Annual meeting of the Croatian Immunological Society 2020

Online Only

01-2.10.2020

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2020 ANNUAL MEETING OF THE

CROATIAN IMMUNOLOGICAL SOCIETY

Online Only, OCTOBER 1st-2nd 2020

ORGANIZED BY

CROATIAN IMMUNOLOGICAL SOCIETY
University of Rijeka Faculty of Medicine

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Vice President: Alenka Gagro, Zagreb
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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, unfortunately this year online only.

The beginning of 2020 was marked by an event that the world had not seen since the Spanish Flu: a worldwide pandemic with a deadly virus, SARS-CoV-2. Whereas infectious diseases seemed all but forgotten one year ago, almost the entire field of medical science has united in its quest to purge this blight. Since Immunology has always been a strong field of science in Croatia, I am proud to say that the members of HID have played a vital role in both the medical and scientific fight against Corona. **Prof. Alemka Markotić** is one of the leading clinicians in Croatia to battle this disease. In addition, four of our members (**Dr. Beata Halassy, Prof. Astrid Krmpotić, Doc. Ilija Brizić** as well as **myself**) received a unique HRZZ grant dedicated to the research on COVID-19. This is a clear sign that members of HID not only do good science but are also able to deploy their knowledge to address urgent issues of our society. Corona has brought us loss and great suffering, but the members of HID are doing their part to counter this threat.

But the world is still bigger that Corona alone and our scientists have made a great contribution to the field of immunology despite its challenges. Again, our members published their work in top-ranking journals, such as *The Journal of Experimental Medicine*, *Cancer Letters, eLife* and *PLoS Biology*. Moreover, two of our members, Dr. Marko Šestan and Vedrana Jelenčić received the **national award for science**. Finally, I myself was presented with a great honor this year, as I received the **HAZU award for the greatest scientific achievement of 2019 in the field of medical sciences**. These are all evidence that the field of immunology is stronger than ever in Croatia and that the members of HID are spearheading its research.

I do regret that we cannot have a real-live meeting this year. People joining each other in friendship and in a shared passion for immunological research are even more important than the sharing of data. Alas, it was irresponsible to do so. Nevertheless, I hope that we can present you these two days with an interesting program, with great national and international speakers, interspaced with talks of some of our greatest young talents.

Therefore, as always, I do hope that you will all have a splendid meeting!

Felix M. Wensveen

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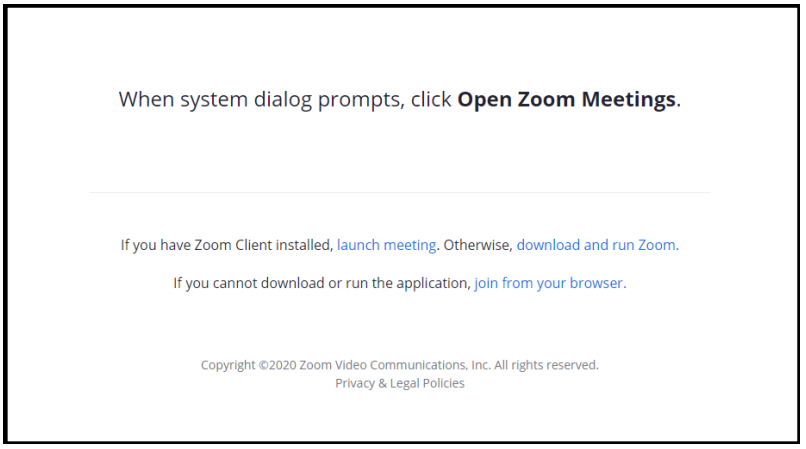
PROTOCOL FOR JOINING THE MEETINGS:

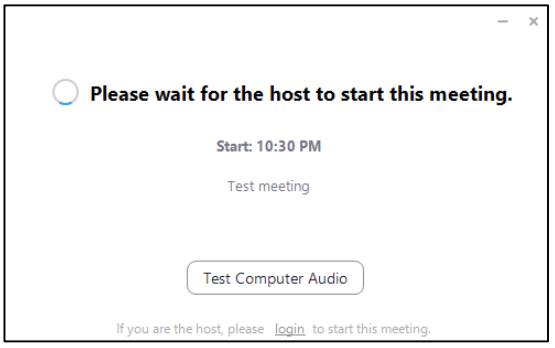
Joining the meeting

The annual meeting this year will be held on the ZOOM platform. In order to join this meeting, click on the meeting links below and published on our website. The following site will open:

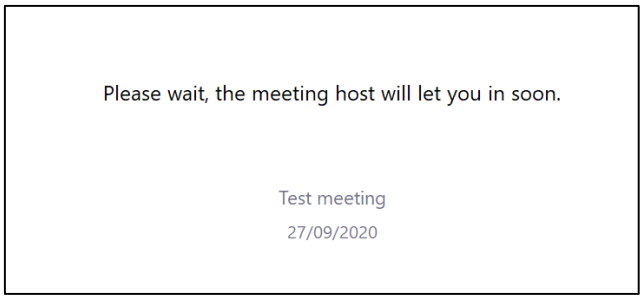


You are advised to install a small program on your computer which allows you to login. This program is automatically downloaded if you click on the meeting links below. You can also do this beforehand on the download page of ZOOM: https://zoom.us/download

There will be two sessions, one on Thursday and one on Friday. We will send an email to all registered participants with these links, but the link to the meeting will also be made available on the website of HID. Once you click the link, you will be prompted to install the program if you have not already done so. If the session has not started yet, the following prompt will appear:



Once the meeting starts, you will first the enter the `waiting room`, with the following window:

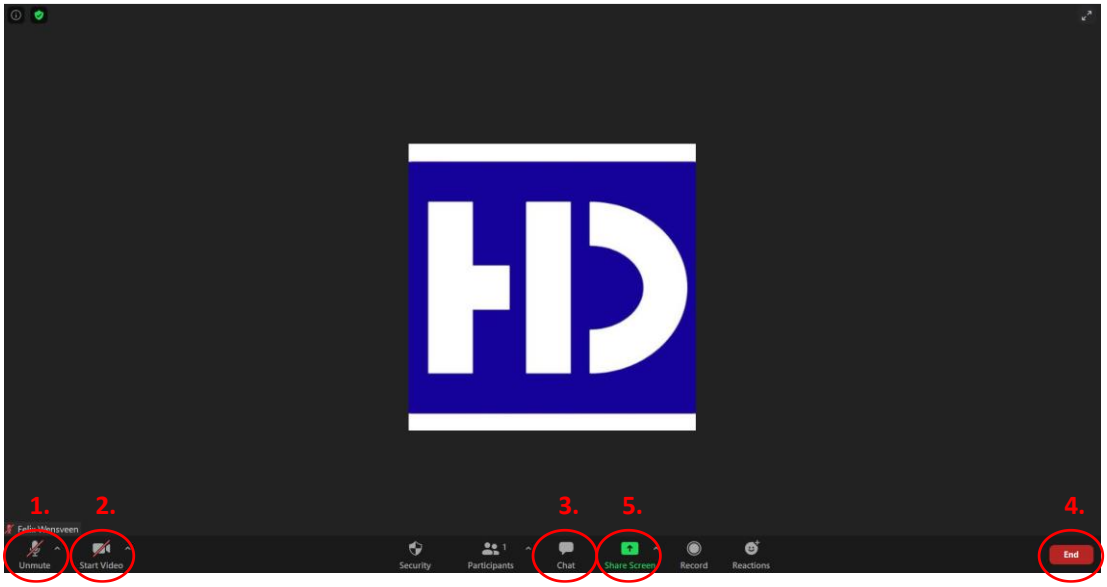


When the meeting hosts (i.e. HID organizers) are ready, they will let you in the meeting.

In the meeting

If you are a participant in the meeting, you can decide to join with or without microphone (1.) and with/without video (2.) by selecting this at the bottom left of the screen. We ask that all participants except the session leader (chair) mute the microphone during the session. At the end of a presentation, the session leader will indicate that it is time for questions. If you have a question, please write 'question' in the meeting chat (3.). The session leader will give you the word. When you want to leave the session, simply click 'end' (4.).

Example of the ZOOM screen:



When presenting

When you are giving a presentation, please join on time for the meeting. Prepare your PowerPoint presentation on your computer. The session leader will indicate that your presentation can start. At that time, you should select 'share screen' (5.), after which you can decide which screen you want

to share. You can share your entire screen, or just the powerpoint that you prepared. Please take care that when sharing your screen, no personal data is visible. Do not forget to unmute your microphone. At the end of your talk, after the questions, select ‘stop share’ at the top of your screen to end your session.

Voting

After the Bright Spark session, we will open a pool with the three names of the speakers. The poll will be open for 5 minutes. Please select the speaker that you considered was best. After you select your choice, the prompt will disappear, and your vote has been counted. The voting is anonymous, and results will be presented at the end of the meeting on Friday 02.10.20 at 15.45h.

Meeting link, Thursday 01.10.2020:

https://us02web.zoom.us/j/86765991054?pwd=TTVIaTcyOVphcFFQQU11NXNjc0Yrdz09

Meeting ID: 867 6599 1054

Passcode: 372456

Meeting link, Friday 02.10.2020:

https://us02web.zoom.us/j/82551339422?pwd=Umw2TGtQLzZRYjhUb1hjS205SXNlUT09

Meeting ID: 825 5133 9422

Passcode: 938132

PROGRAM

THURSDAY October 1st 2020

Location: ZOOM

11:30-11:40 OPENING

Assoc.Prof. Felix M. Wensveen, President of the Croatian Immunological Society

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11:40-13:00 SESSION 1 – Invited lectures & Short Talks

Chair: *Vanda Juranić Lisnić*

11:40-12:15 **Prof. Melanie Brinkmann**

Helmholtz Centre for Infection Research, Braunschweig, Germany

Digging deep into the innate immune response to human cytomegalovirus infection

12:15-12:30 **Marina Babić** - *NK cell receptor NKG2D enforces pro-inflammatory features and pathogenicity of Th1 and Th17 cells*

12:30-12:45 **Ljerka Karleuša** - *Multistep cytokinesis failure in MCMV infection*

12:45-13:00 **Tina Jenuš** - *Molecular and functional characterization of M116 region in mouse cytomegalovirus*

13:00-13:20 BREAK

13:20-14:55 SESSION 2 – Invited lectures & Short Talks

Chair: *Stipan Jonjić*

13:20-13:55 **Prof. Alemka Markotić**

University of Rijeka, Faculty of Medicine, Rijeka, Croatia

COVID-19 – Challenge for Croatia and the World

13:55-14:10	**Agneza Marija Pasini** - *Serum and synovial fluid concentration of vascular endothelial growth factor and ultrasound-detected synovial angiogenesis in juvenile idiopathic arthritis*
14:10-14:25	**Branka Popović** - *Dynamic RNA binding protein interactions to cytokine mRNA govern human T cell effector function*
14:25-14:40	**Mia Krapić** - *IFN-γ produced in infection down-regulates PPAR-γ and changes adipose tissue biology*
14:55-15:10	BREAK
15:00-16:00	SESSION 3 - EFIS-LECTURE AWARD SPEAKER Chair: Felix Wensveen
15:00-16:00	**Prof. Yenan Bryceson** Karolinska Institute, Stockholm, Sweden *Regulation of NK cell differentiation and function*
16:00	END

PROGRAM

FRIDAY October 2nd 2020

Location: ZOOM

11:30-12:00 GENERAL ASSEMBLY OF THE CROATIAN IMMUNOLOGICAL SOCIETY

Chairs: **Inga Kavazović** & **Felix Wensveen**

12:00-12:30 SESSION 4 – Invited Lecture

Chair: Alan Šučur

Prof. Janoš Terzić

Faculty of Medicine, University of Split, Split, Croatia

Bladder cancer – an unfinished immunological story

12:30-13:35 SESSION 5 - `BRIGHT SPARKS` ORAL PRESENTATIONS

Chair: *Bojan Polić*

12:30-12:50 **Mirna Jurković -** *Obtaining a mumps virus with altered fidelity polymerase in order to reduce its virulence*

12:50-13:10 **Daria Kveštak -** *Pathogenic role of NK cells in congenital CMV infection*

13:10-13:30 **Maša Filipović -** *Increased expression of Notch receptors on osteoclast progenitors induced by rheumatoid arthritis*.

13:30-13:35 Voting for the 'Brightest Spark'

13:35-14:00 BREAK

14:00-14:30 SESSION 6 – Invited Lecture & Short Talks

Chair: Alenka Gagro

Prof. Isabelle Meyts, President of ESID

UZ Leuven, Leuven, Belgium

What's new in ADA2 deficiency

14:30-14:45 **Marko Šestan** - *Ménage à trois: Neuro-endocrino-immune regulation of metabolic homeostasis*

14:45-15:00 **Barbara Tomić** - *DNA damage pathway contributes to monocytic differentiation*

15:00-15:15 **Maya Lenartić** - IL-17A producing γδ T cells initiate development of non-alcoholic steatohepatitis through NKG2D engagement on metabolically stressed hepatocytes

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15:15-15:45 SESSION 7 – Invited Lecture

Chair: Danka Grčević

Prof. Sylvia Knapp

Medical University, Vienna, Austria

Protective Type-2 Responses in Pulmonary Immune-Homeostasis.

15:45-16:00 AWARD CEREMONY & CLOSURE

Assoc.Prof. Felix M. Wensveen

INVITED LECTURES

DIGGING DEEP INTO THE INNATE IMMUNE RESPONSE TO HUMAN CYTOMEGALOVIRUS INFECTION

Prof. Melanie Brinkmann, PhD

Technical University Braunschweig, Braunschweig, Germany

Interferon-stimulated gene products (ISGs) play a crucial role in early infection control. The ISG zinc finger CCCH-type antiviral protein 1 (ZAP/ZC3HAV1) antagonizes several RNA viruses by binding to CG-rich RNA sequences, whereas its effect on DNA viruses is largely unknown. Here, we decipher the role of ZAP in the context of human cytomegalovirus (HCMV) infection, a β-herpesvirus that is associated with high morbidity in immunosuppressed individuals and newborns. We show that expression of the two major isoforms of ZAP, the long (ZAP-L) and short (ZAP-S), is induced during HCMV infection and that both negatively affect HCMV replication. Transcriptome and proteome analyses demonstrated that the expression of ZAP decelerates the progression of HCMV infection. SLAM-sequencing revealed that ZAP restricts HCMV at early stages of infection by destabilizing a distinct subset of viral transcripts with low CG content. In summary, this report provides evidence of an important antiviral role for ZAP in host defense against HCMV infection and highlights its differentiated function during DNA virus infection.

COVID-19 – CHALLENGE FOR CROATIA AND THE WORLD

Prof. Alemka Markotić

University of Rijeka, Faculty of Medicine, Rijeka, Croatia

REGULATION OF NK CELL DIFFERENTIATION AND FUNCTION

Prof. Yenan T Bryceson

Karolinska institute, Stockholm, Sweden

NK cells are generally considered a subset of innate cytotoxic lymphocytes initially discovered for their ability to spontaneously recognize and kill tumor cells. They play a role in decidual vascularization during pregnancy and express a number of receptors implicated in recognition and killing of infected cells. In addition, they contribute to regulation of immune responses and maintenance immune homeostasis. My lab studies cytotoxic lymphocytes in relation to human health and disease, in part by studying patients with rare, inherited defects in immunity. We recently uncovered phenotypically diverse subsets of NK cells that emerge in individuals following cytomegalovirus infection. Adaptive NK cells display altered signaling and target cell recognition, as well as decreased responsiveness to innate cytokine cues and increased *in vivo* persistence. To decipher epigenetic landscapes and gain insights into regulation of gene expression and cellular diversity, we have examined the transcriptional and epigenetic profiles of distinct human blood NK cell populations. The *BCL11B* gene, encoding a transcription factor (TF) essential for T cell development and function, was the most extensively regulated TF, with expression increasing throughout NK cell differentiation including adaptive NK cells. Several Bcl11b-regulated genes associated with T cell-signaling were specifically expressed in adaptive NK cell subsets. Regulatory networks revealed reciprocal regulation at distinct stages of NK cell differentiation, with Bcl11b repressing *RUNX2* and *ZBTB16* in canonical and adaptive NK cells, respectively. A critical role for Bcl11b in driving NK cell differentiation was corroborated in patients with *BCL11B* mutations. Together, our findings define TF regulatory circuitry of human NK cells and uncover an unexpected role for Bcl11b in promoting NK cell differentiation and regulating function.

BLADDER CANCER – AN UNFINISHED IMMUNOLOGICAL STORY

Prof. Janoš Terzić

Faculty of Medicine, University of Split, Split, Croatia

Bladder cancer (BC) is the most common malignant disease of the urinary tract. Recurrent high-grade BC carries a serious risk for progression and subsequent metastases accompanied by a high mortality rate. N -butyl-N -(4-hydroxybutyl) nitrosamine induced tumors in mice recapitulate the pathology of human muscle-invasive BC. Activation of the proinflammatory IL-6 / Stat3 axis promotes the development of different cancers by acting on cancer cells and cancer microenvironment. Using a genetic and pharmacological approach in a mouse model, we demonstrated the importance of IL-6 and Stat3 signaling in bladder cancer. Our findings show that inhibition of Stat3 effectively delays the progression and invasiveness of bladder cancer. Moreover, either IL-6 blockade or Stat3 inhibition sensitized bladder cancer to anti-PD-L1 immune therapy. Taken together, our study demonstrates an important role of IL-6/Stat3 signaling in bladder cancer and creates a rationale for testing the therapeutic potential of Stat3 inhibitors in human muscle-invasive BC.

WHAT'S NEW IN ADA2 DEFICIENCY?

Prof. Isabelle Meyts

UZ Leuven, Leuven, Belgium

PROTECTIVE TYPE-2 RESPONSES IN PULMONARY IMMUNE-HOMEOSTASIS.

Prof. Sylvia Knapp
Medical University, Vienna, Austria

Type-2 immune responses within the lungs are best understood for their disease promoting role in allergic diseases like asthma. Increasing evidence highlights the importance of type-2 immune cells in shaping the immune environment, thereby playing instrumental roles in the prevention of unwanted inflammation. I will present our data on the beneficial role of innate type-2 immune cells in the postnatal establishment of pulmonary immune homeostasis and a new finding about the role of the “allergy module” in antibacterial host defense.

ORAL PRESENTATIONS
Session 1

NK CELL RECEPTOR NKG2D ENFORCES PRO-INFLAMMATORY FEATURES AND PATHOGENICITY OF TH1 AND TH17 CELLS

Marina Babic[1,2], Christoforos Dimitropoulos[1], Quirin Hammer[1], Christina Stehle[1], Frederik Heinrich[3], Mir-Farzin Mashreghi[3], Nicola Gagliani[4], Samuel Huber[4], Bojan Polic[5], Chiara Romagnani[1,2]

[1]Innate Immunity, German Rheumatism Research Centre – a Leibniz Institute, Berlin, Germany
[2]Division of Gastroenterology, Infectiology and Rheumatology, Medical Department I, Charité-Universitätsmedizin Berlin, Berlin, Germany
[3]Therapeutic Gene Regulation, German Rheumatism Research Centre – a Leibniz Institute, Berlin, Germany
[4]Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[5]Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia

Effector functions of T helper (Th) cells can be shaped not only by receiving the T cell receptor and costimulatory signal but also by sensing cytokine cues or signals transmitted via a myriad of activating receptors. NKG2D is a molecular sensor of stressed cells expressed on different subsets of innate and adaptive lymphocytes. Despite its established role as potent stimulator of the immune system, particularly of NK and CD8+ T cells, NKG2D-driven regulation of CD4+ Th cell-mediated immunity remains unclear.

By fate mapping of Il17a-expressing cells in a mouse model of antigen-induced arthritis (OIA), we could show that under inflammatory conditions, NKG2D enriched the population of T-bet-expressing Th17 cells. During arthritis, NKG2D was associated with modulated expression of GM-CSF and IFNγ in antigen-specific Th1 and T-bet+ Th17 cells. T cell-specific deletion of NKG2D impaired the ability of antigen-specific CD4+ T cells to promote inflammation during OIA, resulting in significantly reduced knee swelling and tissue immunopathology and improved disease score. Moreover, mice with T cell-deficiency of NKG2D showed significant resistance to the development of experimental autoimmune encephalomyelitis, further highlighting an important role for NKG2D in promoting Th1/Th17 pathogenicity.

Altogether, our results suggest that triggering of NKG2D, by stress-ligands induced during inflammation, modulates the effector functions of Th1 and Th17 cells in vitro and in vivo. We demonstrate that conditional deletion of NKG2D in T cells has a clear impact in vivo in selected inflammatory disease models and imply that NKG2D might serve as an important target for the amelioration of inflammatory diseases mediated by a mixed Th1 and Th17 response.

MULTISTEP CYTOKINESIS FAILURE IN MCMV INFECTION.

Ljerka Karleuša[1], Silvija Lukanović Jurić[1], Natalija Jug Vučko[1], Valentino Pavišić[1], Hana Mahmutefendić Lučin[1,2], Gordana Blagojević Zagorac[1,2],Berislav Lisnić[3], Pero Lučin[1,2]

1Department of physiology, immunology and pathophysiology, Medical Faculty, University of Rijeka, Rijeka, Croatia
2University North, University Center Varaždin, Varaždin, Croatia
3Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

Mouse cytomegalovirus (MCMV) is a member of *Herepesviridiae* family. Once it infects the host cell, it alters its life cycle and functions to promote viral replication and virion production. The infection interferes with normal cellular processes including cell division. Here we investegated the changes that may lead to cytokinesis failure in cells infected with MCMV.

Balb3T3 fibroblasts were infected with recombinant MCMV, Δm138-MCMV, and the samples were taken at different time points during the infection. Intracellular distribution and expression of protein markers was analysed by immunofluorescent confocal microscopy. Percentage of binucelar cells was calculated in comparison to total cell number. Transcriptome analysis of the genes connected to cytokinesis was performed at 3 and 18 hours post infection. Cells infected with MCMV were recorded on Nanolive 3D Cell Explorer-fluo.

MCMV infection leads to formation of binucelar cells in up to 8% of the infected cells during the first hours of infection. Transcritome analysis of MCMV infected cells shows downregulation of several genes responsible for different phases of cytokinesis. Dysregulation of Golgi apparatus can be seen as transformation of Golgi ribbon into Golgi stacks. Probable dysregulation of cleavage plane formation is demonstrated by Arf6 and Epi64 accumulation. The formation of the γ tubulin rings occurs in more copies than in cells that undergo regular cytokinesis with no detectable mitotic spindles.

MCMV infection leads to cytokinesis failure probably by several mechanisms: downregulation of transcription of genes with role in different phases of cytokinesis, dysregulation of Golgi apparatus and dysregulation of cleavage plane formation.

This work was supported in part by the Croatian Science Foundation (grants IP-2014-9-9564 and IP-2019-04-3582 to PL) and by the University of Rijeka (grants uniri biomed-18-88 6546 to PL, uniri biomed-18-180 1333 to HML, and uniri biomed-18-229 1392 to GBZ).

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

Tina Jenuš[1], Vanda J. Lisnić[1,2], Barbara Adler[3], Hana Mahmutefendić Lučin[4], Deni Oreb[1], Ana Vrbanović[1], Pero Lučin[4], Stipan Jonjić[1,2] , Berislav Lisnić[1,2]

[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] Virology Department, Max-von-Pettenkofer Institute, Ludwig-Maximilians University Munich, Munich, Germany
[4] Department of Physiology and Immunology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

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 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 glycosylated protein and have generated a monoclonal antibody that specifically recognizes that protein. Additionally, we have shown that M116 is localized within the virion assembly compartment and it interacts with gH, one of the entry-complex proteins of MCMV. 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. Finally, we have shown that M116 is affecting the spread of MCMV when administered via natural route of infection, intranasally. 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.

ORAL PRESENTATIONS
Session 2

SERUM AND SYNOVIAL FLUID CONCENTRATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND ULTRASOUND-DETECTED SYNOVIAL ANGIOGENESIS IN JUVENILE IDIOPATHIC ARTHRITIS

Agneza Marija Pasini[1], Goran Roić[1], Alenka Gagro[1,2]

[1]Children's Hospital Zagreb, School of Medicine, University of Zagreb, Zagreb
[2]Faculty of Medicine, Josip Juraj Strossmayer, University of Osijek, Osijek, Croatia

Aim: Synovial angiogenesis is an important early step in the pathogenesis of juvenile idiopathic arthritis (JIA). Vascular endothelial growth factor (VEGF) has been shown to play a crucial role in vessel neoformation. In this study we assessed levels of VEGF in serum and synovial fluid and its possible relevance to disease activity, response to therapy and degree of ultrasound signs of synovial inflammation and angiogenesis in early JIA (at the begining, and after three and six months of treatment).

Patients and methods: Study included 30 patients with oligo- and polyarticular JIA. Each patient underwent clinical and laboratory (ESR, CRP, VEGF in serum/synovial fluid) evaluation. VEGF was determined by quantitative enzyme-linked immunosorbent assay. Synovial angiogenesis was assessed by means of Power-Doppler ultrasound (PD-US).

Results: Significant positive correlation between VEGF levels with CRP and ESR was found by the analysis of serum cytokine concentrations and classical inflammatory markers. Positive correlation was found among serum levels of VEGF and response to therapy after six months. Positive correlation was also found between vascular signals with the disease activity but negative correlation with response to therapy after six months. There was no correlation between vascular signals with the levels of VEGF in serum/synovial fluid.

Conclusion: VEGF in serum and vascular signals determined by PD-US may serve as markers of disease activity or severity and in evaluating treatment efficacy. Negative correlation of PD-US vascular signals and response to therapy may propose vascular signals intensity as negative prognostic factor.

DYNAMIC RNA BINDING PROTEIN INTERACTIONS TO CYTOKINE MRNA GOVERN HUMAN T CELL EFFECTOR FUNCTION

Branka Popovic[1], Benoit P. Nicolet[1], Sander Engels[1], Aurelie Guislain[1], Anouk Jurgens[1], Julian J. Freen-van Heeren[1], Floris van Alphen[2], Maartje van den Biggelaar[2], Fiamma Salerno[1,3], Monika C. Wolkers[1]

[1]Department of Hematopoiesis, Oncode Institute, Sanquin Research-AMC Landsteiner Laboratory, Amsterdam, The Netherlands
[2]Department of Molecular and Cellular Hemostasis, Sanquin Research, Amsterdam, The Netherlands
[3]present address: Laboratory of Lymphocyte Signalling and Development, The Babraham Institute, Cambridge, UK

T cells are critical in killing infected and malignant cells. The clearance of target cells depends on the capacity of T cells to produce ample amounts of effector molecules. We recently showed that the production levels and kinetics of the key cytokines IFN-γ, TNF-α and IL-2 rely on post-transcriptional mechanisms. Which post-transcriptional regulators modulate the cytokine production in T cells is however not well understood. RNA-binding proteins (RBPs) are critical mediators of post-transcriptional events, but their binding to cytokine mRNA is not well studied. Here we employed an RNA-aptamer-based capture assay with human T cell lysates to map RBP interactors with the 3'untranslated regions (3'UTRs) of IFNG, TNF and IL2. We found both promiscuous and cytokine-specific binding of RBPs. Intriguingly, the composition of RBP binding to cytokine 3'UTRs altered upon T cell activation, indicating that RBP interactions may rapidly respond to external stimuli. Genetic deletion of confirmed mRNA-binders in primary T cells uncovered RBP-specific activity in modulating the protein output in response to target cells. For instance, the RBPs ZFP36L1, ATXN2L and ZC3HAV1 dampen the production of all three cytokines, whereas ELAV-L1 enhances the protein production. Intriguingly, only ZFP36L1 destabilizes cytokine mRNA. ZC3HAV1 and ATXN2L employ an mRNA degradation-independent mechanisms to block cytokine production. In fact, ZFP36L1 and ATXN2L deletion shows synergistic effects on the protein production. In conclusion, identifying the RBPs that fine-tune cytokine production in T cells, and unraveling their mode of action should help define novel targets to improve T cell responses against pathogens and malignant cells.

IFNγ PRODUCED IN INFECTION DOWN-REGULATES PPARγ AND CHANGES ADIPOSE TISSUE BIOLOGY

Mia Krapić[1], Tamara Turk Wensveen[2,3], Felix M. Wensveen[1]

[1]Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia.
[2]Department of Endocrinology, diabetes and metabolic disorders, KBC Rijeka, Rijeka, Croatia
[3] Center for diabetes, endocrinology and cardiometabolism, Thallasotherapia, Opatija, Croatia

Infection has a major impact on physiology and systemic metabolism which is often manifested as fatigue, fever and loss of appetite. Modulations in systemic metabolism could be the result of metabolic changes in adipose tissue. However, the importance and mechanisms of adipose tissue and immune system interactions in viral infection are poorly defined. Here, we investigate how the immune system induces changes in adipose tissue metabolism and how this response benefits the organism during viral infection. We find in vitro that IFN-γ, which is a key cytokine produced upon viral infection, strongly impairs lipid metabolism in 3T3-L1 cells. Using total transcriptome analysis of IFN-γ treated cells we could show that PPAR-γ which is a key regulator of adipogenesis and adipocyte metabolism, as well as several of its target genes, were significantly down-regulated. In accordance with these findings, infection of mice with cytomegalovirus caused a reduction in adipocyte size, which was most pronounced three days after infection. Also, serum levels of free fatty acids and tryglicerides were increased in infection, suggesting the release of metabolites from fat. In vitro treatment of B cells with adipose tissue derived nutrients showed to be beneficial for the response of germinal centers. Altogether, these results indicate that immune-adipose interactions may benefit the humoral response against infection and identify an important role for IFN-γ mediated changes in PPAR-γ expression.

ORAL PRESENTATIONS
Session 5 – Bright Sparks

OBTAINING A MUMPS VIRUS WITH ALTERED FIDELITY POLYMERASE IN ORDER TO REDUCE ITS VIRULENCE

Mirna Jurković[1], Jelena Ivančić-Jelečki[1], Anamarija Slović[1], Dubravko Forčić[1], Tanja Košutić-Gulija[1], Renata Jug[1], Maja Jagušić[1]

[1]Center for research and knowledge transfer in biotechnology, University of Zagreb, Zagreb, Croatia

Mumps virus is an RNA virus that causes respiratory infection usually associated with parotid glands swelling. In approximately 1-10% of cases infection can lead to occurence of encephalitis or meningitis due to highly neurotropic nature of the virus.

All vaccines administrated against mumps are based on live attenuated strains. Some of them are not sufficiently attenuated while others are overly weakened or do not protect against currently circulating strains. One possibility for developing more efficient and safe vaccine could be altering viral population content due to the fact that viral population diversity is the main reason of severe virulence of RNA viruses. Identifying a high-fidelity polymerase by passaging virus in vitro with mutagens potentially results in decreased population diversity and attenuated phenotype. We applied this method on wild type mumps virus which was passaged over the course of 19 passages in A549 cells in the presence of ribavirin. Results obtained from three different passages unexpectedly show increase in the number of variants present in viral population. Understanding complexity of mumps virus variants, their selective fitness and impact on the host immune response will further help to determine if this approach can be used for design of new mumps vaccine.

PATHOGENIC ROLE OF NK CELLS IN CONGENITAL CMV INFECTION

Daria Kveštak[1,2], Vanda Juranić Lisnić[1,2], Berislav Lisnić[1,2], Jelena Tomac[1], Mijo Golemac[1], Ilija Brizić[2], Daniela Indenbirken[3], Adam Grundhoff[3], Astrid Krmpotić[1], William J. Britt[4], Stipan Jonjić[1,2]

[1]Dept. of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[2]Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[3]Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany
[4]Dept. of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama, United States of America

Human cytomegalovirus (HCMV) is the major viral cause of congenital infection with severe developmental and functional sequelae even in asymptomatic patients. Since HCMV is strictly species-specific, studies of the pathogenesis of congenital cytomegalovirus (cCMV) infection rely on animal models. Infection of newborn mice with mouse cytomegalovirus (MCMV) recapitulates many of the hallmarks of congenital HCMV infection including brain inflammation and microglia activation as well as altered cerebellar development. We have shown previously that mitigation of inflammation prevented these developmental defects, indicating that the host inflammatory factors are key drivers of altered cerebellar development. Here, we found not only that viral replication in brain is required for activation of microglia, but also that microglial transcriptome is dramatically altered in response to MCMV infection. Moreover, we have shown that microglia-derived chemokines recruit natural killer (NK) cells into the infected brain in CXCR3-dependent manner. Interferon gamma (IFN-γ) released by highly activated brain infiltrating NK cells contributes to the increased thickness of external granular layer (EGL). Importantly, NK cells, although unable to control virus infection in the brain, orchestrated early local inflammatory responses which lead to altered cerebellar development. Our results identify NK cells as one of the major mediators of immunopathology in response to virus infection in the developing CNS which can be prevented by anti IFN-γ antibodies.

INCREASED EXPRESSION OF NOTCH RECEPTORS ON OSTEOCLAST PROGENITORS INDUCED BY RHEUMATOID ARTHRITIS

Maša Filipović[1,2], Alan Šućur[1,2], Darja Flegar[1,2], Zrinka Jajić[3], Marina Ikić Matijašević[4], Nina Lukač[1,5], Nataša Kovačić[1,5], Tomislav Kelava[1,2], Dino Šisl[1,2], Katerina Zrinski Petrović[1,5], Vedran Katavić[1,5], Danka Grčević[1,2]

[1]Laboratory for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia
[2]Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia
[3]Department of Rheumatology, Physical Medicine and Rehabilitation, Clinical Hospital Center "Sestre Milosrdnice", University of Zagreb School of Medicine,Zagreb, Croatia
[4]Clinical Hospital "Sveti Duh", Department of Clinical Immunology and Allergology, Zagreb, Croatia
[5]Department of Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia

As Notch signaling has been implicated in osteoclast differentiation and activation, we investigated the expression of Notch receptors and ligands on osteoclast progenitors (OCP) in rheumatoid arthritis (RA) patients and mouse collagen-induced arthritis model. Peripheral blood mononuclear cells (PBMC) of RA patients and controls were phenotyped by flow cytometry, and an increase in Notch receptors on OCP was observed. During osteoclast differentiation in culture, there is an increase in Notch1, Notch3, Jag1 and DLL1 relative mRNA level in OCP, specifically in RA patients. By comparing the gene expression levels of Notch system components in sorted progenitors vs PBMC or synovial fluid cells we concluded that Notch receptors are generally more abundantly expressed on OCP, with an increase of Notch3 and DLL1 in arthritis. Likewise, in mice there is a significant increase in all the Notch receptors on flow cytometry, both in periarticular bone marrow (PBM) and spleen OCP. Although with initially higher levels of Notch1 and Notch2, during osteoclast differentiation levels of Notch3 and Notch4 as well as DLL4 mRNA increased in arthritic mice. Compared with controls, arthritic mice express more Notch2 mRNA in PBM. Immunohistochemistry of mice hind paws confirmed Notch receptor expression, with Notch1 and Notch2 on chondrocytes and synovial tissue, as well as Notch2 in PBM. Osteoclastogenic culture of sorted progenitors in wells coated with Notch ligands showed Jag1 inhibited, while DLL1 stimulated osteoclast formation. In conclusion, there is an increase in Notch expression on OCPs which may contribute to enhanced bone resorption in arthritis.

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ORAL PRESENTATIONS
Session 6

MÉNAGE À TROIS: NEURO-ENDOCRINO-IMMUNE REGULATION OF METABOLIC HOMEOSTASIS

Marko Šestan[1], Henrique Veiga-Fernandes[1]

[1]Champalimaud Research, Champalimaud Centre for the Unknown, Champalimaud Foundation, Lisbon, Portugal.

It has now been acknowledged for decades that the immune system is implicated in the control of metabolism, a function not linked to the classical immune response of the defence. While inflammation has long been considered as a significant pathogenic feature of diabetes development, recent studies have shown that immune cells are also important for maintenance of metabolic homeostasis in a steady state. Nevertheless, how immune cells integrate local and systemic cues to regulate metabolic processes remains elusive. Herein, we hypothesise that neuro-endocrine cues can regulate innate lymphocytes, forming an organismal neuro-endocrine-immune circuit that ensures metabolic homeostasis and prevention of obesity. By using combined cutting-edge immunology, neuroscience and endocrinology approaches we were able to decipher how this unconventional multi-organ, neuro-endocrine-immune circuit is controlled to regulate the host euglycemia in the health and how it can derail in obesity.

DNA DAMAGE PATHWAY CONTRIBUTES TO MONOCYTIC DIFFERENTIATION

Barbara Tomić[1], Vilma Dembitz[1], Dora Višnjić[1]

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

Our previous studies showed that 5-aminoimidazole-4-carboxamide ribonucleoside (AICAr), a known metabolic modulator, induces differentiation of monocytic cell lines by pyrimidine depletion and subsequent activation of the ataxia telangiectasia and RAD3-related (ATR)/checkpoint kinase 1 (Chk1)-mediated signaling pathway. Apart from nucleotide pool depletion, DNA damage response network can also be activated due to replication stress following integration of nucleotide analogues. Cytarabine or cytosine arabinoside (ara-C), an antimetabolite analogue of cytidine, interferes with DNA replication by multiple mechanisms, primarily by incorporation into DNA molecule. Cytarabine is widely used as a chemotherapeutic drug in acute myeloid leukemia (AML) treatment for more than several decades. It is well known that, apart from its cytotoxic effects, cytarabine stimulates differentiation of leukemic cells, but the exact mechanism remains to be elucidated. The aim of the present study is to test for the possible role of ATR/Chk1 in cytarabine-mediated effects and to further define DNA-damage pathway responsible for monocytic differentiation, in particular the involvement of Cyclin-dependent kinase 1 (Cdk1). Our results show that cytarabine dose-dependently mimics the effects of pyrimidine synthesis inhibitors on the number of viable cells and the expression of differentiation markers in monocytic cell lines. Moreover, cytarabine dose-dependently increased the level of Ser-345-phosphorylated Chk1, and all agents tested increased the level of Tyr-15- phosphorylated Cdk1. Pharmacological inhibition of ATR/Chk1 pathway by Torin2 and VE-821, as well as siRNA-mediated Chk1 knockdown, diminished the effects on differentiation. Therefore, our preliminary data suggest that cytarabine-mediated monocytic differentiation occurs via ATR/Chk1 signaling pathway.

IL-17A PRODUCING γδ T CELLS INITIATE DEVELOPMENT OF NON-ALCOHOLIC STEATOHEPATITIS THROUGH NKG2D ENGAGEMENT ON METABOLICALLY STRESSED HEPATOCYTES

Sonja Marinović[1,2*], Maja Lenartić[1*], Marko Šestan[1], Inga Kavazović[1], Ante Benić[1], Karlo Mladenić[1], Mia Krapić[1], Tamara Turk Wensveen[2], Dora Fučkar Čupić[4], Lidija Bilić-Zulle[5], Adrian Hayday[6], Felix M. Wensveen[1#], Bojan Polić[1#]

[1]Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia
[2]Division of Molecular Medicine, Institute Ruđer Bošković, Zagreb, Croatia
[3]Department of internal medicine, Faculty of Medicine, University or Rijeka, Croatia
[4]Department of General Pathology and Pathological anatomy, Faculty of Medicine, University or Rijeka, Croatia
[5]Clinical Department of Laboratory Diagnosis, CHC Rijeka, Croatia
[6]Department of Immunobiology, King's College London, UK
[*,#] these authors contributed equally to this work

Non-alcoholic fatty liver disease (NAFLD) is a hepatic manifestation of obesity-related metabolic syndrome characterized by occurrence of steatosis in absence of alcohol abuse. This relatively benign condition can progress to fibrosis and loss of liver function upon initiation of inflammation. The key mediators that induce early events of immune activation are still unknown. To investigate the mechanism leading from NAFLD to non-alcoholic steatohepatitis (NASH) we developed a steatosis-steatohepatitis dietary (SSD) model which mimics an unhealthy western lifestyle. After 16 weeks of SSD feeding we noticed several clinical hallmarks of liver damage such as hepatomegaly and elevated ALT levels when compared to chow-fed mice. Moreover, histological quantification revealed formation of micro- and macrovesicular steatosis at early timepoints upon SSD onset and development of inflammatory foci, hepatocyte degeneration and hepatic stellate cells (HSC) activation at later stages. This closely resembles NAFLD to NASH transition in humans. *Ex vivo* stimulation of liver leukocytes revelead IL-17A as the most prominent cytokine that is increased early upon SSD feeding. We identified hepatic γδ T cells as its dominant source. Significantly reduced levels of fibrosis and HSC activation in TCRδ$^{-/-}$ mice confirmed the importance of γδ T cells and IL-17A in disease progression. Interestingly, we found that metabolically stressed lipid-accumulating hepatocytes express high levels of NKG2D ligands on their surface. This suggested a mechanism through which γδ T cells are activated to secrete IL-17A which we confirmed by ELISA on stimulated cells. The importance of NKG2D signalling for the early activation of γδ T cells was confirmed by the striking reduction of fibrosis and HSC activation in SSD-fed NKG2D$^{-/-}$ mice. Our results indicate an importance of early events in immune cell activation in the progression of liver disease and provide a potential new target for NAFLD treatment.

ABSTRACTS

AUTOMATED IMAGE ANALYSIS OF BRIGHTFIELD MICROSCOPY WITH CELLPROFILER

Ivan Kodvanj[1], Barbara Tomić[1], Hrvoje Lalić[1], Vilma Dembitz[1]

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

Automated image analysis is a high-throughput and quantitative approach to microscopy analysis. In this study we investigated the accuracy of the CellProfiler and CellProfiler Analyst software in distinguishing 3 phenotypes of cells on brightfield microscopy. Mononuclear cells were isolated from the bone marrow of patients with acute myeloid leukemia were cultured and treated with AICAr (0.1, 0.2, 0.4 mM), ATRA (1 µM), combination of AICAr (0.2 mM) and ATRA (1 µM), and brequinar (500 nM). Cytospin prepared microscope slides were stained with May – Grunwald and Giemsa. Several images were acquired from each slide at 10x magnification. Image segmentation was performed with a CellProfiler pipeline and nuclei and cytoplasm were identified. Measurements were taken with Measure Object Size Shape module, followed by the analysis in CellProfiler Analyst where random forest classifier model was created by manually sorting identified cells into three categories: blasts, macrophage-like and dead cells. Testing the model on the training set revealed 90% overall accuracy. In further validation of the model we compared its results with the results of a person manually inspecting and classifying objects in whole images and observed a strong congruence in the results. Additionally, classifier yielded similar results as flow cytometry regarding the ratio of macrophage-like and blast cells; however, dead cell count estimated with the model did not correlate with the trypan blue exclusion counting and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In conclusion, automated image analysis of brightfield microscopy with CellProfiler generates quite accurate results when substantial morphological differences between the groups are present.

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RELATIVE HYPOGLYCEMIA DUE TO VIRAL INFECTION PROMOTES THE INNATE ANTI-VIRAL IMMUNE RESPONSE

Marko Šestan[1], Ante Benić[1], Felix M. Wensveen[1], Bojan Polić[1]

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

Viral infection has a major impact on systemic metabolism. We have recently shown that mild viral infection alters endocrine regulation of systemic blood glucose levels, even though this did not lead to dysglycemia. In humans, severe infection may lead to hypo- or hyperglycemia, but how this is regulated on a molecular level and how it benefits the host is mostly unknown.

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. This effect was dependent on IFNγ secreted by γδ T cells, as mice without δ TCR or those treated with anti-IFN□ do not develop hypoglycemia. We have shown that IFNγ causes insulin resistance in muscle, but not in liver which leads to compensatory hyperinsulinemia. As a result of hyperinsulinemia, glycogenolysis and thus liver glucose output is reduced, leading to relative hypoglycemia. Low glucose in turn, activates metabolic stress response which results in higher production of type I interferons that help with resolving the infection.

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

THE ROLE OF NK CELLS IN DIFFERENT MOLECULAR SUBTYPES OF BREAST CANCER: FRIEND OR FOE

Alma Starčević[1], Damir Grebić[2], Manuela Alvirović[3], Milijana Danilović[3], Petra Valković Zujić[4], Danijela Veljković Vujaklija[4], Tamara Gulić[5]

[1]Tissue Typing Laboratory, Clinical Institute for Transfusion Medicine, Clinical Hospital Center Rijeka
[2]Department of General and Oncological Surgery, Clinical Hospital Center Rijeka; Rijeka, Croatia
[3]Department of Pathology, School of Medicine, University of Rijeka
[4]Department of Radiology, Clinical Hospital Center Rijeka
[5]Department of Physiology and Immunology, School of Medicine, University of Rijeka

INTRODUCTION: Breast cancer is one of the leading causes of cancer related deaths. In addition, immune infiltration of breast tumors has been shown to be related to clinical outcome. With respect to other infiltrating immune cells in breast cancer, a strong proportion of natural killer cells (NK) have been found in triple negative breast cancer. As part of the innate immunity, NK cells have a key role to control tumour growth by their cytotoxic activity. Based on this the goal of study was to investigate the immune profile of NK cells in order to gain a better understanding of pathological behaviour by different breast cancer subtypes.

MATERIAL AND METHODS: Immunohistology was used to detect presence and localization of CD56 and IL-15 in paraffin embedded normal and tumoral breast tissue sections. The distribution and frequency of NKG2A, NKG2C, NKp46, CD94, CD69 and CD107a, was investigated in population of NK cells in mononuclear cell suspensions from peripheral blood by flow cytometry. Cytolitic mediator's mRNA was detected by quantitative RT-qPCR.

RESULTS: The percentage of IL15+ and CD56+ cells were significantly higher in triple negative breast cancer tissue in comparison to luminal A and luminal B breast cancer. The frequency of NK cell activating receptors was decreased in breast cancer subtypes while inhibitory receptor (NKG2A) increased. This data correlated with decreased NK cell function, most notably cytotoxicity. Gene expression of cytolitic mediators at local level were up regulated in luminal B breast cancer.

CONCLUSION: Our data show that modulation of NK cell activity at local and systemic level could be involved in pathogenesis of breast cancer. This highlights the importance of developing therapies that will be able to restore NK cell cytotoxicity to limit tumor escape from antitumor immunity.

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TARGETING STAT3 SIGNALING IMPAIRS THE PROGRESSION OF BLADDER CANCER IN A MOUSE MODEL

Jelena Korac-Prlic[1], Marina Degoricija[1], Katarina Vilović[2], Benedikt Haupt[1], Tonči Ivanišević[1], Lucija Franković[1], Sergei Grivennikov[3], Janoš Terzić[1]

[1] Laboratory for Cancer Research, University of Split School of Medicine, Split, Croatia.
[2] Department of Pathology, University Hospital of Split, Split, Croatia; University of Split School of Medicine, Split, Croatia.
[3] Cancer Prevention and Control Program, Fox Chase Cancer Center, Philadelphia, USA.

Bladder cancer is the fourth most commonly diagnosed malignancy in men worldwide and has one of the highest recurrence rates of all cancers. This cancer type is unique because chronic inflammation caused by Schistosoma haematobium can cause bladder cancer, while inflammation induced by Bacillus Calmette Guerin is the therapeutic cornerstone for this cancer type. Activation of proinflammatory IL-6/Stat3 axis promotes the development of different cancers by acting on cancer cells as well as by modulating cancer microenvironment. Using a genetic and pharmacological approach in a mouse model, we demonstrated the importance of IL-6 and Stat3 signaling in bladder cancer. Our findings show that pharmacological inhibition of Stat3 with WP1066 effectively delays progression and invasiveness of bladder cancer in N-butyl-N-(4-hydroxybutyl) nitrosamine-induced mouse model. Moreover, either IL-6 blockade or Stat3 inhibition sensitized bladder cancer to anti-PD-L1 immune therapy. Taken together, our study demonstrates an important role of IL-6/Stat3 signaling in bladder cancer and creates a rationale for testing the therapeutic potential of Stat3 inhibitors in human MIBC both alone or in combination with anti-PD-L1 and anti-IL-6 therapy.

AICAR INDUCES DIFFERENTIATION IN A SUBSET OF PRIMARY NON-APL ACUTE MYELOID LEUKEMIA BLASTS

Vilma Dembitz[1,2], Hrvoje Lalic[1,2], Ivan Kodvanj[1,2], Barbara Tomic[1,2], Josip Batinic[3], Klara Dubravcic[4], Drago Batinic[2,4], Antonio Bedalov[5], Dora Visnjic[1,2]

[1]Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia;
[2]Department of Physiology, University of Zagreb School of Medicine, Zagreb, Croatia;
[3]Division of Hematology, Department of Internal Medicine, University Hospital Center Zagreb, Zagreb, Croatia;
[4]Department of Laboratory Immunology, University Hospital Center Zagreb, Zagreb, Croatia;
[5]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, United States of America

All-trans retinoic acid (ATRA)-based differentiation therapy of acute promyelocytic leukemia (APL) is the most successful pharmacological treatment of acute myeloid leukemia (AML). Recent development of inhibitors of mutated isocitrate dehydrogenase and dihydroorotate dehydrogenase (DHODH) has revived interest in differentiation therapy of non-APL AML. Our previous studies demonstrated that 5-aminoimidazole-4-carboxamide ribonucleoside (AICAr), a known AMP-kinase activator, induced differentiation of monocytic cell lines independently of its known AMPK-mediated metabolic effects, but instead by depleting pyrimidines and consequent ATR/Chk1 activation. In that respect, AICAr shared the mechanism of action with DHODH inhibitor brequinar, which differed from that of ATRA pathway. In the present study, we examined the effects of AICAr and brequinar on the viability and differentiation of primary AML blasts from patients with non-APL AML. We found that AICAr induces differentiation in a subset of primary non-APL AML samples, and these effects do not correlate with FAB classification or mutational status of FLT3 or NPM1, but correlate with sensitivity to DHODH inhibition.

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DYNAMIN-2 IS IMPORTANT FOR GENERATION OF VIRAL PREASSEMBLY COMPARTMENT, BUT ALSO FOR SYNTHESIS OF LATE PROTEINS AND FORMATION OF INFECTIVE VIRONS IN MURINE CYTOMEGALOVIRUS (MCMV) INFECTION

Igor Štimac[1], Natalia Jug Vučko[1], Gordana Blagojević Zagorac[1,2], Zsolt Ruzsics[3], Pero Lučin[1,2], Hana Mahmutefendić Lučin[1,2]

1Department of Physiology, Immunology and Pathophysiology, University of Rijeka, Faculty of Medicine, Rijeka, Croatia
2University North, Varaždin, Croatia
[3]Universitätsklinikum Freiburg, Institut für Virologie, Freiburg, Germany

The life cycle of murine cytomegalovirus (MCMV) is shorter, but very similar to that of human cytomegalovirus (HCMV), and lasts around 48-72 hrs.

In this study, we have investigated the role of dynamin on life cycle of MCMV. For that purpose, Balb3T3 cells and murine embryonal fibroblasts (MEF) have been analysed by flow cytometry, confocal microscopy and western blot analysis.

We have shown that dynasore does not influence either MCMV entry into the cells, or synthesis of immediate early (IE1) and early proteins (E1, m06). However, dynasore has affected reorganization of the Golgi apparatus (both cis-Golgi (GM130), and trans-Golgi network –TGN (Golgin 97 and TGN38)), as well as Rab10 and evectin-2 loading - one of the first hallmarks that indicate formation of preassembly compartment (preAC) for synthesis of new viral particles. Importantly, we have found that, after infection of fibroblasts with SCP-Cherry MCMV, dynasore treatment inhibited nucleocapsid assembly. Furthermore, synthesis of late MCMV proteins (M55, M74 and 130 kDa M25) has also be prevented. Interestingly, synthesis of 105 kDa M25 protein, has been normal 6hpi, but has also retarded during late phase of infection. Finally, results of plaque assay have shown that treatment of dynasore during early phase of MCMV infection has significantly decreased the number of infectious virions.

Therefore, we have concluded that dynamin is important not only for generation of viral assembly compartment, but also for synthesis of nucleocapsid proteins and MCMV envelope proteins.

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γδ T CELLS ARE A POSSIBLE LINK BETWEEN GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONISTS AND NON-ALCOHOLIC FATTY LIVER DISEASE

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

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

Non-alcoholic fatty liver disease (NAFLD) is considered to be the hepatic manifestation of metabolic syndrome and encompasses liver abnormalities from simple steatosis to its progressive form (non-alcoholic steatohepatitis (NASH)). The prevalence of NAFLD is increasing worldwide, with an estimated one third of the global adult population being affected. Therapeutic strategies for NAFLD involve lifestyle changes leading to weight loss, insulin sensitizers, antioxidants and lipid lowering medications, but results have been poor. In contrast, recent studies investigating GLP-1 receptor (GLP-1R) agonists for the treatment of NAFLD have shown promising results with reducing liver inflammation, steatosis and fibrosis, but its underlying mechanism is unknown. Our previous research highlighted inflammation as a crucial event in the pathogenesis of NAFLD in mice. The secretion of the pro-inflammatory cytokine IL-17 by γδ T cells was shown to trigger NASH. Considering the fact that GLP-1R is not expressed on human and murine hepatocytes, we hypothesized that GLP-1R agonists act indirectly by reducing inflammatory capability of immune cells, especially γδ T cells. Preliminary data confirmed the beneficial effects of GLP-1R in our dietary mouse model of NASH. In a dietary mouse model of NASH, weekly treatment of mice with the GLP-1R agonist dulaglutide alleviated the development of fibrosis after 16 weeks. Furthermore, GLP-1R was shown to be highly expressed on γδ T cells and weekly dulaglutide treatment reduced their activation. Further investigation of GLP-1R agonists effects on liver γδ T cells in context of NASH will be the main goal of this project.

THE ROLE OF NOTCH SIGNALING IN CARBON TETRACHLORIDE MURINE MODEL OF HEPATIC FIBROSIS

Dino Šisl[1,2], Sanja Novak[4], Ivo Kalajzić[4], Maša Filipović[1,2], Nina Lukač[1,3], Darja Flegar[1,2], Alan Šućur[1,2], Nataša Kovačić[1,3], Danka Grčević[1,2], Antonio Markotić[1], Tomislav Kelava[1,2]

[1]Laboratory for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia
[2]Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia
[3]Department of Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia
[4]University of Connecticut Health Center, Farmington, USA

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 our research, we have studied the expression of Notch-related molecules during fibrogenesis and analyzed contribution of various αSMA+ cell populations to the pool of liver myofibroblasts. Common murine model of liver fibrosis, 6-week carbon tetrachloride (CCl4) treatment, was used. PCR analysis of whole liver tissue showed upregulation of genes for Hey1, HeyL, Notch2 and Jag2, while downregulation of Notch1 and Notch4, and upregulation of Notch2 was shown on isolated HSCs. Notch1 downregulation in HSCs was further confirmed by flow cytometry where it was shown that 42.3% of freshly isolated HSCs from normal mice express Notch1, whereas only 13.9% of those from fibrotic liver express it. We used tamoxifen inducible Cre mice (αSMA-CreERT2/Ai9) to assess 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 clarify its importance in fibrotic processes.

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FANTASTIC THREE: THE ROLE OF CD16, NKG2D AND NKP46 AS "MASTER REGULATORS" OF NK CELL ACTIVATION

Vanna Imširović[1], Bojan Polić[1], Felix M. Wensveen[1], Vedrana Jelenčić[1],

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

The activation of NK cells depends on the shift in balance of signals from inhibitory and activating receptors, in favor of the latter. Each NK cell has its own repertoire of receptors but one key group of activating NK cell receptors made up by NKG2D, NKp46 and CD16 is expressed on all NK cells. In contrast to most other receptors on these cells, for the ligands of NKG2D, NKp46 and CD16 no inhibitory receptor is known to be expressed on NK cells.

Both NKG2D and Nkp46 recognize stress-induced ligands and play an important role in the fight against different types of infections and tumors, whereas CD16 recognizes Fc tails of cell-bound antibodies and is responsible for induction of antibody-dependent cellular cytotoxicity (ADCC).

We hypothesize that these three receptors are "master regulators" of which a signal is requirerd in order to gain maximal NK cell reactivity.

Our preliminary results endorse our hypothesis, as mice lacking both NKG2D and Nkp46 showed reduced survival in murine lymphoma model in comparison to wild type animals. Mice lacking both NKG2D and antibodies, and therefore ADCC, showed further reduction of survival compared to animals lacking NKG2D alone in the B16 model of murine melanoma. This indicates that Nkp46 and CD16 are required for control of B16 by NK cells.In this project, we want to further elucidate how the lack of NKG2D, NKp46 and CD16 influences effector functions of NK cells and their ability to fight tumors and viral infection. Our goal is to gain deeper insight into NK cell biology and use this knowledge for improvement of NK cell-based cancer immunotherapy.

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4-HYDROXY 2-NONENAL IN PATHOGENESIS OF OSTEOARTHRYTIS

Vedrana Drvar[1], Božena Ćurko Cofek[2], Dalen Legović[3], Veljko Šantić[3], Tatjana Kehler[4,5], Marija Rogoznica[4], Daniel Rukavina[2,6], Gordana Laskarin[2,3]

[1]Clinical Department of Laboratory Diagnostics, Clinical Hospital Centre Rijeka, Rijeka, Croatia
[2]Department of Physiology and Immunology, Faculty of Medicine, University of Rijeka, Croatia
[3]Orthopaedic University Hospital Lovran, Lovran, Croatia
[4]Hospital for Medical Rehabilitation of Hearth and Lung Diseases and Rheumatism "Thalassotherapia-Opatija", Opatija, Croatia
[5]Department of Medical Rehabilitation, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[6]Department of Biomedical Sciences in Rijeka, Croatian Academy of Sciences and Arts, Rijeka, Croatia

Stress on joint tissues and metabolic processes leads to low grade inflammation in joint with osteoarthritis (OA). Inflammation generates free radicals, which damage polyunsaturated fatty acids. The most abundant product of free radical reactions is tremendously reactive 4-hydroxy 2-nonenal (HNE), which exerts cytotoxic effects at the site of generation on a dose dependent manner. HNE promotes survival and proliferation by induction of NF□B signalling pathway and downstream pro-inflammatory mediator-expressions, supporting cell activation. At higher concentrations, HNE activates caspases and DNA fragmentation. The aim of our study was to investigate the presence of HNE in synovial tissue of OA patients in respect to M1/M2 polarisation of macrophages. We used immunohistology and/or double immunofluorescence techniques for antigens detection in paraffin embedded mature synovial tissue, obtained during the knee alloarthroplasty.

HNE was homogenously expressed in cytoplasm of accumulated CD68+ macrophages and lymphocytes, mostly of CD56+ phenotype, together with the expression of pro-survival NF□B. Apoptotic protease activating factor 1, which initiates an apoptotic protease cascade, appeared less often as nuclear granular pattern of labelling. In leukocyte infiltrations, we proved more iNOS+IL-15+CCL2+TNF-□+ CD68 expressing M1 macrophages, while M2 polarisation markers arginase-1 and CCL22 were absent. In contrast, CD68+ macrophages scattered in sublining synovial tissue were strongly arginase-1 positive, indicating their M2 polarisation.

In conclusion, HNE might be involved in supporting pro-inflammatory reactions within synovial tissue leukocyte infiltration governing domination of M1 over M2 macrophage polarisation.

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ROLE OF INNATE IMMUNITY IN MCMV INFECTION IN OVARIES

Ana Vrbanović[1,2], Marija Mazor[1,2], Jelena Tomac[2], Darija Kveštak[1,2], Lars Dolken, Melanie Brinkmann[3], Astrid Krmpotić[2], Stipan Jonjić[1,2], Vanda Juranić Lisnić[1,2]

[1]Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[2]Dept. of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[3]Helmholtz Centre for Infection Research, Braunschweig, Germany

Congenital infections with human cytomegalovirus (HCMV), a widespread pathogen, can lead to malformations, long-term sequelae, and induce pregnancy loss via unknown mechanism(s). However, very little is known about the pathogenesis of the cytomegalovirus infection in reproductive organs and its consequences on fertility.

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 is an excellent and common model for studying the biology and pathogenesis of CMV, especially in research that is difficult to conduct in humans.

Upon I.V or I.P infection, CMV very successfully infects the ovaries however the infection is localized to corpora lutea and stroma and completely exclused from follicles even in strongly immunosuppressed mouse strains. The virus is cleared by day 8 PI, indicating a strong role of innate immune system in virus control. We have previously shown that multiple innate immune mechanisms and barriers protect the follicles from the infection: from gap-junctions, absence of vasculature, stronger sensitivity to IFN I signaling to innate immune cells. In this work, we have focused on the role of NK cells and analyzed mechanisms of virus control by NK cells ad well as differentiation between classical NK cells and ILC-1.

ROLE OF ARF PROTEINS DURING THE EARLY PHASE OF MCMV INFECTION

Valentino Pavišić[1], Hana Mahmutefendić Lučin[1,2], Tamara Gulić[1], Ljerka Karleuša[1], Natalia Jug Vučko[1], Pero Lučin[1,2] and Gordana Blagojević Zagorac[1,2]

[1]Department of Physiology and Immunology, University of Rijeka Faculty of Medicine, Braće Branchetta 20, 51000 Rijeka, Croatia;
[2]University North, Jurja Križanića 31b, 42000 Varaždin, Croatia

Arf proteins are small GTPases that, by the recruitment of their effector proteins organize cellular cytoskeleton and change the membrane dynamics, and thus regulate traffic within secretory and endocytic system, cellular division and migration.Cytomegaloviruses (CMVs) are widely spread DNA viruses from herpesvirus family that cause massive reorganization of the cellular functions in the early phase of infection, including reorganization of the membrane and cellular cytoskeleton that finally results in formation of cytoplasmic virion assembly compartment (cVAC). cVAC is a large, cylindrical, juxtanuclear compartment with phenotype characteristics of several intracellular compartments, including early endosomes, recycling endosomes and trans-Golgi network (TGN). The role of Arf proteins in CMV infection is still poorly understood. The aim of this study was to determine expression, activation, and localization of Arf proteins, and their main regulators (GEFs, and GAPs) during MCMV infection. Furthermore, we wanted to see how impaired function of Arfs affects MCMV infection. For that purpose murine embryonic fibroblasts (Balb/3T3) were infected with recombinant murine cytomegalovirus Δm138-MCMV and 0, 6, 16, and 30 hours post infection Arf proteins and their regulators were colocalyzed with markers of intracellular compartments or with viral proteins. Viral replication was monitored by C3X GFP MCMV. We followed cVAC formation by using Rab10 as a marker, and degree of Arf activation using pull-down and wound healing assays. Impaired function of Arfs was achieved by siRNA or by chemical inhibitors. We found that Arf proteins are over activated during MCMV infection and that they accumulate in the area of cVAC formation, but they do not colocalize with any analysed viral protein.

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IMUNOREGULATORY ROLE OF CIRCULATING ENDOTHELIAL VWF POSITIVE CELLS IN PATIENTS AFTER ACUTE MYOCARDIAL INFARCTION

Marijana Rakić[1], Gordana Laskarin[1,2], Viktor Peršić[1,3], Tatjana Kehler[1,3], Daniel Rukavina[2,4]

[1]Hospital for Medical Rehabilitation of Hearth and Lung Diseases and Rheumatism "Thalassotherapia-Opatija", Opatija, Croatia
[2]Department of Physiology and Immunology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[3]Department of Medical Rehabilitation, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[4]Department of Biomedical Sciences in Rijeka, Croatian Academy of Sciences and Arts, Rijeka, Croatia.

Introduction: We hypothesized that an increased proportion of circulating vWF+ endothelial cells in patients with acute myocardial infarction participates in the regulation of the elicited immune response.

Subjects and methods: The study included 34 patients with non-ST elevation myocardial infarction (NSTEMI), and 33 healthy subjects. Peripheral blood (10-20 ml) was sampled from participants. We performed immuno-phenotyping of mononuclear cells using antibodies towards von Wilebrand factor (vWF), CD3, CD56, perforin, interleukin (IL)-15, interferon-gamma (IFN-γ) or CC ligand (CCL) 17 and established a protocol for purification of circulating vWF+ cells (~80%), CD14+ cells (~80%) and untouched CD3 + lymphocytes (~70%) by magnetic isolation. We cultured enriched vWF+ or CD14+ cells with enriched CD3+ lymphocytes.

Results: On the first day after acute coronary events, patients with NSTEMI have higher proportion of viable vWF+ cells in the peripheral circulation (12%), when compared to healthy subjects (1-2%). In NSTEMI patients, the frequency of vWF+ cells expressing HLA-DR (65-70%) did not differ significantly, while vWF+ cells expressing CD80 (1%), CD86 (50%), CCR7 (0%), IL-15, IFN-□, CCL17 were significantly lower than the frequency of CD14+ cells expressing the corresponding markers. Enriched vWF+ cells did not significantly change the proportion of perforin-positive CD3+CD56- T lymphocytes after 18 hour-culture in a ratio of 1: 2.5 and 1:5 compared to T lymphocytes cultured in medium only (10%), while CD14+ cells did.

Conclusion: Circulating vWF+ cells might downregulate cytotoxic potential in autologous peripheral blood T cells on the first day after acute coronary event in NSTEMI patients.

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M2 POLARIZED SYNOVIAL MACROPHAGES FROM PATIENTS WITH OSTEOARTHRITIS KEEP PLASTICITY AND ARE ABLE TO INITIATE THE LPS-MEDIATED MATURATION PROGRAM IN VITRO

Božena Ćurko-Cofek1, Vedrana Drvar2, Marija Rogoznica3, Dalen Legović4, Veljko Šantić4, Tatjana Kehler3,5, Gordana Laskarin1,3

[1]Department of Physiology and Immunology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia; [2]Clinical Department of Laboratory Diagnostics, Clinical Hospital Centre Rijeka, Rijeka, Croatia; [3]Hospital for Medical Rehabilitation of Hearth and Lung Diseases and Rheumatism "Thalassotherapia-Opatija", Opatija, Croatia; [4]Orthopaedic University Hospital Lovran, Lovran, Croatia; [5]Department of Medical Rehabilitation, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

Introduction: Macrophages are cells present in synovial tissue in large number, and they potentiate the progression of osteoarthritis (OA).

Aim: To analyse polarisation and maturation status, cytokine production, and endocytic capability of synovial macrophages under the influence of lipopolysaccharide (LPS).

Material and methods: Mature synovial tissue samples were obtained during the knee alloarthroplasty due to OA. CD68 and arginase-1, IL-15 or TNF-α were labeled in paraffin-embedded tissue sections by double immunofluorescence. Maturation status and FITC-Dextran endocytosis were analyzed in the isolated and cultured synovial mononuclear cells in the medium only or with LPS (10 ng/mL). We developed a purification strategy for CD68+ cells from synovium.

Results: CD68+ macrophages scattered in sublining synovial tissue were strongly arginase-1 positive, while rarely expressed TNF-α and IL-15, indicating their M2 polarisation. Back gating for CD68+ cells revealed ~45% of CD68+ cells in freshly isolated mononuclear cell suspensions, which remained viable after 18-hour culture. The frequency of HLA-DR (60-70%), CD80 and CD86 (50-60%) did not significantly differ between CD68+cells cultured in the medium only and with LPS. LPS decreased frequency of CD91 receptor and FITC Dextran uptake for about 20-30%, compared to the cells cultured in the medium only, suggesting a specific maturation process. CD68+ cells were enriched only up to 85% from the adherent mononuclear cell fraction using anti-CD68 mAb + goat anti-mouse antibody conjugated with nanobeads, due to cell clumping.

Conclusion: The most of synovial macrophages are M2 polarized, but CD68+ cells keep plasticity and are able to initiate maturation program under the influence of LPS in vitro.

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MEMORY CD8 T CELL DYSFUNCTION IN OBESE MICE IS INDEPENDENT OF INSULIN SIGNALING

Inga Kavazović[1], Mia Krapić[1], Felix M. Wensveen[1]

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

People with obesity have a reduced immunological response to viral infection, but the underlying mechanism, especially with regards to memory T cell dysfunction, is currently unknown. Obesity is commonly associated with insulin resistance and subsequent compensatory hyper-insulinemia. Here we wanted to investigate whether obesity-associated hyperinsulinemia affects the anti-viral memory CD8 T cell response. To test this in vivo, we used the murine diet-induced obesity (DIO) model. 18 weeks after the start of high-fat content diet (HFD), mice were transferred with OT-1 cells and subjected to mCMV-N4 infection. Whereas, obesity did not impact the effector CD8 T cell response we showed that it resulted in reduced functionality of memory CD8 T cells. Upon re-ifection with a second N4-carrying pathogen, obesity caused a decreased ability of memory CD8 T cells to produce cytokines and was associated with an increase in viral titers. In addition, OT-1 T cells in obese animals showed a reduced ability to eliminate B16-N4 melanoma cells. To investigate the importance of insulin for the functionality of memory CD8 T cells we generated mice with T cell specific deficiency for the insulin receptor. We observed that IR-deficiency reduces viability of memory CD8 T cells generated in vitro, however it had no impact on memory formation, function and metabolism in vivo. Furthermore, memory CD8 T cells deficient for the insulin receptor showed no changes in functionality in an obese environment. Our findings indicate that changes in insulin signaling are not responsible for dysfunction of memory CD8 T cells in the context of obesity.

THE ROLE OF COSTIMULATORY MOLECULES IN MEMORY CD8 T CELL INFLATION IN THE MOUSE CYTOMEGALOVIRUS INFECTION MODEL

Maja C. Brdovčak[1], Lea Hiršl[1], Anita Gaberščik[1], Suzanne P. Welten[3], Stipan Jonjić[1,2], Annette Oxenius[3], Astrid Krmpotić[2]

[1]Center for proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[2]Dept. of histology and embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[3]Institute of Microbiology, Dept. of Biology, ETH Zürich, Zürich, Switzerland

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 characterized by expansion and maintenance of certain CMV-specific CD8 T cells in peripheral tissues. The majority of CD8 T cells, referred to as “conventional CD8 T cells” show a typical expansion and contraction kinetics followed by central memory formation. In contrast, specific T cell subsets, referred to as “inflationary T cells”, follow different kinetics and continue to expand in blood and peripheral tissues in the latent phase of the infection, a process termed „memory inflation“. The exact mechanisms underlying inflation of these CMV-specific CD8 T cell populations are still poorly understood. In this study we investigated 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 C57BL/6 mice with WT MCMV or recombinant MCMV viruses lacking viral proteins that negatively regulate expression of different CD8 T cell costimulatory molecules (CD80, CD86, CD155, CD48, CD229 and CD270) and recombinant viruses expressing cellular ligands for CD8 T cells costimulatory receptor NKG2D (RAE-1γ and MULT-1) and followed CD8 T cell response over time. Our results show that a higher frequency of memory precursor effector cells (MPECs) is established early during infection with these recombinant viruses and we will investigate whether this effector memory pool serves as a source of inflationary cells in peripheral tissues.

CD8 T CELL RESPONSE IN MICE VACCINATED WITH RECOMBINANT MCMV VECTOR EXPRESSING NKG2D LIGAND RAE-1γ.

Marko Šustić[1], Ilija Brizić[2], Maja Cokarić-Brdovčak[2], Astrid Krmpotić[1] and Stipan Jonjić[1,2]

[1]Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[2]Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[3]Institute for Medical Microbiology, Immunology and Hygiene, Technical University of Munich (TUM), Munich, Germany
[4]DZIF—National Centre for Infection Research, Munich and Hannover, Germany

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 against these antigens. The immunobiology of cytomegalovirus (CMV) infection with various immunomodulatory mechanisms makes this virus a particularly attractive viral vector. CMV establishes life-long persistence which generates strong CD8 T-cell response with a gradual accumulation of CMV specific CD8-T cells in the infected host. In order to minimize the pathogenicity of the virus and thus make it a more promising vector candidate, we inserted RAE-1γ protein, which is a ligand for an activating receptor NKG2D, in place of its viral inhibitor m152. NKG2D is expressed as an activating receptor on NK cells, therefore RAE-1γMCMV proved to be highly attenuated in vivo in a NK cells dependent manner. Surprisingly, when we co-expressed a foreign CD8 T cell epitope with RAE-1γ in CMV vector, we induced superior expansion of epitope-specific CD8 T cells and these cells showed distinct phenotypical features, such as higher expression of KLRG1 molecule and lower expression of TCF-1 transcription factor as compared to the virus lacking NKG2D ligand expression. Upon challenge of vaccinated mice with mouse lymphoma cells expressing the same antigen on their surface, these epitope specific CD8 T cells proved to be highly resistant to T cell exhaustion and conferred superior anti-tumor protection. Overall, our results indicate that modulating the initial conditions during the priming of CD8 T cells has major effects on their phenotype and functionality.

IMMUNOLOGICAL ROLE OF CELLULAR PRION PROTEIN (PRPC) DURING VIRAL INFECTION

Dubravka Karner[1], Tihana Lenac Roviš[1], Daria Kveštak[1,2], Berislav Lisnić[1,2], Stipan Jonjić[1,2].

[1]Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[2]Dept. of Histology and Embriology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

PrPC is a GPI-anchored glycoprotein predominantly expressed in the brain. Its best-described physiological role is a neuroprotective effect through the impact on cell death pathways and neuronal survival. PrPC is also found on the surface of many immune cells. Regarding the immune response to viruses, the role of PrPC in the neuropathogenesis of HIV infection has been proven. Furthermore, PrPC is a significant factor in several mouse models of viral infections that mimic human conditions. In the absence of PrPC, influenza mortality is particularly increased. Our goal is to determine the immune role of PrPC in cytomegalovirus (CMV) infection.

In our preliminary experiments, the amount of PrPC on microglia cells isolated from mouse CMV infected brains was significantly increased. We have shown that mouse CMV in different cell line and primary cell cultures affects the amount of PrPC on the surface and inside the cells. After initial strong induction of PrPC expression, CMV actively removes PrPC from infected cells. Our findings also indicate that the loss of PrPC following infection is not the result of protein degradation pathways activation as the samples treated with lactacystin and leupeptin show no difference from untreated samples. This can be explained by phenomenon of PrPC shedding by metalloproteases and is further supported by a research done by a different group about HIV infection activating PrPC shedding and PrPC in its shed form acting as a chemoattractant to monocytes.

Altogether, mentioned data indicate that PrPC is a potentially underestimated tool in fighting the viral infection.

GOLGI APPARATUS REARRANGEMENT AS A FIRST STEP IN CVAC FORMATION

Natalia Jug Vučko1, Ljerka Karleuša1, Hana Mahmutefendić Lučin1,2, Gordana Blagojević Zagorac1,2, Marina Marcelić1, Igor Štimac1, Pero Lučin 1,2

[1]Department of Physiology and Immunology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[2]University North, University Center Varaždin, Varaždin, Croatia

Cytomegaloviruses (CMVs) as other members of the herpesvirus family, extensively rearrange the cellular membrane system to develop assembly compartment (AC). The AC area is large, approximately size of the nucleus, composed of host-cell-derived membranous elements delimited at its periphery by the Golgi complex.The loss of normal Golgi compartment morphology and its relocalization from a juxtanuclear ribbonlike structure to a series of concentric rings on the periphery of the AC represents one of the earliest landmarks of membranous system reorganization during MCMV infection.The objective of our study was to characterize Golgi transformations as the first step in the formation of the AC.

Mouse fibroblasts, Balb3T3 were infected with recombinant murine cytomegalovirus Δm138-MCMV (ΔMC95.15), 1PFU/cell. Using confocal imaging analysis, we analyzed different Gogli markers, Rab6 (trans – Golgi and TNG), CM130 (cis/medial Golgi), Grasp55 (medial trans Golgi), Grasp65 (cis Golgi), GS15 (medial and trans) and Golgin 97 (trans-Golgi-TGN interface),through several stages of MCMV replication cycle. Also, changes in Golgi apparatus were followed using specific inhibitor of protein trafficking to Golgi, Brefeldin A. In MCMV infected cells, the cis-, medial-, and trans-Golgi were vacuolized, fragmented and displaced from the nucleus to form the outer ring of the AC.Also, the analysis of the Golgi demonstrates that oAC is mainly build by the C2-C7 Golgi cisternae, whereas the iAC is composed of membrane intermediates derived at the interface the Golgi and post-Golgi linker compartments that are oriented toward the cell center. We have shown unlinking od Golgi ribbon and transformation of cis and medial Golgi cisterns in Golgi stacks in the early phase of MCMV infection as a first step of cVAC formation.

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ESTABLISHING OF AN ELISA-LIKE METHOD TO DIRECTLY AND QUICKLY QUANTIFY VIRIONS RELEASED FROM MOUSE CYTOMEGALOVIRUS INFECTED CELLS

Ingrid Šutić Udović[1], Kristina Grabušić[1], Gordana Blagojević Zagorac[1,2], Hana Mahmutefendić Lučin[1,2], Pero Lučin[1,2]

[1]Department of physiology, immunology and pathophysiology, Medical Faculty, University of Rijeka, Rijeka, Croatia
[2]University North, University Center Varaždin, Varaždin, Croatia

Mouse cytomegalovirus (MCMV) is an extensively studied herpes virus. It provides a valuable animal model necessary to learn more about the related human CMV which is dangerous for specific populations, like immunocompromised persons and newborns. Essential part of any viral study is quantification of virions. Currently, MCMV is quantified by two common methods: 1) plaque forming unite (PFU) assay in which the viral number is indirectly determined by viral infectivity of fibroblasts; and 2) polymerase chain reaction (PCR) which determines the number of viral genomes. While PFU assay is time demanding and of low reproducibility, PCR is highly precise in quantifying the viral genome but it does not provide information about infectious virions. Therefore, improved methods for quantification of MCVM are necessary. The aim of this study is to establish immuno-based quantification of MCVM which will be specific, fast, and reproducible.

The quantification is planned to be ELISA-based detection of the capsid protein gB by already available and proven antibody. In the first phase we used C3X-GFP MCMV that expresses GFP immediately after infection. Our data showed that the fluorescent signal could be measured by ELISA-reader in a suspension of Balb 3T3 cells infected with C3X-GFP MCMV. The detected signal was proportional with number of infected cells and infection duration and also confirmed by flow cytometry. In the next phase we will apply recombinant MCMV containing small capsid protein tagged with a fluorescent protein. This should enable direct detection of virions in solutions and finally measurement of anti-gB captured virions.

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INCREASED PREVALENCE OF ALLERGIC RHINITIS AND ATOPIC DERMATITIS SYMPTOMS WITHOUT CHANGES IN ASTHMA PREVALENCE OVER A 16 YEAR-PERIOD IN SCHOOLCHILDREN FROM THE CITY OF ZAGREB

Iva Topalušić[1], Bernarda Jakuš[2], Slavica Dodig[4], Asja Stipić Marković[2]

[1]Children´s Hospital Zagreb
[2]Zagreb Medical School
[3]Special Hospital for Pulmonary Diseases

Aim: To investigate time trends in the prevalence of asthma, allergic rhinitis and atopic dermatitis among schoolchildren of the city of Zagreb in a 16-year period.

Methods: 454 schoolchildren, aged 10 years 0 months-10 years 11 months, from 30 randomly selected schools from the city of Zagreb participated in the study. The study was done using ISAAC (International Study of Allergy and Asthma in Childhood) questionnaire.

Results: Among 454 participants, 225 (49,56%) were girls and 229 (50,44%) were boys. 108 children (23,84 %) had wheezing once in their lives, 26 (5,72%) during last 12 months, 27 (5,06%) reported asthma diagnosis. In comparison with the results from the school-year 2001/2002, there was no statistical difference in the prevalence of asthma variables (p=0.194;0.233;0.808). 190 children (41,94%) had symptoms of allergic rhinitis once in their lives, 162 (35,76%) during last 12 months, 67 children (14,79%) reported diagnosis of allergic rhinitis. There was a statistically significant increase in the prevalence of allergic rhinitis in all three variables (p<0.001;<0.001;0.008) comparing with the earlier study. Finally, 168 children (37,08%) had symptoms of atopic dermatitis once in their lives, 18,1% of them during last 12 months, 140 (31,11%) reported diagnosis of atopic dermatitis. There was also a statistically significant increase in the prevalence of atopic dermatitis in all three variables (p<0.000) comparing with the earlier study.

Conclusion: Our study showed increased prevalence of allergic rhinitis and atopic dermatitis symptoms among schoolchildren from the city of Zagreb in a 16-year period, while the prevalence of asthma symptoms was stable.

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

Carmen Rožmanić[1], Ilija Brizić1, Lea Hiršl[1], Eugene Park[2], Ana Lesac Brizić[1], Berislav Lisnić[1], Vanda Juranić Lisnić[1], Kristina Gotovac[3], Fran Borovečki[3], Wayne M. Yokoyama[2], Astrid Krmpotić[1], Stipan Jonjić[1]

[1]Center for proteomics and Department for histology and embryology, Faculty of medicine, University of Rijeka, Croatia
[2]Division of Rheumatology, Department of Medicine, Washington University School of Medicine, St. Louis, USA
[3]Department for functional genomics, Center for translational and clinical research, School of medicine, University of Zagreb, Croatia

Infections during the early life can have substantially different outcomes as compared to infections of adults. In this study, we have used a mouse model in which new-born mice are infected with the mouse cytomegalovirus (MCMV). We found that viral infection in the early life drives major shift towards terminal maturation of NK cells. Importantly, we found that NK cell function is severely compromised following infection, as observed by their reduced ability to produce cytokines and perform cytotoxic function. This effect was specific for infection during the early life, as the NK cell function of infected adult mice was comparable to control NK cells. Furthermore, active virus replication was required for these effects; however, even infection with heavily attenuated CMVs induced NK cell hyporesponsiveness suggesting that NK cell dysfunction is not due to impaired control of virus in new-born mice. Mechanistically, infection induces suppression of major transcription factors governing NK cell fate and function, such as TCF-1 and Eomes, resulting in impaired NK cell function. Altogether, our data indicate that NK cells are strongly affected by the congenital CMV infection.

CYTOMEGALOVIRUS LIMITS T CELL ACTIVATION AND VIRUS CONTROL VIA ICOS:ICOSL PATHWAY BY DONWREGULATION OF ICOSL FROM THE SURFACE OF ANTIGEN PRESENTING CELLS

Guillem Angulo[1], Jelena Zeleznjak[2,3], Pablo Martínez-Vicente[1,4], Joan Puñet-Ortiz[1,4], Martin Messerle[5], Annette Oxenius[6], Stipan Jonjic[2,3], Astrid Krmpotic[3], Pablo Engel[1,4], and Ana Angulo[1,4*]

[1]Immunology Unit, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain
[2]Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[3]Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia
[4]Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain
[5]Institute of Virology, Hannover Medical School, Hannover, Germany
[6]Institute of Microbiology, Department of Biology, ETH Zürich, Zürich, Switzerland

The inducible co-stimulator (ICOS) is an activating co-stimulatory molecule expressed on activated T cells. Interaction with its ligand – ICOSL, a cell surface protein expressed mostly on antigen-presenting cells (APCs), triggers number of activities including enhanced T cell activation and optimal germinal center (GC) formation. Because of this role, inhibition of ICOS:ICOSL pathway is of utmost importance to many pathogens, including viruses. Here we show that ICOSL is strongly downregulated during murine cytomegalovirus (MCMV) infection of APCs. Concretely, MCMV employs an immunoevasion protein m138 to directly bind ICOSL inside the cell where it drives ICOSL towards lysosomal degradation, therefore preventing its maturation and cell surface expression. As a result, host's ability to control the virus is limited. Importantly, we found that the presence of m138 not only restrain ICOSL-dependent antigen presentation and subsequent ICOS-dependent T cell response, but also regulates the production of MCMV-specific antibodies due to a reduction of T follicular helper and GC B cells. Altogether, these findings reveal a novel tactic of MCMV to limit T cell activation using ICOS:ICOSL pathway. What is more, the importance of above mentioned interaction for the host defence against the viruses was supported by the observation that the complete loss of cell surface ICOSL was followed after the infection of both human CMV (HCMV) and two α-herpesviruses, HSV-1 and HSV-2.

THE INFLUENCE OF IMMUNOLOGICAL CHANGES IN FOLLICULAR FLUID OF WOMEN WITH THYROID AUTOIMMUNIY TO FEMALE INFERTILITY

Silvana Petreić Majnarić[1], Neda Smiljan Severinski[2], Ivana Šutić[3], Ines Mrakovčić-Šutić[4]

[1]Department of Nuclear Medicine, University of Rijeka, Medical Faculty, Rijeka, Croatia
[2]Department of Ginecology and Obstetrics, University of Rijeka, Medical Faculty, Rijeka, Croatia
[3]Department of Family Medicine, University of Rijeka, Medical Faculty, Rijeka, Croatia
[4]Department of Physiology, Immunology and Pathophysiology, University of Rijeka, Medical Faculty, Rijeka, Croatia

INTRODUCTION: Hyper-and hypothyroidism in women in reproductive age are very often accompanied with increased risk for infertility, as well as, adverse pregnancy outcomes. It is still unclear how euthyroid or hyper and hypo thyroid function and thyroid autoimmunity (thyroid antibodies) influence in vitro fertilization (IVF) cycle outcome.

OBJECTIVES: To estimate the influence of the serum concentration of thyroid hormones, antithyroid antibodies, ultrasonographic examination of thyroid gland, the presence of thyroid antibodies and changes in immunological status in follicular fluid on conception and reproductive outcome in infertile couples treated with methods of assisted reproduction.

MATERIALS AND METHODS: The reproductive outcomes were compared between the infertile patients and the patients with normal hormone levels, negative thyroid antibody tests and normal ultrasound findings. The flow cytometry was used to analyse the blood and follicular fluid mononuclear cells in patients treated with IVF.

RESULTS: Patients with autoimmune thyroid disorders treated with IVF, significantly less women conceived and significantly more women experienced spontaneous abortions compared to the women with normal thyroid findings. There was a significantly lower proportion of natural killer and natural killer T cells in the blood and in the follicular fluid of the patients with positive thyroid autoantibodies compared to patients with no thyroid autoantibodies.

CONCLUSION: IVF procedures significantly increase probability of conception in patients with subclinical autoimmune thyroid disorder, but not probability of delivery. The presence of anti-thyroid antibodies and diminished percentage of innate immune cells in follicular fluid may play a critical role in female infertility related to thyroid autoimmunity.

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RANK/RANKL/OPG AXIS DEREGULATION IN A B-CLL PATIENT WITH HYPERCALCEMIA

Rajko Kusec[1,2], Zdravko Mitrovic[1,2], Martina Sedinic[1], Tajana Stoos Veic[1,3], Ana Livun[1], Ozren Jaksic[1,2], Danka Grcevic[2]

[1]Dept of hematology, University hospital Dubrava Zagreb, Croatia
[2]School of medicine University of Zagreb, Croatia
[3]School of medicine University of Osijek, Croatia

A 63 years old female patient presented with severe clinical symptoms of hypercalcemia, and B-lymphocytosis of CD20, Pax5, CD79a, CD5, CD23-positive immunophenotype corresponding to chronic lymphocytic leukemia (B-CLL). Osteolyses were detected inTh12, L1 and L2 vertebrae and diffuse infiltrate with skull parietal bones destruction were found. Parathyroid pathology was excluded. Treatment was initiated with glucocorticoids and bisphosphonates and obinutuzumab (anti-CD20 therapeutic antibody) was given. Patient responded rapidly with normalization of the blood counts, gradual calcemia normalization and significant improvement in clinical performance status. After 4 cycles of obinutuzumab hypercalcemia recurred preceding hematological relapse. Response was again achieved with tyrosine kinase inhibitor ibrutinib when, after 5 months, hypercalcemia with clinical deterioration and dropping blood counts were noted. As the third line therapy a BCL-2 inhibitor, ventoclax was given, but with limited effect marked by low blood counts and therapy refractory hypercalacemia. Ischemic cerebral insult further complicated the clinical course with the fatal outcome. Hypercalcemia accompanying B-CLL has been rarely described. PTHrP as the frequent malignant hypercalcemia causing mediator was excluded in previous studies. We looked at the osteoclast regulating gene expressions of RANK/RANKL/OPG in the peripheral blood of the patient with clonal B-lymphocytosis and hypercalcemia in comparison to a normocalcemic B-CLL and multiple myeloma patient. RANK and RANKL were upregulated in comparison to normocalcemic B-CLL, even at the time of normalizing blood counts and persisting elevated calcium, with higher values at relapse. OPG was increased at relapse. In addition to known involvement of the RANK/RANKL/OPG axis in MM it seems involved in our patient with B-CLL accompanied with osteolysis and hypercalcemia.

ANNOUNCEMENT

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