuses on the role of cellular prion protein (PrP), known for its critical functions in neuroprotection and immune regulation. The latest research suggests that PrP plays a role in dampening immune responses across various organs, thereby preventing immune-related pathologies. Our main objective is to elucidate the role of the PrP protein and its impact on the progression of perinatal cytomegalovirus infection and resulting brain abnormalities.

Using a murine model, we demonstrate the role of PrP in modulating perinatal T cell immunity during CMV infection. PrP-deficient mice exhibit enhanced viral control through elevated virus-specific CD8 T cell responses, resulting in decreased viral titers without causing excessive immunopathology. We also uncover the underlying molecular mechanisms, showing that the initial CMV-induced upregulation of PrP expression is subsequently followed by its release via the metalloproteinase ADAM10, which specifically impairs the CD8 T cell response in CMV-infected neonatal mice. Furthermore, we confirm PrP downregulation in human CMV-infected cells, underscoring the broader relevance of our findings beyond the murine model.

Overall, our study reveals how PrP protein affects the modulation of the perinatal immune response in the context of viral pathogenesis.

S4-O1 ***Immune cell crosstalk during neuroinflammation - role for cellular stress sensor NKG2D***

Christoforos Dimitropoulos[1,2], Timo Rückert[1,2], Alba Del Rio Serrato[3], Carmen Infante Duarte[3], Bojan Polic[4], Chiara Romagnani[1,2,*], Marina Babic[4,*]

[1]German Rheumatism Research Centre (DRFZ), Berlin, Germany; [2]Institute of Medical Immunology, Charité – Universitätsmedizin Berlin, Germany; [3]ECRC Experimental and Clinical Research Center, a Joint Cooperation between the Charité- Universitätsmedizin Berlin and Max-Delbrück-Center for Molecular Medicine, 13125 Berlin, Germany; [4]Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia.
*equal contribution

Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system (CNS) associated with inflammation, demyelination, oligodendrocyte loss and axonal injury. Various immune cells have been described to contribute to the inflammatory processes that underlie MS and experimental autoimmune encephalomyelitis (EAE), a mouse model thereof. However, the very early events in this process during MS are still poorly understood. Moreover, the upstream signals that drive the activation as well as multifaceted nature and pathogenicity of effector cells as well as the unique features thereof are still poorly defined. We performed single cell transcriptome analysis of splenic versus CNS CD4+ T cells at disease peak revealing a transcriptional continuum within CNS CD4+ T cells with distribution skewed by the expression of key effector cytokines and activation markers. One prominent feature associated to CNS as compared to splenic CD4+ T cells was the expression of innate receptors, particularly *Klrk1*, coding for Natural Killer Group 2, Member D (NKG2D), a key innate sensor of cellular danger signals. Moreover, expression of NKG2D ligands was detected in CNS-derived activated microglia and monocyte subsets. CNS derived antigen-specific CD4+ T cells from mice with *Klrk1*-deficiency in the T cell compartment (*Klrk1*ΔCD4) were impaired in the production of inflammatory cytokines as well as in the recruitment of inflammatory myeloid cells. Importantly, we could demonstrate that *Klrk1*ΔCD4 mice had significantly lower EAE clinical disease score when compared to wild-type littermates. Altogether, our findings suggest the role for the stress-sensing innate receptor NKG2D in the modulation of Th cell-mediated neuroinflammation.

S4-O2 ***Immunization against SARS-CoV-2 using cytomegalovirus as a vaccine vector***

Maja Cokarić Brdovčak[1], Jelena Materljan[1,2], Marko Šustić[1], Sanda Ravlić[3], Tina Ružić[1], Berislav Lisnić[1], Karmela Miklić[1], Marina Pribanić Matešić[1], Beata Halassy[3], Federico Bertoglio[4], Maren Schubert[4], Luka Čičin-Šain[5], Stipan Jonjić[1], Astrid Krmpotić[2]

[1]Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia; [2]Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia; [3]Centre for Research and Knowledge Transfer in Biotechnology, University of Zagreb, Zagreb, Croatia; [4]Institute for Biochemistry, Biotechnology and Bioinformatics, Technische Universität Braunschweig, Braunschweig, Germany; [5]Department of Viral Immunology, Helmholtz Center for Infection Research, Braunschweig, Germany.

Conventional strategies of vaccination which induce protective humoral immune response have not successfully dealt with the issue of different infectious agents against which T cells play an important role in adaptive immunity. A promising approach against these microbial pathogens are the vaccines which can generate potent and long-lasting cellular immunity against infectious agents based on the robust $CD8^+$ T cell response. Cytomegaloviruses (CMVs) are excellent inducers of antigen specific $CD8^+$ T cells, which accumulate over time. Therefore, CMVs genetically engineered to express foreign antigens are attractive live replicating viral vaccine vectors due to their large genomes and numerous immunomodulatory genes which can be manipulated to modulate their vaccine properties. We have previously constructed a murine CMV (MCMV) vector expressing an NKG2D ligand RAE-1γ (RAE-1γMCMV). RAE-1γMCMV proved to be highly attenuated compared to the control vector, but at the same time induced and maintained potent $CD8^+$ T cell response to vectored foreign antigens. These vectors elicited excellent protection against bacterial and tumor challenges, substantially increasing the survival rate of mice compared to animals immunized with vectors lacking NKG2D ligand expression.

We have now generated recombinant MCMV vaccine vectors expressing full-length S (spike) and M (membrane) protein of SARS-CoV-2. Immunization with these vectors resulted in potent and long-lasting antigen-specific $CD8^+$ T cell response in mice, which was maintained for 16 months. Moreover, our recombinant vectors expressing SARS-CoV-2 S protein elicited excellent anti-S IgG antibody response and antiviral antibodies of strong neutralizing capacity against several SARS-CoV-2 variants. Importantly, not only immunization via systemic route but also via intranasal application of our recombinant vectors resulted in the induction of protective immune response in mice.

Overall, our results indicate that herpesviruses are promising vaccine vectors against SARS-CoV-2 due to their capacity to induce exceptional and long-lasting antibody and cellular immune response.

S4-O3 ***Is there a need to develop new procedures for the preparation of high-quality human immunoglobulin G, in addition to the registered ones?***

Tihana Kurtović[1], Sanda Ravlić[1], Adela Štimac[1], Sanja Mateljak Lukačević[1], Ana Hećimović[2], Saša Kazazić[3], Beata Halassy[1]

[1]Centre for Research and Knowledge Transfer in Biotechnology, University of Zagreb, Zagreb, Croatia
[2]Croatian Institute of Transfusion Medicine, Zagreb, Croatia
[3]Division of Physical Chemistry, Ruđer Bošković Institute, Zagreb, Croatia

Current core plasma fractionation technology largely relies on a well-established backbone process encompassing cryoprecipitation and cold ethanol precipitation. Over the years its complexity has remarkably increased due to the implementation of additional steps, not only to improve product's purity, enhance its recovery and assure viral inactivation or removal, but also to isolate new clinically useful plasma proteins from the existing fractions. Consequently, the highest possible IgG extraction efficacy might not be achieved in such complex and lengthy fractionation protocols.

However, there are situations, which will be discussed, when registered technologies are not suitable for their preparation, but more efficient, faster and modern ones are needed. During COVID-19 epidemics, very simple technological platform for the purification of immunoglobulins G from human convalescent plasma, enriched with SARS-CoV-2-specific antibodies1, was developed on the laboratory scale. It consisted of caprylic acid precipitation of the majority of albumin, followed by 100 kDa diafiltration of the IgG-enriched supernatant for the removal of precipitating agent and low Mw proteins, and final AEX chromatography polishing in the flow-through mode which appeared very effective in depletion of unwanted immunoglobulins of other classes from the IgG fraction, as well as aggregates. Overall IgG yield of 75%, with removal of 95% of IgA and 100% of IgM, was achieved.

[1]Kurtović et al. Frontiers in Immunology 2022;13:889736;
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S4-O4 ***The IL-17A and IFN-γ dichotomy in MASLD compromises the immune response to mCMV infection***

Karlo Mladenić[1], Felix M. Wensveen[1], Bojan Polić[1]

[1]Faculty of Medicine, University of Rijeka, Rijeka, Croatia

MASLD (Metabolic dysfunction-associated steatotic liver disease), previously known as NAFLD, is a liver condition linked to metabolic syndrome, ranging from simple fat accumulation to cirrhosis. Our research identifies chronic inflammation as a key factor in MASLD progression. Although chronic inflammation is linked to immune dysfunction and poor viral control in metabolic diseases like type 2 diabetes, its effect on viral infections in MASLD is not well understood. Recent studies report a higher incidence of severe infections in MASLD patients, but the underlying molecular mechanisms remain unclear.

In our previous work, we found an increase in IL-17A-producing cells in the liver and blood of both mice and MASLD patients, indicating a systemic shift towards a type 3 immune response. We therefore hypothesized that the MASLD hampers type 1 polarization of the immune response following viral infection. MASLD was induced by feeding mice with a steatosis and steatohepatitis diet (SSD). Upon mCMV infection, IL-17A production was elevated in the liver and spleen of SSD-fed mice compared to those on a normal chow diet (NCD). This was associated with a concomitant decrease in IFNγ production by Natural Killer cells and an increase in viral titers in the organs of SSD-fed animals. NK cells are essential for the early control of mCMV and may functionally be impaired by IL-17A. Indeed, we observed that IL-17R is present on NK cells, particularly in SSD-fed animals. Notably, in vitro stimulation of these cells with IL-17A diminished IFN-γ output.

Our findings indicate that Type-3 polarization of the immune system in people with MASLD impairs its ability to form an optimal type 1 immune response following viral infection. Our findings may therefore explain why people with MASLD are more prone to viral infection.

POSTER SESSION

Number	Presenter	Title
P1	Sanja Mikašinović	*Adiponectin increases CD8 memory cell function by promoting mitochondrial metabolism*
P2	Inga Kavazović	*NK cell derived IFN-γ mobilizes free fatty acids from adipose tissue to promote B cell activation during viral infection*
P3	Vanda Juranić Lisnić	*Cytomegalovirus in the adrenal glands*
P4	Maja Lenartić	*Immune aspects of thermorestriction mechanism of thermoregulation upon MCMV infection*
P5	Lucija Šakota	*The role of STING in a mouse model of congenital cytomegalovirus infection*
P6	Andrea Mihalić	*Primed microglia limit reactivation of latent herpesvirus at expense of synaptic connectivity*
P7	Vedrana Jelenčić	*Role of CD16 receptor in development of diabetes mellitus type 2*
P8	Marta Radošević	*Increased osteoclastogenesis and bone resorption in a mouse model of type 1 diabetes mellitus*
P9	Pavao Planinić	*The effect of 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) on bone biology in a murine model of cholestatic liver disease*
P10	Pavao Planinić	*High risk PNPLA3 rs738409 polymorphism is associated with higher concentrations of CCL2 in alcoholic end-stage liver disease*
P11	Ivo Krešić	*Functional and phenotypic characteristics of peripheral blood monocyte subsets*
P12	Erika Gamulin	*Finding the optimal administration route (i.m. or i.v.) of antibody-based therapeutics against snakebite envenoming*
P13	Barbara Anđelić Dmitrović	*Ongoing WNV genome mutations driven by prolonged infection caused by B lymphocytes depletion*
P14	Ruža Frkanec	*Design and synthesis of new peptidomimetics of bacterial peptidoglycan monomer for combating drug-resistant bacterial infections*
P15	Mariastefania Antica	*Development of thymus organoids from epithelial stem cells*
P16	Lidija Milković	*Monitoring the development of human thymospheres in vitro*

P1 ***Adiponectin increases CD8 memory cell function by promoting mitochondrial metabolism***

Sanja Mikašinović[1], Inga Kavazović[1], Karlo Mladenić[1], Tamara Turk Wensveen[2-4], Felix M. Wensveen[1]

[1]Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia; [2]Center for Diabetes, Endocrinology and Cardiometabolism, Thalassotherapia Opatija, Opatija, Croatia; [3]Department of Internal Medicine, Faculty of Medicine, University of Rijeka, Rijeka, Croatia; [4]Department of Endocrinology, Diabetes and Metabolic Diseases, Clinic of Internal Medicine, Clinical Hospital Center Rijeka, Rijeka, Croatia

Activated immune cells are subject to a wide range of external signals that control their activity and this includes regulation of their metabolism. Adiponectin is a hormone produced by adipose tissue which restricts systemic nutrient use and its receptors are expressed on activated CD8 T cells. However, how adiponectin regulates CD8 T cell biology is currently unknown.

Here we investigated the impact of adiponectin on CD8 T cell function in vitro. We found that AdipoR1/2 are amongst the most highly expressed hormone receptors on CD8 T cells. Upon activation, their expression is further increased during the formation of CD8 T cell memory. In vitro stimulation of activated CD8 T cells with adiponectin showed that this hormone promoted cytokine production in a dose-dependent manner, whereas it did not affect viability, proliferation, or cytotoxicity. Analysis of CD8 T cell metabolism showed that adiponectin selectively promoted energy production by the mitochondria during the memory phase but not the effector phase of the response, whereas glycolytic metabolism was not affected.

Thus, our findings show that memory CD8 T cell biology is under control of a key endocrine hormone. Our findings may have implications for immune cell functionality in people with metabolic disease in whom adiponectin levels are dysregulated.

P2 ***NK cell derived IFN-γ mobilizes free fatty acids from adipose tissue to promote B cell activation during viral infection***

Mia Krapic[1], Inga Kavazovic[1], Sanja Mikašinović[1], Sabine Helmrath[2], Marc Schmidt-Supprian[2], Fran Krstanović[3], Ilija Brizić[3], Bojan Polić[1], Tamara Turk Wensveen[4,5], Felix Wensveen[1]

[1]Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia; [2]Institute for Experimental Hematology, Center for Translational Cancer Research (TranslaTUM), School of Medicine, Technical University Munich, Munich, Germany; [3]Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia; [4]Center for Diabetes, Endocrinology and Cardiometabolism, Thalassotherapia Opatija, Opatija, Croatia; [5]Department of Internal Medicine, Faculty of Medicine, University of Rijeka, Rijeka, Croatia.

Viral infection typically causes us to lose fat, but how and why this happens is unclear. The immune system plays a major role in the regulation of adipose tissue biology during homeostasis and in context of metabolic disease. Here, we investigated whether immune cells also modulate adipocyte metabolism during viral infection. We find that visceral adipose tissue transiently decreases adiposity following viral infection. Upon pathogen encounter, adipocytes upregulate surface expression of ligands for the receptor NCR1 on NK cells, which drives their secretion of IFNγ. This cytokine directly stimulates adipocytes to downregulate PPARγ, which leads to their release of lipids in circulation, most notably of free fatty acids (FFAs). The FFA oleic acid stimulates the early activation of B cells by promoting oxidative phosphorylation. Oleic acid induced their expression of costimulatory B7 molecules and promoted their ability to prime CD8 T cells. Prevention of lipid release by adipocytes during infection impaired B cell activation, leading to reduced CD8 T cell responses and increased viral replication. Our findings uncover a new mechanism of metabolic adaptation to infection and provide a physiological background for the activation of immune cells in adipose tissue in context of metabolic disease.

P3 ***Cytomegalovirus in the adrenal glands***

Jelena Železnjak, Marija Mazor, Magdalena Medved, Tina Ružić, Maja Cokarić Brdovčak, Jelena Tomac, Stipan Jonjić, Berislav Lisnić, Vanda Juranić Lisnić

Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia

Cytomegalovirus (CMV) is a highly relevant and widespread human pathogen and an excellent model virus for studying antiviral immune responses. Due to its wide tropism, CMV infects nearly every tissue, making it an excellent model for studying organ and tissue-specific immunity. However, our knowledge about immune responses to viral infection is often restricted to large and readily accessible organs, such as the spleen, liver and lungs. Nonetheless, we have recently shown that murine CMV readily and strongly infects smaller organs, such as ovaries and adrenal glands (AGs), with the peak of infection at day 4-6 and virus clearance by day 8-10 indicating important role of innate immune mechanisms in the control of virus infection. We have utilized depleting antibodies as well as mice genetically deficient for various subsets of the innate and adaptive immunity and demonstrated an important role for NK cells and CD8 T cells in the early control of the virus in the adrenal gland, whereas CD4 T cells regulate the ability of the virus to establish latency in this organ. Interestingly, despite the high infection rate, we have observed no detrimental effects on AG's ability to secrete major hormones, demonstrating the incredible adaptability of this organ to resist virus infection.

P4 ***Immune aspects of thermorestriction mechanism of thermoregulation upon MCMV infection***

Maja Lenartić, Marko Šestan, Marko Ljesar, Sarah Furjan, Fran Krstanović, Felix Wensveen and Bojan Polić

Faculty of Medicine, University of Rijeka, Rijeka, Croatia

Fever is a crucial immune response that creates an inhospitable environment for pathogens and enhances immune cell function. However, excessive fever can cause hyperinflammation, tissue damage, and organ failure. While the molecular mechanisms of fever induction are well understood, how the body limits excessive temperature increases and physiological mechanisms restricting temperature at the later stages of infection are less clear. We investigated thermoregulation in C57BL/6J mice infected with MCMV and found a strong thermorestriction response at 6 dpi, coinciding with the peak of T cell activity. Depletion of CD4 and CD8 T cells resulted in severe thermoregulatory perturbations, indicating T cells play a critical role in infection-induced thermorestriction. Notably, effector T cells differentiated *in vitro* at higher temperatures (39°C) produced more IFN-γ and TNF, leading us to hypothesize that thermorestriction may prevent immune overactivation and subsequent immunopathology. IFN-γ signaling, but not TNF, was essential for proper thermoregulation, as IFN-γ-deficient mice failed to exhibit strong thermorestriction upon infection. We further explored the role of brown adipose tissue (BAT) and found that BATectomy abolished thermorestriction, highlighting its key role in infection-induced temperature regulation. However, CNS-specific IFN-γ receptor knockout mice did not show altered thermoregulation, suggesting IFN-γ does not act directly on the CNS. Moving forward, we aim to explore the physiological importance of thermoregulation during infection and its potential clinical relevance for managing immune responses.

P5 The role of STING in a mouse model of congenital cytomegalovirus infection

Lucija Šakota[1,2], Fran Krstanović1, Andrea Mihalić[1], Stipan Jonjić[1], Ilija Brizić [1]

[1]Center for proteomics, Faculty of Medicine, University of Rijeka, 51000 Rijeka, Croatia; [2]Faculty of Pharmacy, University of Mostar, 88000 Mostar, Bosnia and Herzegovina.

Congenital cytomegalovirus (CMV) infection is a major cause of transplacentally transmitted congenital viral infections that can cause a wide range of structural and functional impairments and permanent neurological sequelae such as hearing loss, mental retardation, or cerebral palsy. Inflammatory response contributes to the pathogenesis of cytomegalovirus infection in the central nervous system (CNS), however, the underlying mechanisms are still largely unknown. The aim of this study is to determine the role of stimulator of interferon genes (STING) in the pathogenesis of CMV infection in the brain.

STING is one of the main elements in the activation of type I interferons (IFN I). We are using a mouse model of congenital CMV infection to answer these questions.

We found that IFN I has a crucial role in the control of MCMV infection in newborn mice, as mice lacking receptor for IFN I succumbed to infection and had increased levels of MCMV in the brain and other organs. It was previously described that STING is not essential for the control of MCMV in adult mice; however, STING contributed to the control of MCMV in the brain and other organs of neonatal mice. Interestingly, STING deficiency affected microglial and T-cell responses in neonatal mice. Thus, IFN I and STING are important players in immune response in early life, affecting both innate and adaptive immunity.

P6 ***Primed microglia limit reactivation of latent herpesvirus at expense of synaptic connectivity***

Andrea Mihalić[1#], Daria Kveštak[1#], Berislav Lisnić[1#], Fran Krstanović[1], Shirin Hosseini[2,3], Katarzyna Sitnik[4,5], Mijo Golemac[1], Vanda Juranić Lisnić[1], Ahmad S. Rashidi[6], Isabell Bochow[2], Alessia Arossa[7], Milena Furione[8], William J. Britt[9], Georges M.G.M. Verjans[6], Martin Korte[2,3], Luka Čičin-Šain[4], Stipan Jonjić[1,10] and Ilija Brizić[1]

[1]Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia; [2]Department of Cellular Neurobiology, Zoological Institute, Technische Universität Braunschweig, Braunschweig, Germany; [3]Helmholtz Centre for Infection Research, Research Group Neuroinflammation and Neurodegeneration, Braunschweig, Germany; [4]Department of Viral Immunology, Helmholtz Centre for Infection Research, 38124 Braunschweig, Germany; [5]Department of Biological Sciences and Pathobiology, University of Veterinary Medicine Vienna, 1210 Vienna, Austria; [6]HerpeslabNL of the department of Viroscience, Erasmus Medical Center, 3015 Rotterdam, the Netherlands; [7]Department of Obstetrics and Gynecology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; [8]Microbiology and Virology Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; [9]Department of Pediatrics, University of Alabama at Birmingham, Birmingham, USA.

Infections of the central nervous system (CNS) represent a significant health burden. Microglia are myeloid cells that reside within the CNS, carrying out numerous homeostatic activities and executing immune functions upon different pathogenic stimuli, including infections. Upon primary infection, herpesviruses can establish a lifelong latency from which they can reactivate intermittently upon waning of immune control. The role of microglia in preventing herpesvirus reactivation is unclear. Here, we studied infection with cytomegalovirus (CMV), the prototypical β-herpesvirus, which infects and establishes latency in the CNS if the infection occurs in early life. We show that mouse CMV (MCMV) latency in the CNS is associated with lifelong microglial priming, enhancing responsiveness to secondary challenge. These characteristics of microglia included continuous activation and extensive transcriptional reprogramming at the single-cell level, resulting in the

expansion of a subpopulation of microglia associated with latent infection. Lifelong microglial activation depended on the local production of interferon-gamma. Notably, the maintenance of primed microglia enhanced control of latent infection and superior recall response but was associated with excessive loss of synaptic dendritic spines mediated by primed microglia. Altogether, our results indicate that latent CMV infection in the brain leads to perturbation of microglial homeostasis, leading to chronic neuroinflammation limiting virus reactivation but simultaneously compromising synaptic connectivity in the brain.

P7 ***Role of CD16 receptor in development of diabetes mellitus type 2***

Vanna Imširović, Felix Wensveen, Maria Molnar, Bojan Polić, Vedrana Jelenčić

Department of Histology and Embryology, Faculty of Medicine Rijeka, Rijeka, Croatia

CD16 is an activating receptor expressed on NK cells, macrophages, monocytes, and neutrophils. This receptor was mostly investigated in the context of ADCC while its role in other aspects of immune responses remained poorly investigated. We noticed that mice lacking CD16 receptor have higher body weight than control animals, leading us to our hypothesis that this receptor might play a role in adipose tissue homeostasis. To investigate how CD16 mice cope with metabolic stress we used a model of obesity induced diabetes mellitus type 2 (DM2). In this model, mice were fed ad libitum with a normal diet (NCD) or high-fat diet (HFD) in which 50% of calories were derived from animal fat. After 12 weeks on HFD diet mice develop insulin resistance and glucose intolerance. We noticed that CD16 deficient mice gained more weight after HFD and were more prone to the development of DM2, as seen from greater glucose intolerance and insulin resistance than control mice.

P8 ***Increased osteoclastogenesis and bone resorption in a mouse model of type 1 diabetes mellitus***

Marta Radošević[1,2], Sara Aničić[1,2], Maša Filipović[1,2], Darja Flegar[1,2], Ivo Krešić[4], Pavo Planinić[4], Tomislav Kelava[1,2], Nataša Kovačić[2,3], Alan Šućur[1,2], Danka Grčević[1,2]

[1]Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia; [2]Laboratory for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia; [3]Department of Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia; [4]University of Mostar School of Medicine, Mostar, Bosnia and Herzegovina

The autoimmune destruction of insuling-producing pancreatic beta-cells is the underlying cause of chronic hyperglycemia in type 1 diabetes mellitus (T1D), causing multi-organ complications. Although bone loss has been associated with T1D, mechanisms driving increased bone resorption by osteoclasts are not fully explained. Recent studies link

inflammatory cytokines, chemokines and advanced glycation end-products (AGEs) to increased osteoclast activity. Therefore, our study aimed to investigate osteoclast progenitor (OCP) populations and their bone-resorbing capacity in the context of T1D. Upon Ethical approval, T1D was induced by streptozotocin administration (STZ, 50 mg/kg) to male C57BL/6 mice (9-11 weeks). Bone structure was analyzed by micro-CT 3 weeks after STZ administration, compared to control (CTRL) mice. Bone marrow (BM) and spleen (SPL) phenotyping was performed using flow-cytometry. Sorted OCPs were stimulated with osteoclastogenic factors (M-CSF/RANKL) to promote in vitro differentiation of tartrate-resistant acid phosphatase (TRAP)-expressing osteoclasts. STZ-treated mice exhibited severe hyperglycemia (T1D 25.6 mmol/L vs CTRL 8.6 mmol/L, $p<0.05$) and weight loss (T1D 21.5 g vs CTRL 23.3 g, $p<0.05$), alongside a slight reduction in femoral trabecular bone volume (T1D 3.95% vs CTRL 4.77% BV/TV, $p>0.05$). Additionally, these mice showed an increased frequency of peripheral (SPL, CD45+CD3-B220-NK1.1-Ly6G-CD11b+CD115+) and medullar (BM, CD45+CD3-B220-NK1.1-Ly6G-CD11bloCD115+) OCPs expressing the chemokine receptor CX3CR1, as well as a higher number of TRAP+ osteoclasts. Our findings suggest expansion of OCPs in T1D, with the enhanced differentiation capacity. Future experiments will focus on understanding the mechanisms driving OCP stimulation and their increased recruitment to bone surfaces.

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P9 ***The effect of 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) on bone biology in a murine model of cholestatic liver disease***

Pavao Planinić[1], Sara Aničić[2,3], Ivo Krešić[1], Darja Flegar[2,3], Alan Šućur[2,3], Nataša Kovačić[2,4], Tomislav Balen[2,4], Danka Grčević[2,3], Antonio Markotić[1], Ivan Ćavar[1], Tomislav Kelava[2,3]

[1]Department of Physiology, School of Medicine, University of Mostar, 88000 Mostar, Bosnia and Herzegovina; [2]Laboratory for Molecular Immunology, Croatian Institute for Brain Research, School of Medicine, University of Zagreb, 10000 Zagreb, Croatia; [3]Department of Physiology, School of Medicine, University of Zagreb, 10000 Zagreb, Croatia; [4]Department of Anatomy, School of Medicine, University of Zagreb, 10000 Zagreb, Croatia.

Mice fed with 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) containing diet are a common model for the investigation of cholestatic liver diseases. In humans, these diseases are frequently accompanied by osteoporosis. Therefore, we aimed to investigate the effects of DDC-diet-induced biliary cirrhosis on bone biology.

Female C57BL/6 mice were fed with 0.025% DDC supplemented standard food for 4 or 8 weeks. Controls were fed standard diet. Femora and lumbar spines were sampled for uCT scanning to evaluate trabecular bone changes (bone volume/total volume ratio (BV/TV) and trabecular number) and cortical bone changes (BV/TV and cortical thickness of femoral

cortex). To determine whether the effect on the bone structure is exerted directly by DDC or it emerges due to biliary cirrhosis, we also sorted CD45+Ly6G−CD3−B220−NK1.1−CD11b−/loCD115+ osteoclast progenitor cells and cultured them in RANKL and M-CSF supplemented medium with addition of different concentrations of DDC or vehicle. Differentiated osteoclasts in cultures were identified by TRAP staining.

Bone analysis revealed significantly higher BV/TV and trabecular number of spinal trabecular bone in DDC-fed mice compared to controls. Conversely, femoral cortical thickness was significantly lower in DDC-fed mice compared to controls. *In vitro* experiments showed that DDC dose-dependently decreases number of TRAP positive osteoclasts and total TRAP positive surface area, suggesting the direct effect of DDC as a potential explanation for the observed bone phenotype.

In further experiments, we intend to define and elucidate the molecular mechanism behind the observed phenotype.

P10 ***High risk PNPLA3 rs738409 polymorphism is associated with higher concentrations of CCL2 in alcoholic end-stage liver disease***

Ivan Budimir Bekan[1], Pavao Planinić[2,3,4], Dino Šisl[3], Alan Šućur[3,4], Ana Bainrauch[1], Sara Aničić[3,4], Katerina Zrinski Petrović [4, 5], Nataša Kovačić[4, 5], Danka Grčević[3,4], Vibor Šeša[6], Anna Mrzljak[5], Tomislav Kelava[3,4]

[1]Merkur University Hospital, Zagreb, Croatia; [2]University of Mostar School of Medicine, Mostar, BiH; [3]Department of Physiology University of Zagreb School of Medicine, Croatia; [4]Croatian Institute for Brain Research, Zagreb, Croatia; [5]Department of Anatomy, University of Zagreb School of Medicine, Croatia; [6]University Hospital Center Zagreb, Zagreb, Croatia.

PNPLA3 rs738409 single nucleotide polymorphism (G allele) has been pointed out as the most robust genetic predictor of hepatocellular carcinoma (HCC) occurrence in chronic liver disease (ESLD). In the present research, we have analysed the association between the PNPLA3 genotype and the concentration of proinflammatory cytokines in patients with alcoholic end-stage liver disease (ESLD). Upon Ethical approval, DNA was isolated from the whole blood, and patients (106 transplant candidates with alcoholic ESLD, without HCC as determined by explant histology, and sex and age matched control patients) were genotyped for PNPLA3 rs738409 by PCR using the TaqMan assays. Concentrations of 13 selected cytokines (IL-1β, IFN-α2, IFN-γ, TNF-α, CCL2, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IL-18, IL-23, IL-33) were determined in plasma by flow cytometry using the commercially available assay (LEGENDplex™ Human Inflammation Panel 1). Comparison of patients with ESLD and healthy controls revealed that there is a statistically significant ($p<0.001$) difference in concentration of 11 cytokines (IL-1β, IFN-α2, IFN-γ, TNF-α, CCL2, IL-6, IL-8, IL-10, IL-12p70, IL-23, IL-33). There was no statistically significant difference in the concentration of IL-17 and IL-18. Forty patients with ESLD had CC genotype, 40 were heterozygotes and 26 had GG

genotype, there was no difference in sex and age between the genotypes. Patients with the GG genotype had a significantly higher concentration of CCL2 (213 (136 - 265) pg/mL, median with interquartile range) than patients with GC (150 (93 - 238)) and CC genotype (139 (117 - 186)), $p<0.001$ (Kruskal Wallis test followed by Mann Whitney). No significant association was found for the remaining 12 measured cytokines ($p>0.05$ for all analyses). The possible contribution of CCL2 to the development of liver-related events in alcoholic liver disease will be further evaluated.

P11 ***Functional and phenotypic characteristics of peripheral blood monocyte subsets***

Ivo Krešić[1], Marina I. Matijašević[2], Sara Aničić[3], Pavao Planinić[1], Katerina Z. Petrović[3], Danka Grčević[3], Alan Šućur[3]

[1]University of Mostar School of Medicine, Mostar, Bosnia and Herzegovina; [2]"Sveti Duh" University Hospital, Zagreb, Croatia; [3]University of Zagreb School of Medicine, Croatian Institute for Brain Research, Zagreb, Croatia.

Introduction: Monocytic lineage is crucial in pathogenesis of autoimmune diseases, including inflammatory arthritides, as monocytes can differentiate into macrophages and dendritic cells (DC). Macrophages are main proinflammatory cytokine producers, while DC are hallmark antigen-presenting cells. Thus, both cells contribute to inflammatory arthritis onset and progression by activation of effector cells - lymphocytes and osteoclasts.

Aim: We aimed to analyze expression of macrophage and dendritic markers on peripheral blood monocytes, measure their change upon stimulation with inflammatory mediators, and assess differentiated macrophage or DC functionality.

Methods: Healthy individuals were sampled for blood and PBMC were isolated from buffy coat after centrifugation in Ficoll gradient. Cells were stained for monocytic (CD14, CD16), dendritic (CD1a, CD1c, CD209), macrophage (CD206, CD163), costimulatory (CD80, CD86) and antigen presentation (MHCII, CD40) markers at baseline. Classical monocytes (CD3-CD19-CD56-CD14+) were isolated with FACS and cultured in vitro no stimulus, MCSF, LPS, IFN-γ or a combination (MCSF+TNF-α+IL-4) for 5 days, then analyzed for expression of noted markers and functionally tested for phagocytic ability using pHrodo dye.

Results: Freshly isolated classical monocytes (CD14+CD16-) generally did not express noted activation and differentiation markers, with exception of highly expressed CD1a (58%), HLAII (79%) and CD86 (46%), while non-classical monocytes (CD14-CD16+) had notable but lower expression of all three markers. In vitro culture resulted in increased expression of all analyzed markers, with several markers expressed on almost all cells (80-90%) (namely CD1a, CD206, CD209, CD40, HLAII). Inflammatory stimulation with TNF-alpha resulted in an increase of CD86 expression with reduced CD1a expression. Phagocytosis was inhibited by IFN-γ or TNF-α stimulation (30% and 47% of nonstimulated), whereby LPS enhanced it (120% of nonstimulated). We plan to expand this preliminary work by enrolling

rheumatoid and psoriatic arthritis patients to assess presence and disease-specificity of activation and differentiation marker expression, as well as test antigen presenting functionality.

P12 ***Finding the optimal administration route (i.m. or i.v.) of antibody-based therapeutics against snakebite envenoming***

Erika Gamulin[1], Sanja Mateljak Lukačević[1], Maja Lang Balija[1], Dražen Vnuk[2], Ana Smajlović[2], Jadranka Gulan Harcet[3], Maja Tomičić[3], Ana Hećimović[3], Beata Halassy[1], Tihana Kurtović[1]

[1]Centre for Research and Knowledge Transfer in Biotechnology, University of Zagreb, Zagreb, Croatia; [2]Clinic for Surgery, Orthopaedics and Ophthalmology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia; [3]Croatian Institute of Transfusion Medicine, Zagreb, Croatia.

Antivenoms are antibody-based products, the only ones providing specific therapy against venomous snakebites. There is no standardized protocol for their administration, but there is a prevailing opinion that the *i.v.* route is more effective than the *i.m.,* based on monitoring of venom/antivenom pharmacokinetics in the systemic circulation only. Following *i.v.* administration, the entire antivenom fraction is immediately available in the bloodstream, allowing for rapid neutralization of venom components that have already entered the circulation. When antivenoms are administered *i.m.,* their entry into the bloodstream is slower and less efficient, resulting in a considerably longer time to peak concentration and lower bioavailability. Recent findings indicate that *i.m.* antivenom-mediated venom neutralization, not only in the bloodstream, but also in the lymphatic system may be important for achieving a favorable clinical outcome. Therefore, the need to reconsider the (dis)advantages of each therapeutic principle has emerged.

We aimed to determine the optimal administration route by gaining insight into the venom/antivenom interplay in both blood and lymph of experimentally envenomed sheep. For the first time, the antivenom effect on decrement of venom quantities in both body compartments was investigated comparatively to reveal how the route affects distribution and elimination of toxic components. This allowed the most effective envenoming treatment to be identified. The study also included monitoring of coagulation and hematological parameters to confirm a more effective route for resolution of clinical signs. Additionally, measurements of anti-antivenom IgGs provided information on the approach leading to a weaker humoral response and, consequently, a better safety profile.

P13 ***Ongoing WNV genome mutations driven by prolonged infection caused by B lymphocytes depletion***

Barbara Anđelić Dmitrović[1], Ivan-Christian Kurolt[1], Kristian Bodulić[1], Mihaela Kordun[1], Alemka Markotić[1,2,3]

[1]Research Department, University Hospital for Infectious Diseases "Dr. Fran Mihaljević", Zagreb, Croatia; [2]School of Medicine, Catholic University of Croatia, Zagreb, Croatia; [3]Faculty of Medicine, University of Rijeka, Rijeka, Croatia.

West Nile Virus (WNV; *Flaviridae*; *Flavivirus*) is an emerging zoonotic pathogen transmitted by mosquito' species from genus *Culex*. The majority of infections—roughly 80%—remain asymptomatic, whereas 20% progress to a self-resolving flu-like illness. Here, we report a symptomatic patient infected with WNV during his fourth round of rituximab and bendamustine chemotherapy for the treatment of indolent B-cell non-Hodgkin's lymphoma. WNV replication continued for almost five months, confirmed by successful viral isolation. Virus clearance was observed after detection of specific antibodies, due to B lymphocyte regeneration, by viral neutralization assay. Viral RNA was successfully isolated from clinical samples collected at different time points and virus was isolated on Vero E6 cells. WNV whole genome sequencing from multiple time point samples was successfully performed. Using reference-guided and *de novo* assembly, high-quality reads were assembled into complete viral genomes. A comparative analysis of consensus genomes revealed several nucleotide changes in the coding region, including both nonsynonymous and synonymous mutations. The number of mutations across genomes increased over time, from two changes observed in the second time point, to ten in the final compared to the first time point. As shown by hamster models and previous human infections WNV can adapt to new conditions through nucleotide changes during a long-lasting infection process, due to a viral RNA-dependent RNA polymerase without proofreading capabilities. Prolonged viral replication was possible due to B-cell depletion allowing for constant viral genome changes. Differentiation of WNV infections in immunosuppressed patients is complex and dependent on the reason of immunosuppression.

P14 ***Design and synthesis of new peptidomimetics of bacterial peptidoglycan monomer for combating drug-resistant bacterial infections***

Marcela Šišić[1], Leo Frkanec[2], Ruža Frkanec[1]

[1]Centre for Research and Knowledge Transfer in Biotechnology, University of Zagreb, Rockefellerova 10, 10000 Zagreb, Croatia; [2]Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institut, Bijenička cesta 54, 10 000 Zagreb, Croatia.

With the continuing emergence and spread of multidrug-resistant bacteria, there is an urgent need for the development of new antimicrobial agents. One possible source of new antibacterial targets is the biosynthesis of the bacterial cell wall peptidoglycan. Cyclic glycopeptides, such as vancomycin and teicoplanin, bind to the terminal two residues (acyl-D-alanyl-D-Ala) of the pentapeptide peptidoglycan precursor as it is exported across the cell membrane to the cell wall. The binding of D-Ala-D-Ala by the bulky antibiotic is thought to

block the interaction of the transglycosylase with the peptidoglycan, thus preventing the growth of the cell wall peptidoglycan. In recent years, natural antimicrobial peptides, comprising one or more lipid chains (lipopeptides), have been identified with potent antimicrobial activity. Lipopeptides offer several advantages, including better target accessibility on the cell membrane and improved stability. Furtehrmore, the immunotherapeutic agents that stimulate the appropriate immune response and defence functions, which can eliminate the pathogen involved in the infection, are intensively researched. The aim of this work is the synthesis of new peptidomimetics of the peptidoglycan monomer (PGM), GlcNAc-MurNAc-L-Ala-D-isoGln-mesoDAP(ε-NH2)-D-Ala-D-Ala, isolated from the cell wall of the non-pathogenic bacterium *B. divaricatum*, which has confirmed various biological activities, particularly immunostimulating effects. Two synthetic approaches were explored: (i) the synthesis of a cyclic peptidomimetic of PGM and (ii) the synthesis of a lipophilic peptidomimetic. After establishing the synthetic protocol and performing chemical characterization, the antibacterial and immunostimulatory activities of the synthesized peptidomimetics will be tested in the next phase of the research.

The results of this research on the design and synthesis of PGM peptidomimetics with enhanced stability and binding affinity to the target sites of peptidoglycan biosynthesis will serve as valuable tools and could accelerate efforts to develop new antibiotics in the fight against antimicrobial resistance.

P15 ***Development of thymus organoids from epithelial stem cells***

Ildikó Bódi[1], Sabrina Adorisio[2], Domenico V. Delfino[2], Zsolt Prodan[3], Delfa Radić-Krišto[4], Danka Grčević[4], and Mariastefania Antica[5]

[1]Semmelweis University, Budapest, Hungary; [2]Department of Medicine and Surgery, University of Perugia, Italy; [3]Gottsegen National Cardiovascular Center, Budapest, Hungary; [4]University of Medicine, Zagreb, Croatia; [5]Ruđer Bošković Institute, Zagreb, Croatia.

The function of the immune system depends on the ability of the thymus to produce immunocompetent T cells. This ability depends on the diversity of stromal epithelial cells. Since the thymus is the first organ to degenerate, already during puberty and since it is very sensitive to cytotoxic interventions, it is a serious problem for many patients. The aim of our study is to regenerate the function of the immune system in immunocompromised patients. Transplantation of neonatal human thymus can lead to successful regeneration of the immune system, but the supply of tissue limits its use. We analysed by immunohistology the neonatal and adult human thymus and show here the different compartments especially regarding the epithelial cells. Therefore, it is a major challenge to find thymic epithelial stem cells (TESC) capable of generating and expanding in vitro all epithelial subpopulations of the human thymus in order to administer these cells to immunocompromised recipients and thus fully restore the human thymus and T cell development

As we found a novel population of TESCs from the pediatric human thymus, we continue our previous work, and characterize them phenotypically and functionally as epithelial stem cells by 3D *in vitro* cultures that form organoids as the basis for re-aggregation cultures of spheroids leading to differentiation of T lymphocyte from CD34+ hematopoietic precursors.

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P16 ***Monitoring the development of human thymospheres in vitro***

Lidija Milković[1], Josipa Skelin[1], Dražen Belina[2], Ivan Lučić[1], Danka Grčević[2], Domenico V. Delfino[3], Mariastefania Antica[1]

[1]Ruđer Bošković Institute, Zagreb, Croatia; [2]University of Medicine, Zagreb, Croatia; [3]Department of Medicine and Surgery, University of Perugia, Italy

The thymus is a crucial organ in the human immune system, serving as the site for T lymphocyte maturation and differentiation. Aging and certain diseases cause thymic involution, reducing its function and weakening immune responses, thereby increasing susceptibility to infections and immune-related disorders. The thymus has a complex structure, consisting of cortical and medullary cells, with thymic epithelial cells (TECs) playing a critical role in shaping the T lymphocyte repertoire. Therefore, developing new strategies to promote thymic regeneration is essential.

By means of stromal cell enrichment from the human thymus, we found a new population of stem cells forming spheres in 3D *in vitro* conditions. These thymospheres are believed to be formed from thymic epithelial stem/progenitor cells that possess stemness features. The aim of the present study was to optimize the *in vitro* conditions and find the best growth factors for stem cell development and differentiation. We cultured human pediatric thymus samples under low-attachment conditions with specific growth factors to promote thymosphere formation. After 13 days of cultivation, the thymospheres were counted and imaged using an inverted microscope with a digital camera. We measured their surface area using ImageJ software. Calculating the thymosphere surface area proved to be a valuable tool for assessing the efficacy of microenvironmental factors and for optimizing the procedures that would contribute to thymic repair and restoration.

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