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2019 ANNUAL MEETING OF THE

CROATIAN IMMUNOLOGICAL SOCIETY

ROVINJ, OCTOBER 11th-12th 2019

ORGANIZED BY

CROATIAN IMMUNOLOGICAL SOCIETY
University of Rijeka Faculty of Medicine

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Dear Friends and Colleagues,

Hereby I would like to welcome you all to the annual meeting of the Croatian Immunological Society at this great venue of island hotel Istra!

Immunology is one of the most competitive fields of science, but I am proud to say that Croatian researchers continue to uphold a firm position within its ranks. Last year was marked by several key publications of our members in some of the top-ranking journals in immunology, such as *The Journal of Experimental Medicine*, *Nature Immunology* and *Immunity*. Moreover, our member Dr. Ilija Brizić received the **national award for science**, indicating that the accomplishments of our members are recognized and visible at a national level. Finally, I am happy to say that we received a large number of new applications for our society from young researchers, which promises a vibrant new generation of immunologists in Croatia.

2019 was also the start of a new management for HID and a moving of its seat from Zagreb to Rijeka. We are doing our best to continue the high standards set by our predecessors, but new management also marks a few changes. This year, we are introducing a few new concepts at our meeting, of which we hope that they will contribute to its excellence. We have introduced the **Bright Sparks session**, named after a similar setup at the ECI, in which three young scientists with the best evaluated abstract present their findings. We are allowed to hand out the **EFIS-Immunology Lecture award** to Prof. Teunis B.H. Geijtenbeek, as a recognition of his contribution to immunology in Europe. We have a **strategic lecture** of Prof. Bojan Polić on grant application and management, to aid future group leaders in their struggle for sufficient research funds. But the core of our meeting is of course interaction between junior and senior scientists from Croatia and abroad to promote collaboration, science and friendship through lectures or simply a beer at the bar.

Finally, a special word of thanks to our sponsors, without whom the meeting would not have been possible. I recommend that all of you attend their booths in the poster area to see how they can help you with your work.

I hope that you will all have a splendid meeting!

Felix M. Wensveen

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INVITED LECTURES

THE COMPLEX OF MCMV PROTEINS AND MHC CLASS I EVADES NK CELL CONTROL VIA MISSING-SELF AND DRIVES THE EVOLUTION OF VIRUS-SPECIFIC ACTIVATING LY49 RECEPTORS

Assis. Prof. Vanda Juranić Lisnić, PhD

School of Medicine, University of Rijeka, Croatia

Cytomegaloviruses (CMVs) are widespread dsDNA viruses that efficiently downregulate MHC I molecules to avoid recognition by cytotoxic T cells. However, absence of MHC I on the cell surface renders the infected cell susceptible to NK cell killing upon missing-self recognition. To counter this, mouse CMV (MCMV) rescues some MHC I molecules to engage inhibitory Ly49 receptors. Here we identify a new viral protein, MATp1, that is essential for MHC I surface rescue together with m04. Rescued altered-self MHC I molecules show increased affinity to inhibitory Ly49 receptors resulting in inhibition of NK cells despite substantially reduced MHC I surface levels. This enables the virus to evade recognition by licensed NK cells. Interestingly, MATp1 ORF is highly variable among different virus strains which has prompted us to investigate whether it has been under strong selection pressure by the immune system. Differential sensitivity of various mouse strains to MCMV has been linked to the capacity of NK cells to recognize infected cells via activating Ly49 receptors. We and others have previously shown specific recognition of MCMV-infected cells by activating Ly49 receptors that requires MHC I, m04 and additional virally encoded factor. Using multiple MCMV mutants we show that the unknown virus factor is also MATp1. Thus, MATp1-mediated MCMV evasion of inhibitory Ly49 receptor could have prompted the evolution of activating, virus-specific Ly49 receptors.

DENDRITIC CELLS IN HIV-1 SENSING AND RESTRICTION

Prof. Teunis B. H. Geijtenbeek, PhD

Amsterdam UMC, Amsterdam, The Netherlands

Sexual transmission is the primary route of infection by HIV-1 and mucosal dendritic cell (DC) subsets are amongst the first targets for HIV-1. Although DCs are paramount to the induction of antiviral immunity to HIV-1, it is becoming evident that HIV-1 subverts submucosal DCs for dissemination to T cells as well as escape from antiviral immunity. HIV-1 productively infects submucosal DCs but this does not lead to an antiviral type I Interferon immune response as HIV-1 escapes viral sensing in DCs. We identified the dead-box helicase DDX3 as a RNA sensor for HIV-1 and our recent data strongly suggest that HIV-1 blocks this viral sensor in DCs, preventing antiviral immunity. Here I will discuss this inhibitory mechanism and how we can prevent this to enhance immunity to HIV-1.

Strikingly, not all DC subsets become infected by HIV-1 as our data show that mucosal Langerhans cells (LCs) are resistant to HIV-1 infection. LCs efficiently capture HIV-1 and route the virus into a autophagy-mediated degradation pathway, which prevents infection. Here I will discuss the molecular mechanisms underlying the distinct functions in DC subsets in HIV-1 infection and how we can harness these mechanisms to prevent infection and enhance antiviral immunity.

STROMAL CELL – IMMUNE CELL INTERACTION

Prof. Burkhard Ludewig, PhD

Medical research center, St. Gallen, Switzerland

Lymphoid organs guarantee productive immune cell interactions through the establishment of distinct microenvironmental niches that are built by fibroblastic reticular cells (FRC). These specialized immune-interacting fibroblasts coordinate the migration and positioning of lymphoid and myeloid cells in lymphoid organs and provide essential survival and differentiation factors during homeostasis and immune activation. In this presentation, I will highlight how different FRC subsets integrate innate immunological signals and molecular cues from immune cells to fulfill their function as nexus between innate and adaptive immune responses.

DESENZITATION OF HEPATOCYTES TO FAS-INDUCED APOPTOSIS DURING THE ACUTE INFLAMMATION

Assis. Prof. Tomislav Kelava, PhD

University of Zagreb School of Medicine, Zagreb, Croatia

During the inflammatory response hepatocytes receive signals from many various cellular and molecular mediators. Complex downstream events are initiated which through interference with the apoptotic pathway can have a decisive influence on survival or death of liver cells. Better understanding of the crosstalk between the inflammatory and apoptotic signals is crucial to elucidate pathogenesis of various hepatic diseases

In the present research we have studied the effects of lipopolysaccharide (LPS), one of the crucial inductors of inflammation in liver, on apoptotic outcome. We show that LPS-induced inflammation diminishes the sensitivity of hepatocytes to Fas stimulus in-vivo at caspase 8 level. Analysis of molecular mechanisms revealed an increased expression of various proinflammatory cytokines in nonparenchymal liver cells and hepatocyte-specific increase in Bcl-xL, associated with signal transducer and activator of transcription 3 (Stat3) phosphorylation. Pretreatment with ruxolitinib, a selective Janus kinase (JAK) 1/2 inhibitor, prevented the LPS-induced Stat3 phosphorylation and restored sensitivity of hepatocytes to Fas-mediated apoptosis. Furthermore, ruxolitinib pretreatment diminished the LPS-induced Bcl-xL upregulation without an inhibitory effect on LPS-induced expression of proinflammatory cytokines. In summary, although there are reports showing that the effects of isolated proinflammatory mediators such as TNF- α or neutrophils are proapoptotic, the overall effect of inflammatory milieu on hepatocytes in-vivo is Stat3-dependent desensitization to Fas-mediated apoptosis.

Funded by HRZZ, grant number: UIP-2017-05-1965

ASSOCIATION BETWEEN AUTOANTIBODIES AND MUSCLE PATHOLOGY IN INFLAMMATORY MYOPATHIES: FROM BENCH TO BEDSIDE

Assis. Prof. Mislav Radić, MD, PhD

University hospital Split, Croatia

Idiopathic inflammatory myopathies represent a heterogeneous group of autoimmune diseases with systemic involvement. Even though numerous specific autoantibodies have been recognized, they have not been included, with the only exception of anti-Jo-1, into the 2017 Classification Criteria, thus perpetuating a clinical-serologic gap. The lack of homogeneous grouping based on the antibody profile deeply impacts the diagnostic approach, therapeutic choices and prognostic stratification of these patients. Autoantibodies are considered to be epiphenomenon in autoimmunity, their presence frequently plays a pivotal role for the diagnosis of these diseases. Autoantibodies found in inflammatory myopathies patients have been classified into two main categories: myositis-specific autoantibodies (MSAs), which can be found in IIMs exclusively, and myositis-associated autoantibodies (MAAs), which can also be found in other connective tissue diseases. There is no agreement about the attribution of rare and newly discovered autoantibodies to either MSAs or MAAs group. Anti-synthetase autoantibodies themselves, especially anti-PL-7, PL-12 and KS, often detected in interstitial pneumonia with autoimmune features patients, independently from muscular involvement, are still discussed as MSAs. The MAAs group contains Anti-Pm-Scl, U1/U2RNP and Ku, which are associated with overlap syndromes with muscular involvement. Anti-fibrillarin and anti-U1-snRNP are sometimes considered as MAAs, even though they are more specific for the diagnosis of systemic sclerosis and mixed connective tissue disease. Anti-Ro52 are usually considered a MAA, even though they are more frequently found in association with other MSA (anti-synthetase autoantibodies, anti-MDA5 and anti-SRP, in particular), and define a peculiar clinical spectrum in which the lung involvement is more common than the muscular one. Indeed, several of them exhibit a pathogenethic role in inflammatory myopathies. Despite this, there is still a gap between bench and bedside because the intense basic research efforts have not been translated in clinical practice, as already futuristically underlined more than 20 years ago. As a fact, only anti-Jo-1 have been included into the Classification Criteria for Adult and Juvenile inflammatory myopathies. In the context of heterogeneously grouped diseases such as myositis, they should be even more appreciated as able to clinically stratify patients in terms of diagnostic work-up, histological patterns, peculiar organ involvement, severity, and, therefore, treatment intensity and prognosis. This process could be accomplished by a laboratory auto-immunologist well-trained in recognition of indirect immuno-fluorescence ANA nuclear and, also, cytoplasmic patterns, in strict collaboration with the clinical doctor, as a decision-maker for running in-depth analysis towards the identification of the culprit autoantibody. Multicentric studies with a multidisciplinary approach may help bridging the divide of the selection bias depending on the setting where patients are initially screened (i.e. pulmology vs. dermatology vs. clinical immunology vs. rheumatology vs. neurology outpatient clinics).

IMMUNOLOGICAL MEMORY OF THE TISSUES

Prof. Klaas van Gisbergen, PhD

Sanquin Research, Amsterdam, The Netherlands

Tissue-resident memory CD8 T cells (Trm) constitute a non-circulating memory subset that provides early protection against re-infection. However, many aspects of Trm biology remain unclear such as the developmental pathway of how Trm arise from effector T cells and the contribution of Trm to the generation of secondary memory responses. A substantial impediment for research on Trm is a lack of tools to specifically dissect their function. We have previously defined Hobit as a unique transcriptional regulator of Trm within the CD8 T cell lineage. Therefore, we took advantage of the Trm-restricted expression of Hobit to generate Trm reporter/deleter mice, containing a “knock-in” of the fluorescent protein tdTomato, the CRE recombinase and the diphtheria toxin (DT) receptor within the Hobit locus. The newly developed mice accurately and specifically report tdTomato (Hobit) expression in pathogen-specific Trm of gut, kidney and liver after LCMV or Listeria infection. Therefore, the Trm reporter/deleter mice appear to form a unique model system to tackle important unresolved issues in Trm biology through direct visualization and genetic manipulation of these memory T cells. Indeed, our findings using the Trm reporter/deleter mice provide interesting insights into how effector cells commit to Trm development in the peripheral tissues and to how Trm contribute to local and systemic T cell responses upon pathogen re-challenge.

ORAL PRESENTATIONS

Session 1

THE EFFECT OF CCL2/CCR2 SIGNALING BLOCKADE ON BONE RESORPTION AND OSTEOCLAST PROGENITORS IN COLLAGEN INDUCED ARTHRITIS

Darja Flegar[1,2], Alan Šućur[1,2], Antonio Markotić[1,2], Maša Filipović[1,2], Nina Lukač[3,2], Dino Šisl[1,2], Nataša Kovačić[3,2], Tomislav Kelava[1,2], Vedran Katavić[3,2], Katerina Zrinski Petrović[3,2], 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, Zagreb, Croatia
3 Department of Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia

INTRODUCTION: Osteoclast progenitors (OCPs) originate from myeloid lineage precursors common to macrophages and dendritic cells. Their differentiation to osteoclasts, specialized bone resorbing cells, increases in rheumatoid arthritis (RA) and promotes joint destruction. We investigated effects of CCL2/CCR2 axis blockade on OCPs and osteoresorption in mice with collagen-induced arthritis (CIA), a mouse RA model.

METHODS: Male DBA and B6 mice were immunized with chicken type II collagen to induce CIA. DBA mice developing CIA (day 15-30 after immunization) were treated with methotrexate (MTX) (2mg/kg) and CCR2 receptor antagonist (CRA) (4mg/kg) every 48 hours. CIA severity was assessed by clinical scoring, and osteoresorption by fluorescence imaging using osteoclast-specific probe, micro-CT and histology. Spleen and distal tibia bone marrow (BM) cells were immunophenotyped for hematopoietic markers to assess effects on myeloid lineage cells and OCP frequency. Sorted OCP subsets were assessed for migration potential using Transwell system and osteoclast differentiation by culturing with M-CSF/RANKL.

RESULTS: Frequency of CD45+B220-CD3-NK1.1-Ly6G-CD11b-/loCD115+CCR2+ OCPs was significantly increased in arthritis. CIA severity score and osteoresorption, measured by fluorescence imaging and micro-CT analysis, were decreased in treatment groups. Frequencies of BM neutrophils, macrophages and CD45+B220-CD3-NK1.1-Ly6G-CD11b-/loCD115+CCR2+ OCPs were also decreased with tretment, particularly in CRA treated mice. Sorted OCPs from treated groups generate multinucleated TRAP+ osteoclasts less efficiently compared to untreated CIA group.

CONCLUSION: CCR2 blockade affects myeloid cell populations and OCPs. Therefore, additive therapeutic inhibition of CCL2/CCR2 signaling may help antagonizing enhanced osteoresorption in arthritis.

SUPPORT: Croatian Science Foundation projects IP-2014-09-7406, IP-2018-01-2414 and DOK-2018-09-4276.

Poster number 09

THE DYNAMICS OF THE INFLAMMATORY RESPONSE DURING BBN-INDUCED BLADDER CARCINOGENESIS IN MICE

Marina Degoricija[1], Jelena Korac-Prlic[1], Katarina Vilovic[2], Tonci Ivanisevic[1], Benedikt Haupt[1], Vinko Palada[3], Marina Petkovic[1], Ivana Karaman[2] and Janos Terzic[1]

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2 Department of Pathology, University Hospital Split, 21000 Split, Croatia
3 Department of Physiology and Pharmacology, Karolinska Institutet, 17177 Stockholm, Sweden

The most common preclinical mouse model for bladder cancer (BC) relies on administration of N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) to mice. BBN-induced tumors in mice recapitulate the histology of human BC and were characterized with an overexpression of markers of basal-like cancer subtype in addition to a high mutational burden with frequent mutations in Trp53, similar to human MIBC. High mutational load in human BC was previously associated with response to drugs, including immune checkpoint inhibitors. In this study, we characterized the dynamics of the inflammatory response in the BBN-induced BC in mice. We performed RNA-seq, qPCR and a thorough pathohistological analysis of bladder specimen at different time points during and post BBN treatment of male C57BL/6 mice. We observed that the treatment with BBN, gradually induced a robust inflammation in the first 2 weeks of administration, however, the inflammatory response was progressively silenced in the following weeks of the treatment, until the progression of the primary carcinoma in situ. Tumors at 20 weeks were characterized with a marked upregulation of IL18 when compared to premalignant inflammatory response at 2 weeks. In accordance with this, we observed an increase in expression of IFNg-responsive genes coupled to a pronounced lymphocytic infiltrate during the early stages of malignant transformation in bladder. Similar to human basal-like BC, murine tumors displayed an upregulated expression of immunoinhibitory molecules such as CTLA-4, PD-L1, and IDO1 which lead to cytotoxic resistance and tumor escape, therefore representing an optimal model for preclinical studies on immunomodulation in management of BC.

Poster number 10

NK CELLS ARE MAJOR PLAYERS IN NEUROINFLAMMATION AND BRAIN PATHOLOGY FOLLOWING CONGENITAL MCMV INFECTION

Daria Kveštak[1], Vanda Juranić Lisnić[1,2], Berislav Lisnić[1], Ilija Brizić[2], Jelena Tomac[1], Mijo Golemac[1], Ester Pernjak Pugel[1], Astrid Krmpotić[1] and Stipan Jonjić[1,2]

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

Human cytomegalovirus (HCMV) is the most common causative agent of congenital viral infections in humans, which may lead to long-term central nervous system (CNS) abnormalities such as hearing and vision impairments and disorders of the motor and cognitive functions. Since HCMV is species-specific, we have used a mouse model in which newborn mice are infected intraperitoneally with the mouse cytomegalovirus (MCMV). Following intraperitoneal inoculation of newborn mice with MCMV, the virus disseminates to the CNS, replicates in the brain parenchyma and causes altered cerebellar development, most evident in the smaller size of the cerebellum and thicker external granular layer.

Here we show that MCMV infection in brain of newborn mice leads to activation of the microglia towards proinflammatory phenotype. Moreover, we show that activated microglia secrete CXCL9 and CXCL10 that acts on CXCR3 receptors on NK cells and recruits them from the periphery into the brain. Blockade of CXCR3 with monoclonal antibody not only reduced amount of NK cells in the brain but also reduced cerebellar pathology. NK cells are an early source of IFN-γ which drives the polarization of microglia towards a proinflammatory phenotype associated with altered cerebellar development in infected newborn mice. Depletion of NK cells or neutralization of IFNγ abolished polarization of microglia and notably, normalized neurodevelopmetnal delay in infected newborn mice. Thus this study provides previously unidentified direct evidence that NK cells, that infiltrate into the brain following perinatal MCMV infection, have a detrimental impact that results in altered cerebellar development.

Poster number 18

GUT MICROBIOTA DISPARITIES BETWEEN JUVENILE IDIOPATHIC AND REACTIVE ARTHRITIS PATIENTS AT THE INITIAL STAGE OF THE DISEASE

Rados I[1], Vidovic M[1], Cindric M[3], Hozic A[3], Buljan D[2], Paleka Bosak E[1], Harjacek M[1,2], Lamot L[1]

1 Sestre milosrdnice University Hospital Center, Zagreb, Croatia
2 University of Zagreb, School of Medicine, Zagreb, Croatia
3 Ruder Boskovic Institute, Division of Molecular Medicine, Zagreb, Croatia

INTRODUCTION: Painful joint swelling is a symptom of many childhood diseases, most notably reactive arthritis (ReA) and juvenile idiopathic arthritis (JIA). The growing body of evidence suggests that gut microbiota could orchestrate the immune response and development of those diseases. This study aimed to assess the differences in the presence of Escherichia coli (E coli) subtypes in the stool of JIA and ReA patients at the first occurrence of symptoms.

METHODS: Stool samples of 14 patients with joint swelling were collected during their first visit to Pediatric Rheumatology Clinic in Sestre milosrdnice University Hospital Center in Zagreb, Croatia. The samples were analyzed by mass spectrometry on nanoLC-Synapt G2 Si instrument. To identify the most abundant E coli subtypes, specialized software named Protein Reader with implemented Dust algorithm searched through NCBI nr database.

RESULTS: Various E coli subtypes (P0301867.1-10, O104:H4, O103:H25, O111:H11, KTE and K) were three times more abundant in patients with JIA, while increased abundance of diarrheagenic E. coli (DEC) was detected in children with ReA.

CONCLUSION: This pilot study has shown differences in the subtypes of E. coli present in the stool of children with ReA and JIA in the early stage of the disease. Since E coli is one of the paramount bacteria in gut microbiota, it is reasonable to assume that the differences described in this study can have a potential impact on the gut environment, contributing to the development of the chronic disease in JIA patients, or the resolution of symptoms in children with ReA.

Poster number 27

ORAL PRESENTATIONS
Session 2

CELL CYCLE ARREST AND MONOCYTIC DIFFERENTIATION BY ACTIVATING CHECKPOINT KINASE 1

Barbara Tomić, Vilma Dembitz, Dora Višnjić

Department of Physiology and Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Zagreb, Croatia

Our previous study demonstrated that 5-aminoimidazole-4-carboxamide ribonucleoside (AICAr), a precursor in purine biosynthesis and a widely used activator of AMP-activated kinase (AMPK), promotes differentiation and inhibits proliferation of monocytic U937 cells. Furthermore, AICAr was demonstrated to inhibit pyrimidine synthesis at a step downstream of dihydroorotate dehydrogenase (DHODH), and AICAr-mediated effects on differentiation were prevented by the addition of uridine. Depletion of nucleotide pools is known to activate the DNA damage signaling pathway through activation of the ataxia teleangiectasia and RAD3-related (ATR)/checkpoint kinase 1 (Chk1)-mediated checkpoint in S-phase of the cell cycle. Present studies are aimed to test for the role of ATR/Chk1 in AICAr-mediated effects. Western blot analysis revealed that AICAr increased the level of Ser-345-phosphorylated Chk1, and the effect was abolished by addition of either nucleosides or uridine. The activation of Chk1 was observed in the presence of DHODH inhibitor brequinar, and the increase in the level of Ser-345-phosphorylated Chk1 in response to AICAr, brequinar and their combinations followed the same pattern as the expression of differentiation markers and S-phase arrest. Pharmacological inhibition of ATR/Chk1 pathway by caffeine, Torin2 and VE-821 prevented differentiation and cell cycle arrest in response to AICAr and brequinar. Transfection of U937 cells with siRNA targeting Chk1 decreased the level of Chk1 and significantly reduced the effects of AICAr and brequinar on the expression of differentiation markers and cell cycle arrest. These results demonstrate that AICAr-mediated differentiation of U937 cells is mediated by activation of DNA damage checkpoint kinase Chk1 induced by pyrimidine depletion.

Poster number 01

DECREASE IN BLOOD SUGAR LEVEL DUE TO VIRAL INFECTION PROMOTES THE INNATE ANTI-VIRAL IMMUNE RESPONSE

Marko Šestan, Ante Benić, Felix M. Wensveen, Bojan Polić

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

Viral infection has a major impact on systemic metabolism. We have recently shown that viral infection impacts endocrine regulation of systemic blood glucose levels. However, it is not known how strength of infection reflects on blood sugar levels during infection, and if these changes are beneficial to the host. Here we investigated how different viral loads impact regulation of blood glucose levels.

We showed that infection of mice with high, but non-lethal titers of mCMV or LCMV causes transient relative hypoglycemia. Low blood glucose levels were beneficial to the host as enforced hyperglycemia during infection resulted in significant increase in viral titers in peripheral organs. With LCMV, relative hypoglycemia was the result of IFNγ secretion by $\gamma\delta$T cells, as δ deficient mice, and mice treated with anti-IFNγ antibodies did not develop this condition. Using mice without expression of IFNγ receptor on myocytes, we showed that IFNγ causes insulin resistance in muscle, which causes compensatory hyperinsulinemia. This, in turn, impairs glycogen utilization as a source of blood glucose, resulting in low blood glucose levels. In vitro, we could show that low glucose concentrations in medium causes an increase in cellular stress, which made cells less receptive for viral replication and reduced viral titers.

In summary, we found that infection with high, non-lethal titers of virus causes relative hypoglycemia. This causes systemic cellular stress, which makes the organism less receptive for viral replication. Thus, we speculate that reduced blood sugar levels during infection are part of body's natural response to infection.

Poster number 08

THE ROLE OF NOTCH SIGNALING IN MURINE MODELS OF FIBRINOGENESIS

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Hepatic fibrosis is a common feature of various liver diseases characterized by activation of hepatic stellate cells (HSC), a principal source of alpha smooth muscle actin (αSMA) liver myofibroblasts. Recent studies suggested a possible role of Notch signaling pathway in pathogenesis of fibrosis. In the present research we have studied the expression of Notch-related molecules during the fibrogenesis and analyzed contribution of various αSMA positive cell populations to the pool of liver myofibroblasts. Two common murine models of liver fibrosis, carbon tetrachloride (CCL4) treatment for 6 weeks and 0.1% DDC-supplemented diet for 4 weeks were used. PCR analysis showed an upregulation of various Notch-related genes in both models. In CCL4 model, Hey1, HeyL, Notch2 and Jag2 were upregulated, while DDC-induced fibrosis was associated with increased expression of Hey1, Hes1, HeyL, Notch2, Notch3, Jag1 and Jag2. We used tamoxifen inducible Cre mice (αSMA-CreERT2/Ai9) to asses contribution of various αSMA+ cell populations to myofibroblast pool. In normal, nonfibrotic liver, only vascular smooth muscle cells (VSMCs) were labeled after tamoxifen application. In further experiments, tamoxifen was given either before (to label VSMCs) or after (to label activated HSCs and VSMCs) the initiation of CCL4 treatment. Immunohistochemical analysis excluded VSMCs as a major source of myofibroblasts in fibrosis and confirmed that majority of myofibroblasts stem from activated HSCs. In the upcoming experiments we aim to modulate Notch signaling pathway specifically in αSMA+ cells to elucidate its importance in fibrotic processes.

Funded by HRZZ, grant number: UIP-2017-05-1965

Poster number 34

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF *M116* REGION IN MOUSE CYTOMEGALOVIRUS

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Human cytomegalovirus (HCMV) is a species-specific herpesvirus that causes severe disease in immunocompromised individuals and immunologically immature neonates. Murine cytomegalovirus (MCMV) is biologically similar to HCMV and it serves as a widely used model for studying the infection, pathogenesis and immune responses to human HCMV. We have previously identified M116 as one of the most extensively transcribed regions of MCMV genome, indicating that it must play an important role for the virus' life cycle. Interestingly, very little is known about this genomic region in MCMV or its positional homolog in HCMV, UL116, and their protein products.

Our molecular characterization revealed two 5' co-terminal transcripts in M116 region. We have further shown that one of these transcripts encodes a glycosilated protein and have generated a monoclonal antibody that specifically recognizes that protein. In order to study *in vitro* and *in vivo* properties of M116, a MCMV mutant with deletion in M116 open reading frame (ΔM116-MCMV) was constructed. By comparing *in vitro* replication kinetics of ΔM116-MCMV and WT-MCMV, we observed comparable kinetics in primary mouse embryonic fibroblasts. However, ΔM116 was attenuated in bone marrow-derived dendritic cells and macrophages. Since CMVs display a broad tissue tropism, they employ various strategies to ensure their survival and propagation in different cell types. Our results indicate that M116 might have an important role in MCMV infection of myeloid cells.

Poster number 23

ORAL PRESENTATIONS
Session 3 – Bright Sparks

MOUSE CYTOMEGALOVIRUS VECTOR EXPRESSING RAE-1γ IN TUMOR IMMUNOTHERAPY

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In recent decades there have been various attempts in trying to harness body's own immune system in the fight against neoplastic cells by modulating different stages of the "cancer-immunity cycle". One of the approaches is to construct a vaccine vector against tumor antigens which would induce a strong CD8 T-cell response. The immunobiology of cytomegalovirus (CMV) infection makes CMV a particularly attractive viral vector. CMV establishes life-long persistence which generates strong CD8 T-cell immune response with a gradual accumulation of CMV specific CD8-T cells in the infected host. We have constructed mouse cytomegalovirus vector expressing NKG2D ligand RAE-1γ in place of its viral inhibitor m152 which proved to be highly attenuated in vivo as compared to wild type virus, while simultaneously inducing strong CD8 T-cell response. Co-expression of foreign CD8 T-cell epitope with RAE-1γ in CMV vector induced an expansion of KLRG1+ CD8 T-cells with enhanced effector properties. These CD8 T-cells proved to be highly protective in both melanoma and lymphoma models, not only in prophylactic setting but also in therapeutic protocol. CD8 T cell protection was long lasting as epitope-specific CD8 T-cells were maintained at high frequency throughout life, exhibited enhanced effector function and ensured long term protection against secondary melanoma challenge. The vaccination of neonatal mice was shown to be even more efficient, resulting in expansion and long-term maintenance of epitope specific CD8 T cells conferring protection against melanoma challenge. Overall, our results indicate that CMV vectors expressing NKG2D ligand RAE-1γ have the potential for prevention and treatment of CD8 T-cell sensitive tumors.

Poster number 30

THE RIBONUCLEOSIDE AICAR INDUCES MONOCYTIC DIFFERENTIATION VIA PYRIMIDINE DEPLETION

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Changes in metabolic pathways have been shown to regulate myeloid differentiation both in physiologic and pathologic conditions. Our previous work demonstrated that 5-aminoimidazole-4-carboxamide ribonucleoside (AICAr), a commonly used AMP-activated kinase (AMPK) modulator, induced monocytic differentiation in U937 monocytic leukemia cell line in an AMPK-independent manner. The aim of this study was to determine the metabolic pathways necessary for AICAr-mediated differentiation. Here, using metabolic assays, flow cytometry and LC/MS/MS analyses we showed that AICAr-mediated differentiation was independent of the known metabolic effects of AMPK, including glycolysis, but instead depended on glutamine metabolism and pyrimidine synthesis. The addition of nucleosides or uridine alone completely abolished AICAr-induced differentiation and arrest of U937 cells in the S-phase of the cell cycle. LC/MS/MS analysis revealed that AICAr increased the level of orotate and decreased the level of uridine monophosphate (UMP) consistent with inhibition of UMP synthesis, at a step downstream of dihydroorotate dehydrogenase (DHODH). Low concentrations of AICAr and brequinar, an inhibitor of DHODH, had synergistic effects on differentiation and S-phase arrest. Similar effects of AICAr were observed in other cell lines, including monocytic MOLM-14 and THP-1, where they were also uridine dependent. In conclusion, AICAr induces differentiation via inhibition of UMP-synthesis and pyrimidine depletion.

This work has been funded by Croatian Science Foundation under the projects IP-2016-06-4581 (to D.V.), and DOK-2018-01-9599 by the European Union through the ESF Operational Programme Efficient Human Resources 2014-2020, and co-financed by the Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience (project “Experimental and clinical research of hypoxic-ischemic damage in perinatal and adult brain”; GA KK01.1.1.01.0007 funded by the European Union through the European Regional Development Fund).

Poster number 06

EOMES BROADENS THE SCOPE OF CD8 T CELL MEMORY BY INHIBITING APOPTOSIS IN LOW-AFFINITY CELLS

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The memory CD8 T cell pool must select for high-affinity clones to efficiently counter re-infection yet must retain a level of clonal diversity to allow recognition of pathogens with mutated immunodominant epitopes. How the level of diversity within the memory pool is controlled is unclear, especially in the context of a selective drive for antigen-affinity. We find that low-affinity memory formation depends on the transcription factor Eomes in the first days after antigen encounter. Eomes is induced at low activating signal strength and directly drives transcription of the pro-survival protein Bcl-2. At higher signal intensity T-bet is induced which suppresses Bcl-2 and causes a relative survival advantage for low-affinity cells. High-affinity clones form memory largely independent of Eomes and have a proliferative advantage over low-affinity cells, which causes them to be dominant in the memory pool, despite their relative survival deficit. Genetic or therapeutic targeting of the Eomes/Bcl-2 axis reduces the clonal diversity of the memory pool, which increases its specificity and reactivity against the original pathogen but reduced its ability to respond to viral mutants. Thus, we demonstrate on a molecular level how sufficient diversity of the memory pool is established in an environment of affinity-based selection.

Poster number 28

ORAL PRESENTATIONS
Session 4

THE DIFFERENCES IN TREG CELLS SUBPOPULATIONS AMONG JUVENILE IDIOPATHIC AND REACTIVE ARTHRITIS PATIENTS

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BACKGROUND: Juvenile idiopathic arthritis (JIA) is characterized by chronic joint inflammation lasting longer than six weeks as opposed to the acute reaction in reactive arthritis (ReA) that develops in response to an infection, lasts shorter and usually ends with full resolution of symptoms. The objective of this study was to examine the differences in occurrence of regulatory T (Treg) and regulatory B (Breg) cells, type 3 innate lymphoid cells (ILC3) and Th17 derived Th1 cells in JIA and ReA patients.

METHODS: Treg cells, Breg cells, ILC3 and Th17 derived Th1 cells were analyzed in whole blood of ten JIA and six ReA patients by flow cytometry using directly conjugated monoclonal antibodies. The blood samples were collected during the first visit to Pediatric Rheumatology Clinic in Sestre milosrdnice University Hospital Center in Zagreb, Croatia, while the final diagnosis of JIA or ReA was determined three months after. Juvenile arthritis disease activity score (JADAS-CRP) for each patient was calculated during each visit. The median ages of the JIA and ReA patients were 6.41 and 7.22 respectively.

RESULTS: In patients with JIA, the CD3+CD45+CD25+CD4+CD127-CD28- subpopulation of Treg cells was significantly abundant compared to ReA patients (P=0.04). No other significant differences in cell subpopulations among different patient groups were observed.

CONCLUSION: This proof-of-concept study has shown that patients with JIA have significantly higher levels of anergic Treg cells in peripheral blood than those with ReA, which could possibly result in failure of their immunosuppressive effect and development of a chronic disease.

Poster number 03

EXPRESSION OF NKG2C AND TIGIT ON CD56^{DIM}CD16+ NK CELLS IN PATIENTS WITH HEMORRHAGIC FEVER WITH RENAL SYNDROME

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Hemorrhagic fever with renal syndrome (HFRS) is an emerging rodent borne disease caused by negative-sense single-stranded RNA orthohantaviruses (HTV). Clinical characteristics of HFRS infection are acute kidney injury, thrombocytopenia, hemorrhages and hypotension. HTV causes complex immune reactions, which contributes to the pathogenesis. The liver is involved in HFRS pathogenesis, with no enough research evidence about it so far.

Natural killer (NK), are found to be activated in HFRS patients. In Cytomegalovirus seropositive HFRS patients, activating receptor NKG2C positive CD56^{dim}CD16+ NK cells were persistently elevated throughout 60 days after onset of symptoms.

Our aim was to explore some phenotype changes in NK cells with special focus on TIGIT and NKG2C receptors and their relation to HFRS clinical parameters.

We collected blood samples from 15 HFRS patients during early acute (upon hospital admission) and late acute (at discharge) stage of disease, and 9 healthy controls.

Using flow cytometry we analysed CD56^{dim}CD16+ NK cells. We observed higher percentage of this population in patients with severe clinical picture compared to patients with mild disease. In addition, percentage of NKG2C positive cells was positively correlated with percentage of inhibitory receptor TIGIT positive cells during the early stage of disease. Interestingly, low percentage of both parameters were detected in patients with hepatomegaly and significantly high percentage in patients with no hepatomegaly.

Our results suggest possible important role of NK cells driven immune reaction in liver in HFRS patients and special research focus should be directed to the liver involvement in HFRS pathogenesis in the future

Poster number 05

URBAN-RURAL DIFFERENCES IN CONTINENTAL CROATIA

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BACKGROUND: According to biodiversity hypothesis, living in rural environment promotes immune balance and protects from IgE-mediated allergic disorders. Around 50% of schoolchildren express skin sensitivity to at least one inhalant allergen, but the majority of them are asymptomatic. The aim of this study was to compare skin sensitivity in correlation with the disease expression in two populations; urban (city of Zagreb) and rural (Lonjsko Polje).

METHODS: 200 children, 7-14 years old, were randomly selected. Their parents answered questions on allergic symptoms, based on the original ISAAC (International Study of Allergy and Asthma in Childhood) questionnaire. Skin prick tests with common inhalant allergens (Dermatophagoides pteronyssimus, Dermatophagoides farinae, grasses, Ambrosia artemisiifolia, early, late blooming trees, cat dander) were performed using standard procedure. The result was considered positive if the diameter of the wheal was 3 mm or greater. A histamine solution 10 mg/ml as positive control and negative control were used.

RESULTS: There was a significantly higher prevalence of allergic sensibilization to grasses (27,77% versus 9,42%, $\chi 2$ 14,95, $p<0.001$) and early blooming trees (18,01% versus 9,42%, $\chi 2$ 3,86, $p<0.049$) in children living in Zagreb than in those from Lonjsko polje. Positive skin prick tests were good predictors for all three variables of allergic rhinitis (past and current sneezing, medical diagnosis of rhinitis) in the urban (p 0.001-0.022), but not in the rural environment. Nonparametric analysis showed significantly higher ″atopy index″ (Z -2.18, p 0.029) and number of positive skin prick tests (Z -2.05, p 0.04) in the city of Zagreb. Positive skin prick tests were good predictors only for the past atopic eczema in Lonjsko polje (p 0.035).

CONCLUSION: Results confirm changes of immunotolerance to inhalant allergens, with more prevalent positive skin tests and allergic symptoms in the city of Zagreb, and strong connection of positive skin tests with allergic rhinitis. The results should be implemented in the future Croatian strategy against rapid urbanization.

Poster number 13

INVITED LECTURE – Strategic Session

PROJECT ACQUISITION AND MANAGEMENT IN CROATIA: A STRATEGIC PERSPECTIVE

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One of the primary concerns of the senior researcher is the acquisition of sufficient funds to sustain people, consumables and infrastructure. Whereas the ultimate purpose of a grant application is the same (getting money), the requirements may differ greatly between countries, financing bodies and different calls. For example, a national fundamental scientific grant should be set-up differently than a infrastructural grant from the European Union. Not only from the perspective of documents required, but also from the angle in which an application should be written in order to convince the financing body to provide the funds. Similarly, management and reporting of grants can vary greatly between different grants. In this lecture, I will provide some insights from several decades of experience with national and international grant application and management in Croatia. Its purpose is to aid upcoming group leaders to aid in their perpetual struggle to obtain funds for their research group.

POSTER PRESENTATIONS

THE PRESENCE NKG2A, NKG2C AND NKG2D POTENTIAL RECEPTORS FOR HSP70 IN SYNOVIAL TISSUE OF PATIENTS WITH OSTEOARTHRITIS

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INTRODUCTION: The stress-inducible heat shock protein 70 (HSP70) is frequently upregulated in a disease state, therefore it increases in chondrocytes, synovial fluid and sera of patient with osteoarthritis (OA) comparing to healthy control. HSP70 relates to joint inflammation and severity of OA, as well as to the infiltration of synovial tissue with NK cells.

AIM: To analyze the presence and distribution of NK cell receptors of C type lectin family, as potential receptors for HSP70 in synovial tissue.

MATERIAL AND METHODS: Mature synovial tissue samples were obtained during the knee alloarthroplasty. NKG2A, NKG2C and NKG2D were identified in paraffin-embedded tissue sections by immunofluorescence. Two independent observers counted the cells labeled with particular antibody in five different synovial samples at field magnification x400 of the fluorescent microscope and expressed the results as average ± standard deviation of the number of positive cells.

RESULTS: Mature synovial tissue contains 46 ± 13 NKG2A expressing cells, 38 ± 18 NKG2C expressing cells and 28 ± 16 NKG2D expressing cells per field at magnification x400. All three receptors were found in synovial lymphocyte accumulations. Additionally, NKG2A and NKG2C were scattered in sublining synovial tissue, while NKG2D was present on the surface of synovial shoots. The distribution of the receptors resembles to the distribution of synovia NKp46+ NK cells.

CONCLUSION: The presence of NKG2A, NKG2C and NKG2D expressing cells in synovial tissue suggests that they are potentially able to bind HSP 70 and directly support local pro-inflammatory response.

University of Rijeka supported the research by the grants No. Uni-ri-biomed-18-110 to Professor G. Laskarin and No. Uni-ri-biomed-18-160 to Professor T. Kehler.

Poster number 02

THE EXPRESSION OF CD4 AND CD8 IN VARIOUS MOLECULAR TYPES OF BREAST CANCER

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INTRODUCTION: Breast carcinoma is the most common malignant disease in the female population and one of the leading causes of death among women worldwide. At present, various breast tumor classification systems are utilized, more commonly adopted are the TNM classification and immunohistochemistry classification. Hormone receptors-positive carcinomas are the less malignant and have a better clinical prognosis, whereas HER-2 positive and triple negative carcinomas are more aggressive with greater malignancy and a poorer prognosis.

MATERIAL AND METHODS: We conduct an observational retrospective study, processing and analysing patient tissue samples from the archives of the Department of Pathology and Pathological Anatomy (University Hospital Rijeka). The onset of analysis began, with previously immunophenotyped tissue biopsies of the various groups of carcinomas (Luminal A, Luminal B HER2-, Luminal B HER2+, HER2+, triple negative), using “tissue microarray” and later, immunohistochemistry staining of these samples was be performed (using anti CD4 and anti CD8 monoclonal antibodies). After that the number of CD4 and CD8 positive cells was quantified microscopically and the statistical analysis was done using student-t test. These results were compared with clinical data associated with the course and outcome of the disease (age, recurrence of local disease, metastasis and lethal outcome) using hospital’s computer information system.

RESULTS: We have found the lowest CD4 and CD8 expression in the Luminal A and Luminal B HER2- breast cancers group, the highest CD4 and CD8 expression was in HER2+ and tripple negative breast cancers group among the molecular subtypes. The statistical analysis of the results have shown the statistical significance difference among the CD4 and CD8 expression in the HER-2 positive and triple negative breast cancers compared to hormonal positive breast cancers (luminal A group) – $p<0,05$. The women in group of HER-2 positive and triple negative breast cancers had poor prognosis (short time to metastasis and recurrence of the disease) when compared to group of luminal A breast cancers that had better clinical outcome.

CONCLUSION: The goal of this study was to show the difference in the expression of CD4 and CD8 positive cells” among the different groups of breast carcinomas and to incorporate the obtained results with the clinical course and disease outcome in order to gain a better understanding of the clinical and pathological behaviour of various breast cancer immunophenotypes.
This work has been fully supported by the University of Rijeka under the project number 18.07.2.1.02

Poster number 04

THE ANALYSIS OF THE IMPACT OF OMALIZUMAB TREATMENT ON QUALITY OF LIFE IN PATIENTS WITH CHRONIC URTICARIA/ANGIOEDEMA

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INTRODUCTION AND OBJECTIVES: Chronic urticaria/angioedema (CU/AE) usually represents a therapeutic problem, primarily due to the unpredictability of presentation, decreased patient' quality of life and social isolation. The aim of our study was to investigate the impact of omalizumab treatment on quality of life in patients with CU/AE.

MATERIALS AND METHODS: We analyzed the effect of omalizumab treatment in 15 patients with CU/AE during the period of six months with assessment of their quality of life by Dermatology Life Quality Index, both initially and after the sixth administration. The recommended omalizumab (300 milligrams) was administered subcutaneously every four weeks and we determined the impact of CU on each factor: from 0 (not at all) to 3 (very much) – and scores are totalled to give an overall DLQI score from 0-30.

RESULTS: According to our results, omalizumab treatment significantly improved quality of life in the majority of patients with CU/AE. Initial DLQI scores before administration of omalizumab were between 6 and 25, mean DLQI score was 13.4. DLQI scores after sixth administration of omalizumab were between 0 and 9, mean DLQI was 2.3.

CONCLUSIONS: In the majority of patients, omalizumab provided rapid symptom control and reduced the need for systemic corticosteroids, thereby significantly improving the quality of life in patients.

Poster number 29

THE CONTRIBUTION OF COSTIMULATORY MOLECULES IN MEMORY CD8 T CELL INFLATION IN MICE INFECTED WITH MOUSE CYTOMEGALOVIRUS

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Cytomegalovirus (CMV) establishes a lifelong infection of its host, due to the ability of the virus to establish latency. CMV infection in human and mice induces an atypical CD8 T cell response where large numbers of effector-like memory CD8 T cells are induced and continue to expand in blood and peripheral tissues in the latent phase of the infection, a process termed „memory inflation“. The mechanisms underlying inflation of certain CMV-specific CD8 T cells are still poorly understood so we aimed to determine the contribution of costimulatory molecules in CD8 T cell response upon mouse CMV (MCMV) infection and their role in CD8 T cell inflation. We infected WT C57BL/6 mice with WT MCMV or recombinant MCMV viruses lacking viral proteins that negatively regulate expression of different CD8 T cell costimulatory molecules and recombinant viruses expressing cellular ligands for CD8 T cells costimulatory receptor NKG2D, RAE-1y and MULT-1 and followed CD8 T cell response over time. Our preliminary results show that upon infection with these recombinant viruses, a higher frequency of memory precursor effector cells (MPECs) is established early during infection and we will investigate whether this MPEC pool is a source of inflationary cells in peripheral tissues.

Poster number 15

ACTIVATION STATUS, TOLL LIKE RECEPTOR 4 AND CD91 EXPRESSION OF SYNOVIAL TISSUE MACROPHAGES IN PATIENTS WITH MATURE OSTEOARTHRITIS

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The quantity of activated macrophages in synovial tissue is associated with radiographic severity of knee osteoarthritis (OA) and joint symptoms. We aimed to investigate activation status, Toll-like receptor (TLR) 4 and CD91 expression of synovial tissue macrophages in patients with mature osteoarthritis. We sampled synovial tissue during the knee alloarthroplasty. Interleukin 15, interferon-gamma, TLR 4, CD91, lineage and activation markers were labelled by immunohistology and/or immunofluorescence in paraffin-embedded serial tissue sections. Three investigators counted CD68+ cells in synovial sections independently under the microscope.

The average number of synovial CD68+ cells per microscope field x400 was 35 $\pm$ 21 with no statistically significant difference in the numbers among observers. CD68+ macrophages surround the vessels and spread toward the surface of synovial shoots. Their distribution was similar to the distribution of CD14, CD91 and TLR 4. The cells expressing CD68, HLA-DR and interleukin-15 appoint in synovial lymphocyte accumulation, nearby CD3+ and CD56+ cells. Synovial tissue of OA patients nests interferon-gamma+ cells in certain parts.

Conclusion: Peripheral blood derived CD68+ synovial macrophages, equipped with TLR4/CD14 and CD91 pattern recognition receptors possibly interact with synovial fluid antigens, which might support their M1 maturation characterized by intense interleukin-15 and HLA-DR expression, as well as their interaction with synovial lymphocytes, particularly in the parts rich with interferon-gamma.

University of Rijeka supported the research by the grants No. Uni-ri-biomed-18-110 to Professor G. Laskarin and No. Uni-ri-biomed-18-160 to Professor T. Kehler.

Poster number 33

IMMUNOPHENOTYPING OF CHEMOKINE RECEPTORS ON PERIPHERAL BLOOD MONONUCLEAR CELLS IN CHILDREN WITH TYPE 1 DIABETES

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INTRODUCTION: Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by insulin deficiency, hyperglycemia and metabolic disturbances. Chemokines contribute to low-grade inflammation, which predisposes to the development of vascular complications. Our aim was to determine the chemokine receptor phenotype on peripheral blood mononuclear cells (PBMCs) in children with T1D.

METHODS: Mononuclear cells were isolated from peripheral blood of healthy controls (n=13) and children with T1D (n=22). B-cell (CD19+), T-cell (CD3+) and monocyte (CD14+) phenotype was determined using flow cytometry for the following chemokine receptors: CCR2, CCR4, CXCR3 and CXCR4. Frequencies of PBMC subpopulations expressing chemokine receptors were correlated with clinical parameters indicating disease activity (HbA1c and insulin dose), vascular complications (blood pressure and lipid profile) and inflammation (CRP and fibrinogen).

RESULTS: Major immune population frequencies did not differ between groups, however there was a decrease of CCR2+ monocytes in T1D group (ρ=0.019) as well as expansion, though non-significant, of CCR4+ T-cells. Expression of CXCR3 and CXCR4 on PBMCs was similar among T1D patients and controls. Monocytes expressing CCR2 were negatively associated with HbA1c levels (ρ=-0.459) and T-cells expressing CCR4 were negatively associated with systolic blood pressure (ρ=-0.389).

CONCLUSION: Because of the migratory role of CCR2, we propose the decrease in CCR2+ monocyte subpopulation is due to the increased peripheral sequestration that contributes to the disease pathogenesis and its vascular complications. In contrast, T-cells expressing CCR4 may have a protective role and are therefore, decreased, in children with vascular complications.

SUPPORT: Croatian Science Foundation projects IP-2018-01-2414, IP-2014-09-7406 and DOK-2018-09-4276.

Poster number 20

THE ROLE OF *MID1-PP2A AXIS* IN INFLAMMATION-INDUCED JOINT DAMAGE IN ANTIGEN-INDUCED ARTHRITIS

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INTRODUCTION: Rheumatoid arthritis (RA) is a chronic autoimmune joint disease which often causes structural joint damage not reversible by currently available therapeutics, urging discovery of new molecular mediators for therapeutic targeting. In a murine model of RA, antigen-induced arthritis (AIA) we have previously shown that destructive AIA is characterized by accumulation of synovial myeloid cells which up-regulate *Mid1* gene. Due to the previously described involvement of *Mid1* in pathogeneses of immune-mediated diseases, the objective of this study was to evaluate the role of *Mid1* in AIA.

MATERIALS AND METHODS: AIA was induced in C57BL6 mice by immunization with methylated bovine serum albumin (mBSA) and subsequent intra-articular injection of mBSA. Expression of *Mid1* and pro-inflammatory cytokines was assessed by qRT-PCR. Mice were treated with metformin, which prevents Mid1-mediated ubiquitinilation of PP2A, and thus has an anti-inflamatory effect, at a daily dose of 1g/kg, to evaluate the effects of blocking the Mid1-mediated inflammation on arthritis development and subchondral bone resorption (assesed by μ-CT).

RESULTS: *Mid1* was up-regulated in knee joints early after arthritis induction. Its expression positively correlated with severity of arthritis assesed by knee diameter measurement and levels of pro-inflammatory cytokines, IL-1, IL-6 and TNF. Although Mid1 is positioned on the X-chromosome, its expression was up-regulated in arthritic joints of both male and female mice. Metformin treatment ameliorated the severity of arthritis and arthritis-induced subchondral bone resorption.

CONCLUSION: Inhibition of Mid1-mediated PP2A degradation by metformin might aid to therapeutic management of inflammatory arthritides.

SUPPORT: Croatian Science Foundation projects IP-2014-09-7406, DOK-2015-10-9897, IP-2018-01-2414.

Poster number 31

INDUCTION AND CHARACTERIZATION OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE) IN PROGESTERONE RECEPTOR DEFICIENT BALB/C MICE

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A growing body of evidence point that progesterone (P) exerts neuroprotective and immunomodulatory effects in addition to his "canonical" reproductive functions. However, given the large progesterone receptor (PR) pleotropism and redundancy of P and its metabolites, as well as marked tissue specific activity, molecular mechanisms of neuroprotective P effects are very poorly understood.

In this study, we provide the first evidence that PR deficiency attenuates resistance to experimental autoimmune encephalomyelitis (EAE) in BALB/c mice. After challenge with $MOG_{35–55}$ peptide, in contrast to wild type (WT) BALB/c mice, progesterone receptor knock out (PRKO) mice developed clinical picture of EAE. Besides typical signs (tail and hindlimbs paresis / paralysis), immunized PRKO mice also showed weakness of the forelimbs, with substantially pronounced tremor.

Immunohistochemical analysis of CNS tissue obtained at 16th post-immunization (p.i.) day revealed massive demyelinating lesions with inflammatory infiltration and gliosis throughout the brain parenchyma and lumbar spinal cord. Moreover, such pathological changes were also found in the thoracic and cervical spinal cord, which are typically spared in EAE susceptible C57BL/6 mice.

Interestingly, systemic production of classic proinflammatory cytokines (TNFα, IL-6 and IL-12), assessed by qPCR in spleen tissue at 16th p.i. day, was downregulated in both, PRKO snd WT mice. However, immunization in WT mice significantly downregulated COX2 expression, which, in contrast, was increased in immunized PRKO mice.

Results indicate neuroprotective and immunomodulatory roles of PR through the modulation of COX2 expression in lymphatic tissue. Data also underline involvement of COX2 gene repression in inherent resistance to EAE.

This work has been fully supported by the University of Rijeka under the project uniri-biomed-18-187 1341 (Neuroprotective role of progesterone receptors).

Poster number 14

NKG2D SETS ACTIVATION THRESHOLD FOR NCR1 EARLY IN NK CELL-DEVELOPMENT AND CONTROLS SENSITIVITY OF CANCER IMMUNE-SURVEILLANCE

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NK cell activation depends on a change in the balance between signals from inhibitory and activating receptors. The threshold values at which changes in this equilibrium drive NK activation are thought to be set by inhibitory receptor engagement during development. Here, we elucidated how the activating receptor NKG2D controls NK cell responsiveness. NKG2D specifically sets the activation threshold for NCR1 in a process that requires DAP12, but not DAP10. As a result, Klrk1-/- mice better control tumors and cytomegalovirus infection through NCR1-induced IFN-γ production. NKG2D expression before the immature NK cell stage permanently increases protein levels of CD3ζ. Reduced CD3ζ expression in Klrk1-/- mice was associated with enhanced NCR1 signal transduction and CD3ζ deficiency resulted in hyper-responsiveness to NCR1 stimulation. Our findings show for the first time how an activating receptor developmentally controls activity of another activating receptor on NK cells and determines the sensitivity of cancer immuno-surveillance.

Poster number 12

INSULIN RECEPTOR-DEFICIENCY ON CD8 T CELLS DOES NOT AFFECT MEMORY RESPONSES

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CD8 T cells are essential players in adaptive immunity as they protect our body from the occurrence and recurrence of intracellular pathogens and tumors. Recent studies reported an unexpected role of endocrine hormones for their function, as insulin was shown to promote effector T cell responses against viral infection. The role of insulin in the formation and maintenance of memory CD8 T cell responses remained unclear. Here, we investigate how insulin receptor-deficiency on CD8 T cells impacts memory formation and function upon viral infection. CD8 T cells lacking the insulin receptor showed an equal ability to generate memory precursor cells compared to WT cells, following infection of mice with cytomegalovirus. Moreover, the frequencies of central and effector memory cells were comparable both at early and late time points after infection. CD8 T cell-specific insulin receptor deficiency did not impair the ability of memory cells to produce cytokines upon in vitro re-stimulation. Importantly, recall capacity of these cells was not affected since there were no observed differences in cell numbers, activation or cytokine production, nor was there a difference in glucose uptake after secondary infection. Altogether, these results suggest a redundant role of insulin receptor-mediated stimulation in the formation and functionality of memory CD8 T cells.

Poster number 22

COMPARISON OF TWO DOWNSTREAM PROCESSES FOR EFFICIENT AND SUSTAINABLE ANTIVENOM PREPARATION IN TERMS OF YIELD, FINAL PRODUCT PURITY AND VIRUS-INACTIVATING POTENTIAL

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Snakebite envenoming has recently been recognized by a WHO as a highly relevant public health issue. The only validated treatment is immunotherapy with animal-derived antivenoms. Since these life-saving medications have been out of the mainstream of pharmaceutical development/manufacture for decades, number of drawbacks pertaining to their availability, safety and efficacy is becoming increasingly evident. One of the main reasons is associated with low sustainability of current productions and technological innovation is of great need. We have developed two highly efficient antivenom downstream processing strategies at the laboratory scale. Both involve caprylic acid fractionation of horse plasma for purification of IgGs, their pepsin-mediated digestion and final polishing by flow-through chromatography, but differ in number of processing steps which reflects on performance complexity.

Here we provide comparison of two processes in terms of the final product quality. Both F(ab')2-based antivenoms were of high purity (100 and 97%), proving comparable to the purest currently available on the regulated market. As contaminants, minor traces of IgM, transthyretin and inter-alpha-trypsin inhibitor were identified. Each preparation was free from aggregates. Prevention of viral transmission as safety assuring requirement was assessed on mumps and measles vaccine strains. Their infectivity was reduced by more than 5 log during caprylic acid-involved processing steps. Additional inactivation was achieved by digestion step, which appeared more efficient for measles (4.6 log) than for mumps virus (2.6 log).

High purity of the experimental antivenoms, aggregate absence and the virus-inactivating power of processing strategies, together with satisfactory yields, support their further development towards clinics.

Poster number 17

SENSITIVITY OF ACUTE MYELOID LEUKEMIA PRIMARY CELLS TO AICAR-MEDIATED DIFFERENTIATION

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Differentiation therapy is a promising treatment strategy for treating acute myeloid leukemia (AML) and most successful example is all-*trans*-retinoic acid (ATRA)-based therapy of acute promyelocytic leukemia (APL). Nucleotide biosynthesis pathway has been recently identified as a novel differentiation target in AML and the inhibitors of dihydroorotate dehydrogenase (DHODH), a fourth step in de novo pyrimidine synthesis, are currently being tested in Phase I clinical trials. Our previous work demonstrated that 5-aminoimidazole-4-carboxamide ribonucleoside (AICAr) induced differentiation of monocytic leukemia cell line U937 by inhibiting UMP synthesis at the step downstream of DHODH. The aim of this study was to test the effects of AICAr on proliferation and differentiation of mononuclear cells isolated from the bone marrow of patients suffering from de novo AML. In a set of bone marrow samples from 16 patients with non-APL AML grown *in vitro* with the addition of IL-3, IL-6, SCF and FLT3L, AICAr induced a significant decrease in viability as measured by MTT assay. In one sample (FAB-M2, normal male karyotype, $FLT3^{wt}$ $NPM1^{wt}$, primary refractory), the addition of AICAr resulted in a pronounced increase in the expression of differentiation markers CD11b and CD64 as well as accumulation of cells that morphologically resembled macrophages. An increase in the levels of differentiation markers in this sample was observed in response to brequinar, but not ATRA. In conclusion, AICAr exhibits profound antiproliferative effects in primary AML samples *in vitro*. Preliminary data suggest for the existence of a potential subpopulation of AML patients in which both AICAr and brequinar could induce differentiation.

This work has been funded by Croatian Science Foundation under the projects IP-2016-06-4581 (to D.V.), and DOK-2018-01-9599 by the European Union through the ESF Operational Programme Efficient Human Resources 2014-2020, and co-financed by the Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience (project “Experimental and clinical research of hypoxic-ischemic damage in perinatal and adult brain”; GA KK01.1.1.01.0007 funded by the European Union through the European Regional Development Fund).

Poster number 25

MCMV INFECTION AS AN AGGRAVATING FACTOR OF DIABETIC NEPHROPATHY

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Diabetic nephropathy as a complication of diabetes mellitus type II affects 20-40% of patients. It is described by progressive loss of nephronal function which can progress to renal failure. The underlying cause of diabetic nephropathy is hypertension and hyperglycemia but the molecular mechanism that drives the most prominent histopathological changes such as mesangial matrix expansion, tubulointerstitial fibrosis and podocyte loss remain to be determined. Proinflammatory mediators are known to aggravate DM2-associated pathologies. For instance, using a mouse model of DIO our group has shown that infection induces loss of glycemic control in subjects at risk for developing DM2. Here we seek to elucidate whether viral infection also directly affects the severity of diabetic nephropathy. First, we investigated the histopathological changes in kidneys of mice which were put on normal chow (NCD) or high fat diet (HFD), and subsequently infected with MCMV or left uninfected. HE staining of the kidneys revealed that juxstamedullary glomeruli were significantly bigger in the HFD fed MCMV infected group of mice exclusively. In addition, podocyte synaptopodin staining obtained significantly weaker signal in infected HFD fed mice in comparison to the controls. This finding was further confirmed by quantitative PCR. ELISA and western blot analysis of albumin in urine, however, showed only mild levels of albuminuria in HFD fed groups of mice. Nevertheless, immunohistological staining of smooth muscle actin showed significantly more foci of tubulointerstitial fibrosis in infected HFD fed mice in comparison to the infected mice on NCD.

Poster number 19

COMPARATIVE STUDY OF DIFFERENT WHOLE IGG SNAKE ANTIVENOM REFINEMENT STRATEGIES AND THEIR IMPACT ON THE PURIFIED IGG FEATURES

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The main treatment for snake bite envenoming is antivenom consisting of antibodies obtained from hyperimmunised animal plasma. Still, the "refined" preparations commonly cause clinical side effects attributable to contaminating proteins and/or aggregates. According to the literature, certain purification methods induce conformational changes of IgG molecules making them more prone to aggregation while others, leaving them in solution throughout, don't have such an impact. The aim of this study was to verify this hypothesis by comparing IgGs isolated by five different protocols with regard to stability, purity and immunoglobulin subclass composition. The fractionation methods were: ammonium sulphate precipitation (ASP), anion (AEC) and cation (CEC) exchange chromatography, affinity chromatography (AC) and caprylic acid precipitation (CAP).The highest purity was achieved by CAP and AC, while the highest aggregates content was observed in samples from AC, CEC and ASP. Contaminating proteins in IgG preparations were identified by mass spectrometry (MS) analysis. The most prominent impact on the subclass composition had AC, causing the highest loss of IgG(T). Less pure IgG fractions were additionally purified by CAP step prior stability study. Pure IgGs had different melting temperatures (Tm) in thermal shift assay, which might be the consequence of diverse subclass composition. One-month storage of IgGs at 37 °C didn't influence either Tm or aggregates content of analysed preparations. The results indicate that different procedures gain IgGs of variable purity and subclass composition which might affect both safety and effectiveness. However, conformational changes during purification procedures might not be the trigger for increased aggregation.

Poster number 21

INTERRELATION OF SYSTEMIC AND OXIDATIVE STRESS ON LEUKOCYTES BY CONSUMPTION OF EGGS ENRICHED WITH N-3 PUFAS

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Previously, we have demonstrated that 3 weeks of n-3 PUFAs enriched eggs intake reduced the level of oxidative stress due to increased blood antioxidative capacity and reduced levels of oxidative stress in leukocytes.In this study, we wanted to determine whether there was an association between an increased oxidative stress in leukocyte subtypes and systemic oxidative stress.

Fifteen young healthy individuals consumed n-3 PUFAs enriched chicken eggs with a daily intake of 403,10 mg n-3 PUFAs.At the beginning and at the end of the study were measured peripheral leukocytes' oxidative stress by flow cytometry with DCF-DA (2',7'-dichlorofluorescin diacetate) assay from whole blood and oxidative stress markers TBARS (Thiobarbituric Acid Reactive Substances) for lipid peroxidation measurement and FRAP (Ferric Reducing Antioxidant Power) for measuremet of antioxidative capacity from serum samples. Levels of intracellular ROS in leukocytes were assessed prior and 30 minutes after PMA (phorbol-12-myristate-13-acetate) mediated induction of ROS production. Correlation analysis was calculated using Pearson's correlation coefficient at 95% confidence of interval (SigmaPlot v11.2,USA).

By consuming n-3 PUFAs enriched eggs, statistically significant positive correlation was found between oxidative stress level in leukocytes and lipid peroxidation before (r=0.621,P=0.0002) and after (r=0.378,P=0.0393) PMA stimulation. Also, positive correlation was observed between oxidative stress level in monocyes after PMA stimulation and TBARS (r=0.363,P=0.0488) and negative correlation with FRAP (r=-0.389,P=0.0335).Not found significant correlation between the level of oxidative stress on leukocytes and FRAP level.

Results suggest that n-3 PUFAs with decreasing level of systemic oxidative stress has significant effect on decreasing leukocytes oxidative stress level and indirectly on reduction of inflammation in circulation.

This work was supported by European Structural and Investment Funds, grant for the Croatian National Scientific Center of Excellence for Personalized Health Care, University Josip Juraj Strossmayer Osijek, #KK.01.1.1.01.0010.

Poster number 24

THE ROLE OF γδT CELLS IN THE DEVELOPMENT OF NAFLD

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Non-alcoholic fatty liver disease (NAFLD) is defined by the accumulation of fat in liver cells that is not caused by alcohol, and is considered as the hepatic manifestation of metabolic syndrome. Whereas in most people NAFLD manifests itself as simple steatosis, in some people the disease progresses to steatohepatitis (NASH), which may lead to cirrhosis and end-stage liver disease. The immunological mechanism that drives liver inflammation in NASH is currently unknown.

Research starting point was to determine mechanisms that are causing inflammatory process and development of liver fibrosis. After we saw in WT mice that SSD diet caused accumulation of fat in hepatocytes and early increase in number of γδ T cells, change of their phenotype that included increase in expression of NKG2D receptor and production of pro- inflammatory IL-17A cytokine the focus of the research was directed towards γδ T cells. To confirm their role we used TCRδ -/- mice and later NKG2D -/- which showed decreased levels of inflammation and fibrosis, but comparable levels of steatosis to WT mice

For our model we used SSD diet that consist of 40% fat, 20% fructose and 2% cholesterol, and is inducing NAFLD in 16 weeks. To confirm efficiency of model and development of NAFLD hematoxylin and eosin (HE) and Sirius red staining was performed on liver sections.

We concluded that initiation and development of NASH is dependent on NKG2D signaling axis, and is largely mediated by T γδ cells.

Poster number 32

SIGNIFICANCE OF PROINFLAMMATORY CYTOKINES IN MONITORING DISEASE ACITIVITY AND RESPONSE TO THERAPY IN JUVENILE IDIOPATHIC ARTHRITIS

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AIM: To define the role of proinflammatory cytokines IL-1α, TNF-α, IL-6, IL-17A, VEGF and IL-22 in serum/synovial fluid in children with juvenile idiopathic arthritis (JIA) in the assesment of disease activity and response to therapy at the beginning, and after three and six months of treatment.

PATIENTS AND METHODS: Study included 30 patients with oligo- and polyarticular JIA. Each patient underwent clinical (JADAS-10 score, ACR Pedi 70 score) and laboratory (classical markers of inflammation (ESR, CRP), cytokines in serum/synovial fluid (which levels were determined by quantitative enzyme-linked immunosorbent assay)) evaluation.

RESULTS: Serum and synovial fluid TNF-α levels showed positive correlation with disease activity at the beginning of the disease and IL-6 serum levels after three and six months after initiation of treatment. Significant positive correlation between IL-6 and VEGF levels with CRP and ESR was found by the analysis of serum cytokine concentrations and classical inflammatory markers. Significant positive correlation was found between cytokine levels of IL-6, TNF-α, VEGF, and IL-17A in synovial fluid with CRP and also IL-6 levels with ESR. Positive correlation was found among serum levels of IL-6 and VEGF and response to therapy after six months.

CONCLUSION: IL-6, VEGF and TNF-α in serum and TNF-α in synovial fluid may serve as markers of disease activity. IL-6 and VEGF serum levels may serve as markers in evaluating treatment efficacy.

Poster number 11

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

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Congenital human cytomegalovirus (HCMV) infection is the most common viral cause of long-term neurodevelopmental sequelae, including mental retardation, microcephaly and sensorineural hearing loss. NK cells have been shown to play an important role in containing cytomegalovirus (CMV) infection and various adaptive features of NK cells in response to CMV infection are recently being increasingly studied. Despite an increasing body of knowledge the involvement of NK-cell mediated immunity in congenital CMV infection is so far largely unknown. Since HCMV is species-specific, we have used a mouse model in which newborn mice are infected with the mouse cytomegalovirus (MCMV). Here we show that perinatal MCMV infection leads to a persistent alteration of transcriptional activity and strongly affects the maturation and function of NK cells. NK cell expression of T-box transcription factor Eomes, critical for NK cell development, was dramatically impaired after infection. The downregulation of Eomes correlated with major changes in the NK cell phenotype, most notably NK cell exhaustion characterized by an impaired NK cell response to different stimuli. This population of NK cells persisted for several months in infected mice indicating that congenital CMV infection shapes NK cell response over long-term period. Altogether, our data indicate that NK cells also strongly affected by the congenital CMV infection.

Poster number 16

ROLE OF NK CELLS IN THE PATHOGENESIS OF CMV INFECTION IN THE OVARY

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

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

Poster number 07

NOVEL MECHANISM OF MHC I MODULATION BY MCMV AND ITS CONSEQUENCES ON NK AND T CELL RESPONSES

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Cytomegalovirus (CMV) is a widely-spread β-herpesvirus that efficiently downregulates MHC I molecules in order to avoid recognition by cytotoxic T cells. However, complete downregulation of MHC I makes infected cells susceptible to NK cells lysis. Therefore, mouse CMV (MCMV) encodes for m04 protein that binds some MHC I molecules in the infected cells, escorts them to the cell surface where they engage inhibitory Ly49 receptors and inhibit NK cell response. Here we show a new MCMV protein, MATp1, that is crucial for mentioned MHC I surface rescue by enabling proper m04/MHC I complex formation. Such MATp1/m04 altered-self MHC I molecules egress to the cell surface and bind to inhibitory Ly49 receptors stronger then MHC I alone. Consequently, NK cell activation is impaired which leads to inability of NK cells to adequately control the virus despite dramatically reduced surface levels of MHC I. Moreover, we show that these MATp1/m04 altered-self MHC I molecules are also specifically recognized by several activating Ly49 receptors indicating that MATp1 has prompted the evolution of virus-specific Ly49 receptors capable of recognizing CMV altered-self MHC I molecules. Interestingly, such MHC I-modulation by MATp1 also negatively affects the virus-specific CD8+ T cell response during MCMV infection.

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