



European Federation of
Immunological Societies

3rd BELGRADE EFIS SYMPOSIUM ON IMMUNOREGULATION Immunity, Infection, Autoimmunity and Aging



Hotel Izvor, Arandjelovac Spa (Belgrade)

24-27 May 2015



Organizers: Miodrag L. Lukic, Janko Nikolich-Zugich, Stipan Jonjic



Welcome

It is our pleasure to invite you to welcome you in Arandjelovac at the occasion of the 3rd Belgrade EFIS Symposium on Immunoregulation.

As the previous two, the meeting is organized under the auspices and with support of European Federation of Immunological Societies. The meeting will offer the lectures and discussions related to several major topics of contemporary immunology. The list of speakers includes outstanding scientists from Europe, Asia and North America and oral presentations from the participants. There will be also time for poster presentations and discussions with invited speakers.

Arandjelovac Izvor Spa is an ideal place for relaxed and productive scientific meeting and offers pleasant environment for informal discussions.

We have also organized sightseeing of Belgrade.

***Belgrade** is a hospitable city at the confluence of the two major European rivers (Danube and Sava). It is one of the oldest cities in Europe with tumultuous history and vibrant cultural life and entertainment at present.*

Once again, we wholeheartedly welcome you.

**Miodrag L. Lukic
Janko Nikolich-Zugich,
Stipan Jonjic**

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THIS SYMPOSIUM IS SPONSORED BY:

- EFIS European Federation of Immunology Societies
- Center for Molecular Medicine, Faculty of Medical Sciences, University of Kragujevac, Serbia
- Serbian Society of Immunology

PROGRAM

DAY 1: SUNDAY

17.00-17.30 Welcome (M. L. Lukic, J. Nikolich-Zugich, S. Jonjic)

Opening session

Chairs: A. Erdei - J. Nikolich - Zugich

17.30-18.00 Lorenzo Moretta (Institute Giannina Gaslini, Genova, Italy)

- *Natural killer cells: new approaches in the therapy of high risk leukemias*

18.00-18.30 Rene Van Lier (Sanquin Blood Supply Foundation, Amsterdam, The Netherlands)

- *Properties of tissue-resident CD8⁺ memory T cells*

18.30-19.00 Foo Y. Liew (University of Glasgow, Glasgow, UK)

- *The role of cytokines in infections and inflammation*

19.30- **Conference dinner (by invitation)**

DAY 2: MONDAY

07.30-08.30 **Breakfast**

Innate immune response in health and disease

Chairs: R. Van Lier - S. Jonjic

08.30-09.00 Marco Colonna (Washington University, St. Louis, MO, USA) - *Innate lymphoid cells in immunity*

09.00-09.30 Adrian Hayday (Kings College, London, UK) - *Epithelial regulation of intraepithelial T cell repertoires and response*

09.30-10.00 Andrew N. McKenzie (University of Cambridge, Cambridge, UK) - *Type-2 innate lymphoid cells in immunity and disease*

10.00-10.30 Andreas Diefenbach (Spemann Graduate School of Biology and Medicine, Institute of Medical Microbiology & Hygiene, Germany) - *Transcriptional control of innate lymphoid cell fate decisions*

10.30-11.00 **Coffee break**

Lymphocyte repertoire and immune response

Chairs: B. Kyewsky - G. Leposavic

11.30-12.00 Kristin A. Hogquist (University of Minnesota, Minneapolis, MN, USA) - *TCR reactivity in thymic selection*

12.00-12.30 Stephen Jameson (University of Minnesota, Minneapolis, MN, USA) - *The transcription factor KLF2 regulates T cell trafficking and differentiation*

 **3rd BELGRADE EFIS SYMPOSIUM ON IMMUNOREGULATION**
Immunity, Infection, Autoimmunity and Aging

- 12.30-13.00** Yousuke Takahama (Tokushima University, Tokushima, Japan) - *Thymic microenvironments that form T cell repertoire*
- 13.00-13.30** Juan Carlos Zuniga-Pflucker (University of Toronto, Toronto, Canada) - *Initiation of the T-lineage development program in response to Notch signals requires GATA-3 for survival and full commitment of early progenitors*
- 13.30-15.30** **Break and poster viewing**
- 15.30-17.30** **Immunometabolism and glycoimmunology**
Chairs: S. Gay - S. Stosic Grujic
- 15.30-16.00** Marc Donath (University of Basel, Basel, Switzerland) - *Targeting inflammation in treatment of Type 2 diabetes: time to start*
- 16.00-16.30** Yvette Van Kooyk (University of Amsterdam, Amsterdam, The Netherlands) - *Nanotechnology aimed to design DC targeting vaccines for the induction of tumor-immunity*
- 16.30-17.00** Nada Pejnovic (University of Kragujevac, Kragujevac, Serbia) - *Galectin-3 in type 2 diabetes and immunometabolism*
- 17.00-17.30** Bojan Polic (University of Rijeka, Rijeka, Croatia) - *NK cells link obesity-induced adipose stress to inflammation and insulin resistance*
- 17.30-19.00** **Free time**
- 19.00-20.00** **Dinner**
- 20.00-22.00** **Poster discussion with refreshment**

DAY 3: TUESDAY

- 07.30-08.30** **Breakfast**
- 08.30-12.30** **Mechanism of tolerance, autoimmunity and immunopathology**
Chairs: Y. Van Kooyk - M. L. Lukic
- 08.30-09.00** Bruno Kyewski (German Center for Cancer Research, Heidelberg, Germany) - *Self-antigen diversity in the thymus: what thymocytes see and don't see*
- 09.00-09.30** Steffen Gay (University of Zurich, Zurich, Switzerland) - *Epigenetic regulation of inflammation in autoimmune diseases*
- 09.30-10.00** Sergei Nedospasov (Russian Academy of Science, Moscow, Russia) - *Dissecting pathogenic sources of TNF in disease and cell type-restricted cytokine targeting*
- 10.00-10.30** Anna Erdei (Eotvos Lorand University, Budapest, Hungary) - *Crosstalk between complement and Toll-like receptors; regulation of human B cell responses under physiological and autoimmune conditions*
- 10.30-11.00** **Coffee break**
- 11.00-11.30** Burkhard Becher (University of Zurich, Zurich, Switzerland) - *Cytokine networks in autoimmunity: How helper T cells instruct macrophages*



3rd BELGRADE EFIS SYMPOSIUM ON IMMUNOREGULATION
Immunity, Infection, Autoimmunity and Aging

11.30-12.00 Branka Horvat (International Center for Infectiology Research-CIRI, Lyon, France) - *Emerging contagion: immunopathogenesis of henipavirus infection*

12.00-13.30 Short presentations
Chairs: Dj. Miljkovic – M. Colic

Megan Smithey (University of Arizona, College of Medicine, Tucson, USA) - *Aging with MCMV maintains TCR repertoire diversity in late life*

Branka Popovic (Faculty of Medicine, University of Rijeka, Rijeka, Croatia) - *IL-33-dependent immunosuppressive Treg responses to liver damage during MCMV infection*

Vivian Turner (University of Edinburgh, Edinburgh, UK) - *Dysregulated function of marginal zone B cells in aged mice*

Marija Milovanovic (Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia) - *CMV infection in neonatal and adult mice induces susceptibility to EAE in resistant BALB/c mice*

Kamar Sulu Atrekhanly (Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia) - *Systemic TNF ablation results in delayed tumor growth and reduced MDSC accumulation in transplantable tumor model*

Bojan Jevtic (Institute for Biological Research "Sinisa Stankovic", University of Belgrade, Belgrade, Serbia) - *MicroRNA-155 Contributes to Re-activation of Encephalitogenic T Cells*

13.30-14.30 Break and poster reviewing

14.30-17.00 Persistent and long term infections and their impact on aging
Chairs: M. Colonna – B. Polic

14.30-15.00 Beatrix Grubeck-Loebenstern (University of Innsbruck, Innsbruck, Austria) - *Aging and adaptive immunity in the human bone marrow*

15.00-15.30 Arne Akbar (University College London, London, UK) - *The regulation of T cell senescence and metabolism by p38 MAPkinase signaling*

15.30-16.00 Annette Oxenius (Swiss Federal Institute, Zurich, Switzerland) - *Regulation of T cell Immunity during viral infections*

16.00-16.30 Stipan Jonjic (University of Rijeka, Rijeka, Croatia) - *Vaccines and innate immunity: lessons from cytomegalovirus immuno-evasion*

16.30-17.00 Janko Nikolich-Zugich (University of Arizona, Tucson, AZ, USA) - *TGF- β impairs CD4 and antibody responses in old mice to increase Chikungunya virus disease severity and viral persistence*

17.30-24.00 Excursion and conference dinner

 **3rd BELGRADE EFIS SYMPOSIUM ON IMMUNOREGULATION**
Immunity, Infection, Autoimmunity and Aging

DAY 4: WEDNESDAY

07.30-08.30 Breakfast

Short presentation

Chairs: M. Smithey – V. Volarevic

Miljana Momcilovic (Institute for Biological Research “Sinisa Stankovic”, University of Belgrade, Belgrade, Serbia) - *In Vitro Effects of Binuclear (η^5 -p-cymene) Ruthenium (II) Complex Containing Bridging Bis (nicotinate)-Polyethylene Glycol Ester Ligand on Differentiation Pathways of Murine Th Lymphocytes*

Lovro Lamot (University of Zagreb School of Medicine, Zagreb, Croatia) - *TRP channels overexpression contributes to inflammasome activation in clavicular cortical hyperostosis*

Gergely Toldi (Semmelweis University, Budapest, Hungary) - *Impact of aging on calcium influx and potassium channel characteristics of T lymphocytes*

Vladislav Volarevic (Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia) - *Mesenchymal stem cells attenuate acute liver injury mediated by NKT cells*

Microbiota, Autoimmunity Inflammation

Chairs: S. Nedospasov – N. Pejnovic

09.30-10.00 Leo Joosten (University of Nijmegen, Nijmegen, The Netherlands) - *Trained Immunity: consequences for inflammatory diseases?*

10.00-10.30 Hannes Stockinger (Medical University of Vienna, Vienna, Austria) - *Novel macrophage subsets with potential implication in inflammatory diseases*

10.30-11.00 Hartmut Wekerle (Max Planck Institute for Neurobiology, Munich, Germany) - *Ignitions of brain autoimmune diseases in the gut*

11.00-11.30 Ofer Mandelboim (Immunology and Cancer Research Hebrew University, Faculty of Medicine, Jerusalem, Israel) - *Recognition of bacteria by NK cells*

11.30-12.00 Vishwa Deep Dixit (Yale University, New Haven, CT, USA) - *Immunometabolic control of age-related inflammation*

Chairs: V. Pravica-M. Kataranovski

12.00-12.30 N. Avriion Mitchison (University College London, London, UK)

Closing comments

NATURAL KILLER CELLS: NEW APPROACHES IN THE THERAPY OF HIGH RISK LEUKEMIAS

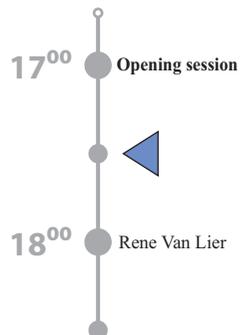
Lorenzo Moretta

Istituto Giannina Gaslini, Genova

NK cells are important effectors of the innate immunity and play a relevant role in tumor surveillance and in defenses against viruses. Human NK cells recognize HLA-class I molecules through inhibitory receptors (KIR and NKG2A) that block NK cell function upon interaction with their HLA ligands. As a consequence, NK cells kill target cells that have lost (or underexpress) HLA-class I molecules as tumors or virus-infected cells. NK cell triggering is mediated by an array of activating receptors and coreceptors that recognize ligands expressed primarily on tumors or virus-infected cells. NK cells have proven particularly useful in the therapy of acute leukemias. Donor-derived “alloreactive” NK cells (i.e. that do not express KIR specific for the HLA-class I alleles of the patient) play a major role in the cure of both adult and pediatrics high risk leukemias. In these patients, donor alloreactive NK cells kill leukemia blasts, thus preventing relapses, and patient's DC, thus preventing graft-versus-host responses. FACS analysis of KIRs expressed by NK cells allows to define the presence and the size of the alloreactive NK subset in potential haploidentical donors (i.e. parents and/or siblings) and to select the best donor. We have recently shown that the expression of activating KIRs, in particular the (HLA-C2-specific) KIR2DS1, may also contribute to donor NK alloreactivity in patients expressing C2 alleles. Importantly, a clear correlation was established between the size of the alloreactive NK cell population and the clinical outcome. In this context, we have also shown that alloreactive NK cells are generated from donor's HSC and persist in patients for long time intervals.

Recently, haplo-HSCT has been further developed with the direct infusion, together with CD34+ HSC, of donor-derived mature alloreactive NK cells and TCRg/d+ T cells (obtained by depletion of TCRA/b+ T cells and CD19+ B cells). Both these cell types contribute to a rapid anti-leukemia effect together with an efficient defense against pathogens during the 6-8 week interval required for the generation of alloreactive NK cells from HSC. The results of this novel approach are particularly promising and further support the usefulness of NK cell-based immunotherapy of otherwise fatal leukemias.

Day 1



René A. van Lier¹, Pleun Hombrink¹ and Klaas van Gisbergen^{1,2}

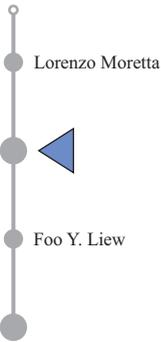
¹Adaptive Immunity Laboratory, Sanquin Research, Amsterdam, Netherlands;

²Div of Molecular Immunology, WEHI, Parkville, Australia

The epithelial cells that line our body are constantly exposed to the external environment. Within the airways, respiratory viruses specifically target epithelial cells as initial site of entry and replication. After infection, a specialized population of tissue-resident memory CD8⁺ T-cells (T_{RM}) resides in the epithelium to maintain constant immune surveillance and protection against recurring respiratory infections^{1,2}. In contrast to other recirculating memory T-cells, Trm in solid tissues do not express CCR7 nor S1P1 that mediate tissue egress allowing Trm retention at peripheral sites. The transcriptional regulation of Trm differentiation is incompletely understood.

We determined the transcriptional profile of T_{RM} from human lung resection samples. A comprehensive set of transcription factors was identified that characterizes lung resident T_{RM}. To this set belongs Hobit (ZNF683), a transcription factor highly homologous to Blimp-1³. Also in mice, Hobit is specifically expressed in CD8⁺ Trm obtained from e.g. gut and skin. As Blimp-1 is co-expressed with Hobit in Trm, we addressed the combined role of Hobit and Blimp-1 in the differentiation of Trm cells using double deficient mice. Hobit and Blimp-1 were essential for the development of skin-resident Trm after infection with HSV-1. In contrast, Hobit and Blimp-1 did not regulate the formation of effector CD8 T-cells within the skin or the formation of memory CD8 T-cells within the spleen. Hobit and Blimp-1 suppressed the expression of CCR7 and S1P1, suggesting that these factors directly regulated tissue retention of Trm. Similar to skin-resident Trm, Hobit was expressed in other tissue-resident lymphocytes including NKT cells, ILC1 and gut Trm after LCMV infection. The maintenance of all of these tissue-resident lymphocytes at peripheral sites required Hobit and Blimp-1. Thus, Hobit is specifically upregulated in tissue-resident lymphocytes in both humans and mice and collaborates with Blimp-1 to maintain these cells within the tissues.

Day 1



¹ Blood and beyond: Properties of circulating and tissue-resident human virus-specific $\alpha\beta$ CD8⁺ T cells. van Aalderen MC, Remmerswaal EB, Ten Berge IJ, van Lier RA. Eur J Immunol. 2014, [Epub ahead of print]

² CD8⁺ T cells with an intraepithelial phenotype upregulate cytotoxic function upon influenza infection in human lung. Piet B, de Bree GJ, Smids-Dierdorp BS, van der Loos CM, Remmerswaal EB, von der Thüsen JH, van Haarst JM, Eerenberg JP, ten Brinke A, van der Bij W, Timens W, van Lier RA, Jonkers RE. J Clin Invest. 2011 Jun;121(6):2254-63

³ Mouse Hobit is a homolog of the transcriptional repressor Blimp-1 that regulates NKT cell effector differentiation. van Gisbergen KP, Kragten NA, Hertoghs KM, Wensveen FM, Jonjic S, Hamann J, Nolte MA, van Lier RA. Nat Immunol. 2012 Sep;13(9):864-71.

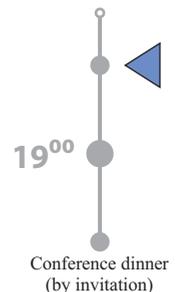
THE ROLE OF CYTOKINES IN INFECTION AND INFLAMMATION

Eddy F Y Liew

Institute of Immunology, Infection and Inflammation, University of Glasgow, Glasgow G12 8AT, UK

Cytokines are hormones of the immune system. Cytokine-targeting represents a major triumph in immunology scientifically, clinically and commercially. There is therefore considerable interest in discovering novel cytokines. I will illustrate the pleiotropic role of some of the novel cytokines by focusing on interleukin (IL)-33. IL-33 is the latest member of the IL-1 family. It is the ligand of ST2, which is expressed mainly on activated Th2 cells, epithelial cells, neuronal cells and mast cells. IL-33 can skew a predominantly Th1 cell population to Th2 cells phenotype in vivo. Furthermore IL-33 potently induces alternatively activated macrophages (M2). IL-33 signals via MyD88, the canonical pathway shared by all members of the IL-1 family. Additionally, IL-33 activates type II cytokines via mTOR (mammalian target of rapamycin). IL-33 mRNA is expressed early during infection of the intestinal-dwelling nematode *Trichuris muris* in mice and IL-33 treatment enhances resistance to *Trichuris* infection by inducing Th2. Furthermore, IL-33 reduces the pathology and mortality of experimental cerebral Malaria infection in mice by activating M2. Importantly, IL-33 also effectively attenuates sepsis by mobilising the innate cells neutrophils, to the site of infection and helps to clear the pathogens. Thus IL-33 is evolutionally preserved for the host defence against infections. However, IL-33 can also induce Type 2 innate lymphoid cells (ILC2), which mediate airway inflammation. Furthermore, IL-33 contributes to the severity of mucocytis accompanying chemotherapy (Irinotecan). Blocking IL-33 prolongs the treatment and effectiveness of chemotherapy in colorectal cancer. In contrast, IL-33 appears to restore the long-term potentiation in a murine model of Alzheimer's disease. Therefore, IL-33 is a double-edged sword and judicious regulation of IL-33 could have considerable potential therapeutic effect.

Day 1



INNATE LYMPHOID CELLS IN IMMUNITY

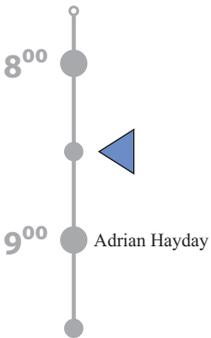
Marco Colonna, M.D.

Washington University School of Medicine
St. Louis, Missouri, USA

SPEAKERS ABSTRACTS

Innate lymphoid cells (ILCs) are a heterogeneous population of immune cells with two defining characteristics: 1) they have lymphoid origin, differentiating from the common lymphoid progenitor (CLP) and dependent on IL-2R γ c signaling. 2) They lack antigen-specific receptors and therefore do not require the RAG proteins for development. Present throughout the body and enriched at mucosal surfaces in both human and mouse, ILCs promptly produce effector cytokines in response to stimulation with STAT-activating cytokines and alarmins of the IL-1 family. Based upon similarities in effector cytokine secretion and developmental requirements, ILCs can be divided into three populations: group 1 ILCs, group 2 ILCs, and group 3 ILCs. IL-12, IL-21, IL-15 and IL-18 activate group 1 ILCs to produce IFN- γ ; this group includes subsets such as T-bet $^+$ Eomes $^+$ NK cells and T-bet $^+$ Eomes $^+$ ILC1. IL-25, thymic stromal lymphopoietin, and IL-33 trigger GATA-3 $^+$ ILC2 to produce type 2 cytokines such as IL-5 and IL-13. IL-23 and IL-1 prompt Ror γ t $^+$ ILC3 to produce IL-22 and/or IL-17. As potent innate cytokine producers that respond to changes in the cytokine microenvironment, ILCs have demonstrated roles in early infection control, adaptive immune regulation, lymphoid tissue development, and in tissue homeostasis and repair. I will illustrate the complexity of ILC subsets, discuss novel functions, focusing on emerging ILCs crosstalk with other immune cells and the microbiota. Furthermore, I will highlight recent insights into the development of ILCs, the common pathways they share as well as points of divergence between ILC groups and subsets.

Day 2



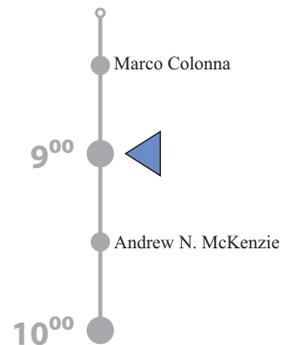
EPITHELIAL REGULATION OF INTRAEPITHELIAL T CELL REPERTOIRES AND RESPONSE

Adrian Hayday

The Francis Crick Institute, CRUK & King's College London.

The thesis of conventional immunology is one of centralised control, whereby the response to infection within tissues is decided within lymph nodes, from which effector T lymphocytes are despatched to quell regional disturbances. But this cannot satisfactorily explain the interaction of the immune system with tissues, since many tissues at steady-state are rich in T cells. Moreover, discrete repertoires of T cells are associated with discrete tissues. These observations raise many questions. For example, what is the biological significance of particular T cells being associated with particular tissues, and how are these mutually compatible immune-stromal compartments determined? To begin to answer these questions, we have adopted a molecular genetic approach, identifying key receptor-ligand axes by which epithelial tissues communicate with their local T cell compartments from their development through to their function at rest and upon tissue dysregulation. These molecular axes offer new insight into immunobiology within tissues in health and disease, with some molecules suggesting new clinical avenues for precisely regulating immune cells in an organ-specific fashion

Day 2



Andrew N.J. McKenzie

MRC Laboratory of Molecular Biology, Cambridge UK

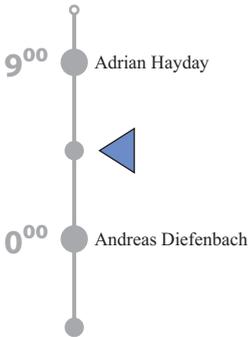
The innate lymphoid cell (ILC) family consists of interferon- γ (IFN- γ -secreting group 1 ILCs (ILC1), type-2 cytokine-producing ILC2s and IL-22 and/or IL-17-positive ILC3s. ILC1 and ILC3 play critical roles in protective immunity against bacteria, intracellular protozoan parasites (ILC1), and fungi (ILC3), and in autoimmune disorders. By contrast ILC2 associate with immune responses to parasitic worms and allergy.

These functionally diverse cytokine-producing cells arise from a common lymphoid progenitor (CLP) under the control of specific transcriptional regulators. These include upstream factors such as inhibitor of DNA binding 2 (Id2), GATA binding protein 3 (GATA3), nuclear factor interleukin-3 (Nfil3), and promyelocytic leukaemia zinc finger (PLZF) that restrict the differentiation of a common helper ILC progenitor (CHILP) from the CLP. Downstream, lineage-specific factors induce lineage commitment: ILC1s require the T-box transcription factor T-bet (*Tbx21*); ILC2s require GATA3 and transcription factor retinoic acid receptor-related orphan nuclear receptor alpha (ROR α); and ILC3s require ROR gamma t (ROR γ t).

Although they comprise only a small proportion of the haematopoietic compartment, ILC2s expand rapidly in response to the epithelium-derived type-2 initiator cytokines IL-25, IL-33 and TSLP, and are a critical innate cellular source of type-2 cytokines. Their secretion of IL-5 and IL-13, but also IL-9, IL-6, IL-4, GM-CSF and amphiregulin induces eosinophilia, mucus production, muscle contractility, and tissue repair responses that are protective against parasitic helminth infection, but also underlie inappropriate allergic inflammation.

Recent studies have shown that ILCs also play roles beyond homeostasis and the acute response to infection or tissue damage, and act additionally to modulate adaptive T cell responses through their expression of MHCII and the presence/absence of additional co-stimulators. Indeed, as new reagents such novel mouse models are developed to study ILCs *in vivo* previously unappreciated roles for ILCs continue to be unearthed. Importantly, the presence of ILCs in human disease offers the possibility of targeting these cells therapeutically.

Day 2



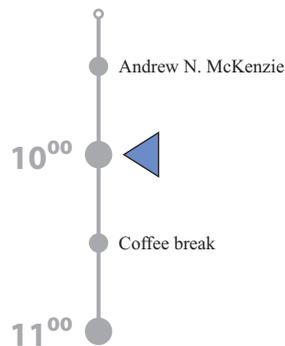
Andreas Diefenbach

Research Centre of Immunology and Institute of Medical Microbiology and Hygiene, University of Mainz
Medical Centre, Obere Zahlbacher Strasse 67, D-55131 Mainz, Germany

Innate lymphoid cells (ILCs) are a recently discovered family of innate lymphocytes that are substantially represented at mucosal surfaces and have been implicated in the protection of epithelial barriers. Various types of ILCs can be discriminated based on the expression of distinct transcription factors controlling the expression of a distinct set of cytokine genes endowing the various ILC subsets with a specific range of effector functions. Currently, three groups of ILCs are being recognized. Group 1 ILCs (ILC1s) are a diverse group of ILCs comprised of natural killer (NK) cells and other, poorly defined subsets of ILCs. It is believed that the ILC1 fate decision is controlled by the T-box transcription factor T-bet endowing ILC1s with the capability to produce large amounts of IFN- γ . ILC2s express high levels of GATA-3, produce IL-5 and IL-13 and have been involved in immunity to helminth infections and in the pathogenesis of allergic diseases. Group 3 ILCs developmentally depend on the transcription factor ROR γ t and produce the cytokines IL-22, IL-17A and IL-17F. ILC3s are believed to be involved in the protection against intestinal bacterial infections and, if inappropriately stimulated, can be important drivers of inflammatory disorders. The transcriptional programs and effector cytokines of the various ILC subsets strikingly resemble those of the various T helper cell effector fates suggesting that such transcriptional circuitry already formed in the evolutionary older innate immune system. The various ILC subsets are developmentally related as all ILC lineages depend on the transcriptional regulator Id2 (inhibitor of DNA binding 2) that interferes with E2 protein-controlled gene expression. This raises the important issue if ILCs may derive from a common ILC progenitor (CILP). Identification of such a progenitor would allow to identify the molecular signals required for the specification of the various ILC lineages. I will discuss progress towards our understanding of the molecular programs regulating ILC fate decisions and our current models of transcriptional stability and plasticity of ILC fates. Finally, I will discuss an unprecedented role of ILC3s in the protection against mucosal virus infections.

Research in my lab is supported by grants from the *European Research Council* (ERC) and *Deutsche Forschungsgemeinschaft*.

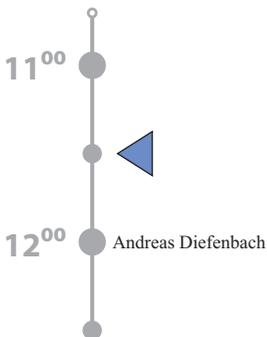
Day 2



Kristin A. Hogquist

Center for Immunology, University of Minnesota, Minneapolis MN

Day 2



The healthy adaptive immune system is responsive to foreign antigens, and tolerant to self. The reactivity of a T cell's receptor for self-peptide MHC plays a determining role in T cell development. T cell signals are integrated with other thymic environmental signals to allow maturation and survival and to facilitate the development of diverse types of T cells, including naïve CD4 and CD8 T cells, nTreg cells, iNKT cells, and nIELs. Cells that receive a low affinity signal during development are positively selected, and migrate to the medulla where they continue to undergo negative selection, functional maturation, and emigration to peripheral lymphoid organs. We sought to gain further insight into the functional maturation process that occurs after positive selection. By comprehensive flow cytometric analysis of RAG2GFP mice, we define the precise stage at which medullary thymocytes acquire the competence to proliferate when stimulated through the TCR. We also show that they become resistant to TNF as they mature. Using microarray analysis we identified gene changes unique to the later stages of thymic maturation. Gene set enrichment analysis suggested these genes are regulated by NF- κ B and IRF transcription factors. We examined a number of mutant mice that exhibit peripheral T cell lymphopenia. Of these, mice deficient in TAK1, a key signaling component of NF- κ B and MAPK signaling pathways, show normal positive selection, but a profound and specific block in functional maturation. Restoration of the NF- κ B signaling arm using a constitutively active IKK β mutant restored the competence to proliferate and emigrate, but did not allow peripheral T cell survival. Alternatively, IFN receptor deficiency resulted in phenotypic abnormality of mature thymocytes, but competence to proliferate was normal. In summary, both NF- κ B dependent and independent signals downstream of TAK1 are required in medullary thymocytes for the final maturation of positively selected thymocytes into naïve T cells that can proliferate and survive outside the thymus.

Funding source: RO1 AI088209

THE TRANSCRIPTION FACTOR KLF2 REGULATES T CELL TRAFFICKING AND DIFFERENTIATION

Stephen C. Jameson

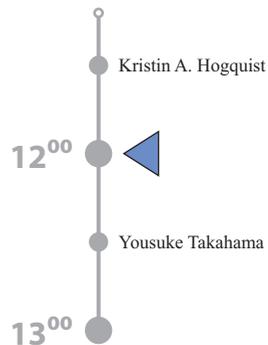
Center for Immunology, Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis MN 55414.

Previous studies showed that the transcription factor Kruppel-like factor 2 (KLF2) is essential for egress of mature T cells from the thymus and for peripheral recirculation of naïve T cells through lymphoid tissues. However, less is known about the function of KLF2 in regulating antigen-specific responses of T cells and the establishment of T cell memory. In studies on CD8+ T cells, we found that KLF2 is important for the generation of prototypical “resident memory” T cells (T_{RM}) – a population that is maintained in non-lymphoid tissues and has been shown to play the major role in protective immunity against infections at barrier tissue sites. Forced expression of KLF2 opposes the maintenance of T_{RM} , and these effects can be mimicked by induced expression of a key transcriptional target of KLF2, S1PR1. In addition, we found that differentiation of CD4+ T cells toward the follicular helper T cell (Tfh) fate is opposed by KLF2. As with T_{RM} generation, S1PR1 expression contributes to inhibition of Tfh generation: however, a more important pathway is KLF2 induction of the Blimp-1 transcription factor (which, through inhibition of Bcl-6 expression, blocks Tfh differentiation). In addition, KLF2 can promote expression of T-bet and GATA3 (involved in differentiation of Th1 and Th2 helper T cell subsets, respectively). Our studies indicate that KLF2 expression is influenced by multiple factors, including cytokine and costimulatory receptors, although regulation of the PI3K pathway is emerging as a core mechanism for control of KLF2 expression.

Hence, for both CD4+ and CD8+ T cells, expression of KLF2 is pivotal for control of distinct but complementary aspects of T cell differentiation, via regulation of T cell localization and expression of lineage defining transcription factors. These data suggest KLF2 serves to direct the fate of post-activated T cells, shaping the distribution and function of effector and memory T cell populations.

This work was supported by NIH award R37 AI-38903 (SCJ)

Day 2



THYMIC MICROENVIRONMENTS THAT FORM T CELL REPERTOIRE

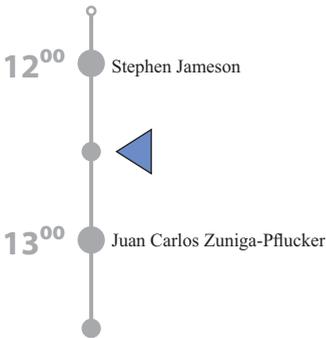
Yosuke Takahama

University of Tokushima, Tokushima, Japan

SPEAKERS ABSTRACTS

During development in the thymus, newly generated repertoire of diverse TCR-ab recognition specificities in immature T cells is selected to form the functionally competent and self-tolerant repertoire of mature T cells. Positive selection supports the survival of potentially useful self-MHC-restricted thymocytes upon low-affinity TCR engagement, whereas negative selection deletes potentially harmful self-reactive thymocytes upon high-affinity TCR engagement. Recent advances in the biology of thymic stromal cells have indicated that T cell formation in the thymus requires positive selection by thymic cortical epithelial cells that express unique protein degradation machineries, including the b5t-containing thymoproteasome. It has also been revealed that the proximal interplay among developing T cells, dendritic cells, and medullary epithelial cells that promiscuously express tissue-specific self-antigens is essential for the establishment of self-tolerant TCR repertoire and the generation of regulatory T cells. These results suggest the vital roles played by self-peptides specifically expressed by multiple thymic microenvironments in the development of functionally competent and self-tolerant T cells.

Day 2



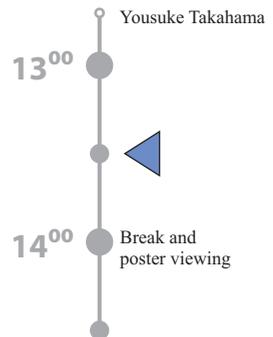
THE TRANSCRIPTION FACTOR KLF2 REGULATES T CELL TRAFFICKING AND DIFFERENTIATION

Juan Carlos Zúñiga-Pflücker

Department of Immunology, University of Toronto, and Sunnybrook Research Institute, Toronto, Ontario, Canada

The zinc-finger transcription factor, GATA-3, plays a crucial role during early T-cell development and also dictates later T-cell differentiation outcomes. However, its role and collaboration with the Notch signaling pathway in the induction of T-lineage specification and commitment have not been fully elucidated. We show that GATA-3 deficiency in hematopoietic progenitors results in an early block in T-cell development despite the presence of Notch signals, with a failure to up-regulate Bcl11b expression, leading to a diversion along a myeloid, but not a B-cell, lineage fate. GATA-3 deficiency results in dysregulated Cdkn2b expression, leading to apoptosis of early T-lineage cells due to inhibition of CDK4/6 function. We also show that GATA-3 induces Bcl11b, and together with Bcl11b represses Cdkn2b expression. Our findings provide a signaling and transcriptional network by which the T-lineage program in response to Notch signals is realized.

Day 2



TARGETING INFLAMMATION IN TREATMENT OF TYPE 2 DIABETES: TIME TO START

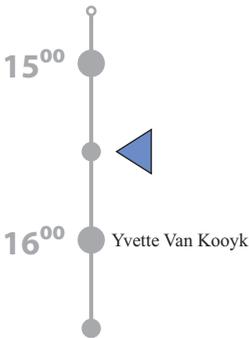
Marc Y Donath

University Hospital Basel, Switzerland

SPEAKERS ABSTRACTS

Onset of Type 2 diabetes occurs when the pancreatic beta-cell fails to adapt to the increased insulin demand caused by insulin resistance. Morphological and therapeutic intervention studies have uncovered an inflammatory process in islets of patients with Type 2 diabetes characterized by the presence of cytokines, immune cells, beta-cell apoptosis, amyloid deposits, and fibrosis. This insulinitis is due to a pathological activation of the innate immune system by metabolic stress and governed by IL-1 signaling. We propose that this insulinitis contributes to the decrease in beta-cell mass and the impaired insulin secretion observed in patients with Type 2 diabetes. Initially, the inflammatory response is probably deployed to promote beta-cell repair and regeneration. Yet, as it becomes chronic activation of auto-inflammatory processes may then become deleterious. Furthermore, IL-6 emerges as an additional regulator of islet secretory function. Indeed, IL-6 mediates a cross talk between fat, muscles and pancreatic islets to adapt to changes in insulin demand via secretion of the incretin glucagon-like peptide-1. Thereby, IL-6 reprograms the alpha cells to produce glucagon-like peptide-1. This implicates IL-1 beta and IL-6 in the regulation of beta cell insulin secretion in both health and disease. It follows that modulation of inflammatory mediators, may present as a possible causal therapy with disease-modifying potential.

Day 2



NANOTECHNOLOGY AIMED TO DESIGN DC TARGETING VACCINES FOR THE INDUCTION OF TUMOR-IMMUNITY

Yvette van Kooyk

Department of Molecular Cell Biology and Immunology, VU University medical Centre, v.d. Boechorststraat 4, 1081 BT Amsterdam, the Netherlands

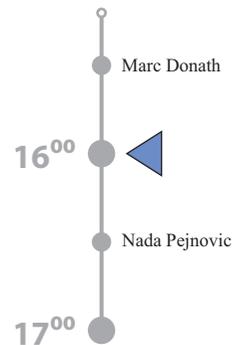
Dendritic cells (DC) are specialized in the recognition of antigen and play a pivotal role in the control of immunity and tolerance. For antigen recognition DC express several C-type lectins, that function as innate receptors that facilitate antigen uptake and cross-presentation. Many of these receptors also modify responses through signalling interference with TLR. The natural ligands of C-type lectins are specific glycan structures that can be found on pathogens but also on tumors. These glycan structures can either contribute to the tumor suppressive programming of the immune system, or oppositely activate the immune system.

Moreover synthetic coupling of glycans to antigens can favour directional targeting of tumor antigens to human DC and Langerhans Cells (LC) in situ, in the skin, facilitating the induction of tumor specific CD4+ and CD8+ T cell responses and Th1 differentiation. Therapeutic vaccination of mice with glycan modified antigen show long term anti-tumor immunity when tumor induced T regulatory cells are temporarily reduced.

We identified that the glycosylation state of tumors may directly regulates immune escape. In particular high sialylation of tumors results in increase of FoxP3 CD4+ T cells (Treg) and lower frequencies of effector T cells. Lowering the sialylation of tumors converts the frequencies Treg/Teff to favourable anti-tumour immunity.

Our work sheds new light on the contribution of glycans to control tumour immunity by either contributing to the induction of anti-cancer immunity or tumour induced immune suppression.

Day 2

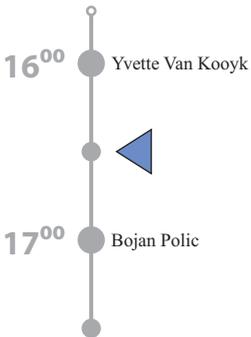


Nada Pejnovic

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Obesity, via gain of ectopic fat and by inducing metaflammation, promotes insulin resistance, hepatic steatosis, and β cell failure thus representing the major risk factor for type 2 diabetes and nonalcoholic fatty liver disease (NAFLD). Galectin-3 (Gal-3), a β -galactoside-binding lectin, modulates immune/inflammatory responses and participates in a spectrum of diseases with sometimes opposite functions. The evidence so far including our own data shows a protective role for Gal-3 in obesity and diabetes. Galectin-3 ablation in a setting of chronic overnutrition induces an increased visceral adipose tissue (VAT) and pancreatic inflammatory response, hyperglycemia and insulin resistance. Enlarged VAT in Gal-3 deficient mice fed high-fat diet contains more numerous proinflammatory myeloid cells, Type-1 T and NKT cells and increased phosphorylated-NF- κ B p65 protein expression. Macrophage infiltration of islets, accumulation of AGE and increased expression of RAGE, NLRP3 inflammasome, IL-1 β and phosphorylated-NF- κ B p65 were present in the absence of Gal-3. Thus, Gal-3 controls excessive activation of NF κ B and NLRP3 inflammasome in islets and VAT and downstream inflammation induced by metabolic stimuli. Gal-3 deficient mice developed more pronounced high-fat diet induced liver steatosis and increased prosteatotic PPAR- γ , Cd36, Abca-1 and FAS in livers. However, hepatocellular damage, inflammation and fibrosis were lower in Gal-3 deficient mice which had less numerous proinflammatory myeloid cells in livers, peripheral blood and bone marrow, and decreased expression of genes related to inflammation, oxidative stress and fibrogenesis. Hepatic IL-33, ST2 and IL-13 mRNA levels were lower in the Gal-3 absence. Exogenous IL-33 failed to induce ST2 upregulation and IL-13 production by CD11b⁺ myeloid cells *in vitro* and *in vivo*. *In vivo* administered IL-33 enhanced liver fibrosis in high-fat diet-fed mice in both genotypes, albeit to a significantly lower extent in Gal-3 deficient mice. Thus, Gal-3 attenuates steatosis, but promotes liver injury, inflammation and IL-33 dependent liver fibrosis. Gal-3 is an important regulator of obesity-associated immunometabolic disorders.

Day 2



NK CELLS LINK OBESITY-INDUCED ADIPOSE STRESS TO INFLAMMATION AND INSULIN RESISTANCE

Felix M Wensveen¹, Vedrana Jelenčić¹, Sonja Valentić¹, Marko Šestan¹, Tamara Turk Wensveen², Sebastian Theurich³, Ariella Glasner⁴, Davor Mendrila⁵, Davor Štimac², F. Thomas Wunderlich³, Jens C Brüning³, Ofer Mandelboim⁴, **Bojan Polić¹**

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

²Department of Internal Medicine, University Hospital Rijeka, Rijeka, Croatia;

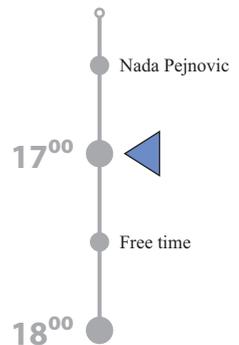
³Max Planck Institute for Metabolism Research Cologne, Cologne, Germany;

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⁵Department of Surgery, University Hospital Rijeka, Rijeka, Croatia

Obesity is an increasingly common health issue that predisposes people to metabolic disorders such as insulin resistance (IR), which can progress to diabetes mellitus type 2 (DM2). An important underlying cause of obesity-induced IR is chronic systemic inflammation derived from accumulating pro-inflammatory macrophages in visceral adipose tissue (VAT). Currently, it is unknown which signal initiates adipose tissue macrophage (ATM) activation in VAT. We find that a phenotypically distinct VAT-resident NK cells provide a crucial link between obesity-induced adipose stress and ATM activation in VAT. Ligands for the NK cell-activating receptor NKp46/Ncr1 are expressed in human and mouse VAT. Feeding with high-fat diet causes up regulation of Ncr1-ligands on adipocytes, leading to localized activation and cellular increase of NK cells. IFN γ produced by these cells drives early pro-inflammatory macrophage differentiation and promotes obesity-induced insulin resistance. Lack of NK cells, Ncr1 or IFN γ prevents macrophage activation in VAT and greatly ameliorates glucose tolerance and insulin sensitivity. Therapeutic blocking of Ncr1-signaling forestalls ATM activation. Our study identifies NK cells as key regulators of macrophage polarization and insulin resistance in response to obesity-induced adipose stress. The NK-ATM axis therefore provides an attractive new target for early treatment of patients with metabolic syndrome to prevent progression to DM2.

Day 2



SELF-ANTIGEN DIVERSITY IN THE THYMUS: WHAT THYMOCYTES SEE AND DON'T SEE

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In the course of central self-tolerance induction a highly diverse repertoire of T cell receptors (TCRs) is probed against a matching array of their ligands, namely self-peptide/MHC complexes. While much has been learnt about the molecular mechanisms underlying the generation of TCR diversity, the cellular and molecular strategies responsible for intra-thymic expression and presentation of self-antigen repertoires are less well understood. The diversity of self-peptide display is, on the one hand, afforded by the remarkable heterogeneity of thymic antigen presenting cells (APCs) and, on the other hand, by their unconventional molecular pathways of antigen expression, processing and presentation (1). Here I will elaborate on one mechanism, namely promiscuous gene expression (pGE). pGE denotes the property of a specific subset of thymic stromal cells - medullary thymic epithelial cells (mTECs) – to express a highly diverse set of tissue-restricted antigens (TRAs) representing essentially all tissues of the body. This allows self-antigens, which otherwise are expressed in a spatially or temporally restricted manner to become continuously accessible to developing T cells. Failure of pGE at the quantitative or qualitative level will result in holes central tolerance and heightened susceptibility for organ-specific autoimmunity, e.g. type 1 diabetes mellitus, rheumatoid arthritis or autoimmune myocarditis.

1. Klein, L, B. Kyewski, P.M. Allen and K.A. Hogquist. 2014. Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). *Nature Rev Immunology* 14, 377-391. Recent studies show that each APC subset appears to carry its specific antigen cargo as a result of cell-type specific features: firstly, transcriptional control (i.e. promiscuous gene expression in medullary thymic epithelial cells); secondly, antigen processing (i.e. proteasome composition and protease sets); thirdly, intracellular antigen sampling (i.e. autophagy in thymic epithelial cells) and fourthly, extracellular antigen sampling (i.e. immigrating dendritic cells sampling extrathymic milieu). The combinatorial expression patterns of these attributes in distinct APC subsets result in a self-peptide display partly unique to the cortex mediating positive selection and to the medulla mediating tolerance induction.

The scope of central tolerance is to a large measure dictated by the pool of promiscuously expressed genes.

Thus even lack of a single TRA can result in spontaneous organ-specific autoimmunity. Promiscuously expressed genes, which have no structural or functional commonality, display two prominent features, they are highly clustered in the genome and show a preference for TRAs. In order to better understand these features, we set out to more precisely define the genomic organization of this gene pool. In particular, we probed to which extent and according to which rules predefined genomic clusters of TRAs are transcribed in mTECs. Our analysis proceeded from the bio-informatic definition of TRA clusters via gene expression analysis in mTECs using whole genome arrays to the in depth analysis of selected TRA clusters by RT-PCR at the population and single cell level. Patterns emerging from these studies will hopefully yield insight into molecular and evolutionary mechanisms responsible for selecting this gene pool. Conceivably, positional cues in the genome and/or particular properties of self-antigens (e.g. immunogenicity) could have been driving forces during the co-evolution of pGE and adaptive immunity.

EPIGENETIC REGULATION OF INFLAMMATION IN AUTOIMMUNE DISEASES

Steffen Gay

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Epigenetics has emerged to be a key regulator of gene expression in health and disease. Most interesting has been the question how epigenetic information might be maintained through DNA replication. In this regard it has been discussed that histone-modifying enzymes might remain associated with the DNA through replication to translate the epigenetic information to the newly-assembled chromatin (1).

Our laboratory has been studying the epigenetic modifications over the past 15 years in inflammation in general and in particular in rheumatoid arthritis (RA) (2,3), progressive systemic sclerosis (scleroderma), pulmonary hypertension and ankylosing spondylitis. This work was supported by EC grants, including FP6 Autocure, FP7 Masterswitch, Osteoimmune, TEAM and the IMI 2 project BeTheCure (BTCure)

The complexity of epigenetics is illustrated by the involvement of multiple regulatory biochemical and biological processes, such as acetylation, methylation, phosphorylation, sumoylation and non-coding RNAs, including microRNAs (miR), and long non-coding RNAs (lncRNA).

The latest results from our laboratory are documented by the findings that bromodomains 2, 3 and 4 are expressed in the RA synovium and can be targeted with selective BRD inhibitors (4) to reduce the production of proinflammatory cytokines and matrix-metalloproteinases (MMP). The earliest studies on hypomethylation of synovial fibroblasts (RASf) (1) have been followed up (2) and most recently resulted in a novel therapeutic concept to silence the aggressive phenotype of RASf (5).

One of our greatest surprises in our current research has been the observation that SF differ in their phenotype depending from the localization in the body through the differential expression of miRs and lncRNAs (6).

Based on the fact that we are not able to cure RA, despite all the impressive results with biologicals and small molecules targeting inflammatory cytokines and signaling, it needs to be stressed that the epigenome of the RASf is an important target to possibly cure RA (7).

- 1) Budhavarapu VN, Chavez M, Tyler JK. How is epigenetic information maintained through DNA replication? *Epigenetics & Chromatin* 6:32, 2013
- 2) Neidhart M et al. Retrotransposable L1 elements expressed in rheumatoid arthritis synovial tissue - Association with genomic DNA hypomethylation and influence on gene expression. *Arthritis Rheum* 43: 2634-2647, 2000
- 3) Klein K, Gay S. Epigenetics in rheumatoid arthritis. *Curr Opin Rheumatol* 27:76-82, 2015
- 4) Klein K et al. The bromodomain protein inhibitor I-BET151 suppresses expression of inflammatory genes and matrix degrading enzymes in rheumatoid arthritis synovial fibroblasts. *Ann Rheum Dis*. 2014 [Epub ahead of print]
- 5) Neidhart M et al. Inhibition of spermidine/spermine N1-acetyltransferase (SSAT1) activity - a new therapeutical concept in rheumatoid arthritis. *Arthritis Rheum* 66:1723-1733, 2014
- 6) Ospelt C et al. Joint specific positional differences in coding and noncoding transcriptome of synovial fibroblasts as a determinant of the susceptibility of synovial joints to rheumatoid arthritis. *Arthritis Rheum* 66:S1216, 2014
- 7) Frank-Bertoncelj M, Gay S. The epigenome of synovial fibroblasts: an underestimated therapeutic target in rheumatoid arthritis. *Arthritis Res Ther* 16:117, 2014

DISSECTING PATHOGENIC SOURCES OF TNF IN DISEASE AND CELL TYPE-RESTRICTED CYTOKINE TARGETING

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²Lomonosov Moscow State University, Moscow, Russia

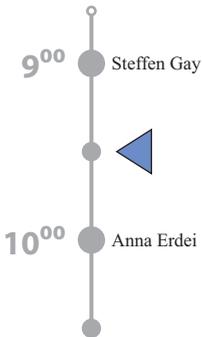
³Lobachevsky University, Nizhni Novgorod, Russia

Proinflammatory cytokines TNF, IL-6 and IL-1 may contribute to pathogenesis of autoimmunity and therefore systemic inhibition of these cytokines became an essential part of the therapy for systemic autoimmune diseases. Our recent studies in experimental arthritis suggest that TNF from particular cellular sources may play an anti-inflammatory role. If so, systemic cytokine inhibition may be like a double-edged sword disrupting both pathogenic and protective signaling.

Based on these findings we are developing an approach to cell type-restricted cytokine neutralization by utilizing bispecific antibody constructs that would attach to the cell surface of a particular type of immune cells, and capture the cytokine released by these cells. Our constructs are based on single domain antibodies (VHH) specific for human TNF and for cell type-specific markers, in particular, for F4/80 surface molecule expressed on macrophages. We find that such antibodies can effectively attach to the cell surface, capture and retain released cytokines preventing their dissemination. Using mice humanized for the TNF system and with macrophages isolated from such mice we assessed activity of these constructs *in vitro* and *in vivo*. Our findings may serve as a basis for bioengineering of new type of cytokine inhibitors.

Supported by Russian Scientific Fund grant 14-25-00160 and Russian Ministry of Science and Education grant 14.Z50.31.0008

Day 3



CROSSTALK BETWEEN COMPLEMENT AND TOLL-LIKE RECEPTORS; REGULATION OF HUMAN B CELL RESPONSES UNDER PHYSIOLOGICAL AND AUTOIMMUNE CONDITIONS

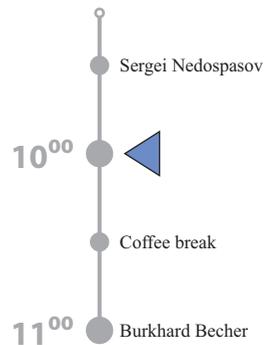
Anna Erdei

Immunology Department, Eötvös University, Budapest, HUNGARY

The complement system and TLRs are two major components of innate immunity which provide a fast first-line defence against harmful agents. Since many pathogen associated molecular patterns are able to activate both arms of the innate immune system, their effect should be investigated simultaneously under physiological as well as pathological conditions. We have shown earlier that complement receptor 1 (CR1, CD35) is a potent inhibitor of the B cell receptor (BCR)-induced functions of human B cells (Jozsi et al, JI, 2002, Kremlitzka et al, Int.Immunol. 2013). Now we found that ligation of CR1 on B cells treated with the TLR9-ligand CpG oligonucleotide strongly reduced the BCR-induced activation, proliferation and Ig-production of the cells. Although in SLE and RA patients the number of CR1 molecules is markedly diminished on B cells, clustering of this complement receptor resulted in efficient inhibition of the major B cell functions similarly to healthy controls.

Our results reveal a novel link between complement and Toll-like receptors in the regulation of humoral immunity and pave the way for developing a new tool for the inhibition of autoreactive B cells.

Day 3



CYTOKINE NETWORKS IN AUTOIMMUNITY: HOW HELPER T CELLS INSTRUCT MACROPHAGES

Burkhard Becher

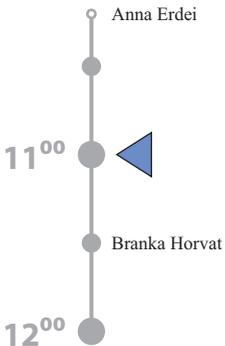
University of Zurich, Switzerland

SPEAKERS ABSTRACTS

There is a long-standing debate as to the role and function of T cell polarization in autoimmune disease. We call pathogenic T cells TH_{path} cells based on their ability to cause tissue inflammation. It is becoming increasingly clear that none of the previously coined TH cell polarization patterns (TH1, TH2 or TH17) fulfil the criteria of defined TH_{path} cells.

In other words, how a pathogenic molecular signature is translated into the ability to trigger autoimmune tissue inflammation is a subject of great debate. I will discuss a few of the features of TH_{path} cells, the role of cytokines in autoimmune disease and propose that TH_{path} cells themselves must recruit myeloid cells into the inflamed tissue to mediate tissue damage. But exactly how TH_{path} cells achieve this feat is the subject of my seminar in which I will discuss a number of recent important observations made by immunologists.

Day 3



EMERGING CONTAGION: IMMUNOPATHOGENESIS OF NIPAH VIRUS INFECTION

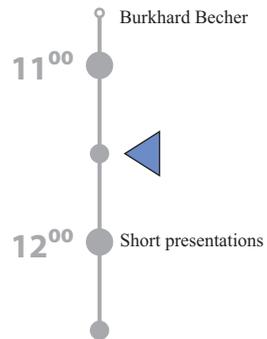
Branka Horvat

International Center for Infectiology Research (CIRI), Inserm U1111, CNRS, UMR5308, ENS-Lyon, University Lyon 1, Lyon, France

Nipah virus (NiV) is a highly pathogenic zoonotic paramyxovirus of Henipavirus genus that causes human outbreaks annually in South-East Asia. Similarly to previous Ebola occurrences, the Henipavirus outbreaks remain sporadic until now and seem to affect only small areas; however, NiV may have a global pandemic potential and is an agent of particular concern in the field of human and agricultural biodefense.

Immunopathogenesis of this recently emerged virus is still poorly understood. Although lymphocytes are not susceptible to Henipavirus infection, they bind efficiently the virus and transinfect endothelial and Vero cells even several days after binding. This transinfection is mediated by heparan sulfate and could be inhibited with heparin, opening thus novel perspectives for the development of heparan sulfate–targeting therapeutic approaches against these emerging infection. We have further analyzed the susceptibility of different type of mice, bearing defects in either innate or adaptive immune system, to NiV infection. In contrast to wild-type, mice deficient for type-I interferon (IFN-I) receptor were shown to be highly susceptible to NiV. Although viral sensing through either TLR or RLR alone was not critical in anti-viral defense, mice devoid in both TLR and RLR signaling succumbed to the infection, with similar survival rate as IFN-I deficient mice. Utilization of mice with tissue-specific deletion of IFN-I receptors suggested that IFN-I signaling in any of single cell population, including macrophages, dendritic cells (DC), natural killer cells and neurons, neither in plasmacytoid DC - specialized in IFN-I production, is not crucial for the protection from lethal NiV infection. Interestingly, presence of T-cell but not B-cell compartment was critical in allowing resistance to the infection, and this effect was independent on perforin production. Finally, depletion of macrophages allowed rapid systemic propagation of NiV infection and high lethality in mice, suggesting their important role at the crossroads between innate and adaptive immunity. Altogether, these results revealed some novel aspects of immuno-regulation of NiV infection, which could help in development of new strategies to control this highly lethal infectious disease.

Day 3



AGING AND ADAPTIVE IMMUNITY IN THE HUMAN BONE MARROW

Beatrix Grubeck-Loebenstein

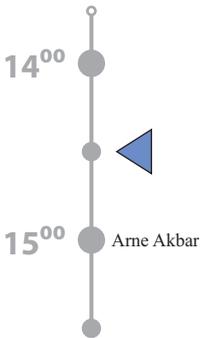
Institute for Biomedical Aging Research, University of Innsbruck, Innsbruck, Austria

SPEAKERS ABSTRACTS

With aging the immune system undergoes significant age-related changes. These age-dependent changes are referred to as immunosenescence and are partially responsible for the poor immune response to infections and the low efficacy of vaccination in elderly persons. Immunosenescence is characterized by a decrease in innate and adaptive cell-mediated immune function in the peripheral blood and the bone marrow.

The aging of bone marrow cells and in particular, of adaptive immune cells in the bone marrow has been addressed relatively rarely. Age-related changes of human bone marrow, T, B and plasma cells will be discussed in this talk.

Day 3



THE REGULATION OF T CELL SENEESCENCE AND METABOLISM BY P38 MAPKINASE SIGNALLING

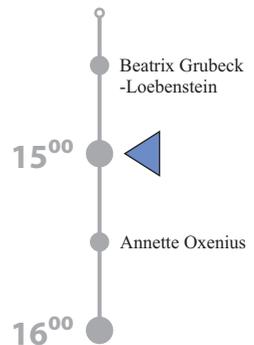
Lanna A, Henson SM and Akbar AN

Division of Infection and Immunity, University College London, U.K.

Persistent viral infections, ageing and inflammatory syndromes induce the accumulation of senescent human T cells. However the mechanism that regulates the function of these end-stage cells is unclear. Human CD8⁺ effector memory T cells that re-express CD45RA (CD27⁺CD45RA⁺; EMRA) express surface KLRG-1 and CD57, exhibit reduced replicative capacity and telomerase activation, decreased survival and high expression of nuclear gH2AX after T cell receptor (TCR) activation. We investigated the involvement of p38 MAP kinase signalling in these senescence characteristics of these cells. The expression of both total and phosphorylated p38 was highest in the EMRA compared to other CD8⁺ T cell subsets. Furthermore, the inhibition of p38 signalling, especially in CD4⁺ EMRA T cells, significantly enhanced their telomerase activity and survival after TCR activation. EMRA T cells preferentially utilize glycolysis to fuel their effector functions. The inhibition of p38 enhances mitochondrial function, reduces reactive oxygen species production and increases proliferation and telomerase activity, however, these cells still utilize glycolysis instead of oxidative phosphorylation for energy. The precursors for glycolysis are generated by autophagy, that is increased after p38 blockade. Thus activation of the p38 MAPK pathway is directly involved in the senescence characteristics of highly-differentiated CD4⁺ T cells and this pathway also regulates the metabolic function in this population.

Henson SM et al J. Clin Invest. 2014.

Day 3



T CELL CONTROL OF CYTOMEGALOVIRUS INFECTION IN GLANDULAR TISSUES

Jenny Thom and Annette Oxenius

Institute of Microbiology, ETH Zurich, Zurich, Switzerland

SPEAKERS ABSTRACTS

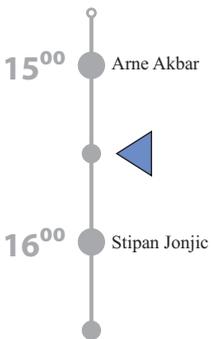
Infecting 60-90% of the world population, human cytomegalovirus (HCMV) is a pathogen of high epidemiological relevance and represents a major cause of morbidity and mortality in immunocompromised individuals. CMV maintains its existence in the population by prolonged replication in and secretion from mucosal tissues, including the salivary glands (SG) which supports viral replication for months after virus is controlled in all other organs.

Experimental infection of mice with murine CMV (MCMV) has served to explore tissue-specific control of CMV infection and reveals that virally mediated MHC class I down-regulation in SG-resident acinar glandular epithelial cells renders this tissue uniquely resistant to CD8 T cell mediated virus control. Instead, CD4 T cells are strictly required to cease virus replication during primary CMV infection. Both CMV-specific CD4 and CD8 T cells gradually acquire a tissue resident memory T (T_{RM}) cell phenotype in the SG, with the inductive requirements differing between the two subsets.

Upon intraglandular reinfection the CMV-specific CD8 T_{RM} cells - and not CD4 T_{RM} cells - confer immediate protection. The reasons for this apparently disparate requirement of CD4 and CD8 T cells conferring protection in SG tissue during primary and secondary CMV infection will be discussed.

Grant funding: This work was supported by ETH Zurich, the Swiss National Science Foundation and the ETH Foundation.

Day 3



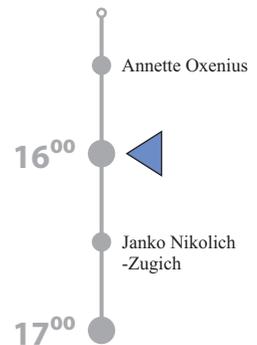
VACCINES AND INNATE IMMUNITY: LESSONS FROM CYTOMEGALOVIRUS IMMUNOEVASION

Stipan Jonjić

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

Cytomegalovirus (CMV) establishes life-long infection of its host, ensuring continuous supply of effector memory CD8⁺ T cells. CMVs possess numerous immunoevasion genes able to modulate basically any part of immune response, including NK cell and CD8⁺ T cell response. It is well established that deletion of these viral inhibitors leads to virus attenuation *in vivo*. These features make CMV a very attractive CD8⁺ T cell vaccine-vector candidate. Control of CMV infection is in great part dependent on NKG2D, an activating receptor when expressed on NK cells and costimulatory one when expressed on CD8⁺ T cells. We have constructed highly attenuated mouse CMV (MCMV) expressing NKG2D ligand RAE-1 γ inserted in place of its viral inhibitor (Slavuljica et al, 2010) and foreign CD8⁺ T cell epitope as well (Trsan et al, 2013). Such a recombinant vaccine-vector provided outstanding and long-lasting CD8⁺ T cell-mediated protection against challenge infections. Moreover, RAE-1 γ MCMV-vector circumvented MCMV interference of antigen presentation, improved antigen presentation to CD8⁺ T cells and potentiated memory CD8⁺ T cell response. Surprisingly, these immuno enhancing properties of RAE-1 γ expressing MCMV vector were retained even in NKG2D deficient mice, pointing to additional NKG2D-independent immune function of RAE-1 γ . In my talk, I will discuss the capacity of MCMV expressing RAE-1 γ as a vaccine vector against other pathogens, as well as tumors.

Day 3



TGF- β IMPAIRS CD4 AND ANTIBODY RESPONSES IN OLD MICE TO INCREASE CHIKUNGUNYA VIRUS DISEASE SEVERITY AND VIRAL PERSISTENCE

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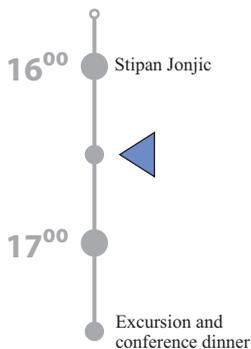
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Chikungunya virus (CHIKV) is a mosquito-borne Alphavirus endemic to Africa and Asia, which causes sudden onset of fever, rash and debilitating poly-arthritis in peripheral joints that can persist for years, particularly in older individuals. In 2013, CHIKV spread to the western hemisphere resulting in more than one million cases in the Caribbean. Autochthonous transmission in the United States was confirmed in 2014. In response to the increased geographic distribution of CHIKV and the likelihood that elderly immune-naïve populations may experience severe and life-threatening disease, we have developed a mouse model of age-related vulnerability to CHIKV infection. We demonstrate reduced ability of old mice to mount effective immune responses to CHIKV and control viremia, leading to increased disease severity and viral persistence in the joints. Ineffective immune responses were due, in part, to dyscoordinated cytokine production. Specifically, CXCL9 and TGF β , were identified as key contributors to impaired CD4 and antibody responses against viral epitopes in old mice. Moreover, neutralization of TGF β reduced acute joint swelling, restored Ab responses, and virtually eliminated chronic joint pathology. These results provide a valuable tool for further mechanistic dissection of age-related vulnerability to CHIKV and point to possible targets in CHIKV disease treatment.

Day 3

ABBREVIATIONS: A - adult mice, 3-5 mo old; CHIKV - chikungunya virus; O - old mice, 18-20 months old



TRAINED IMMUNITY: CONSEQUENCES FOR INFLAMMATORY DISEASES?

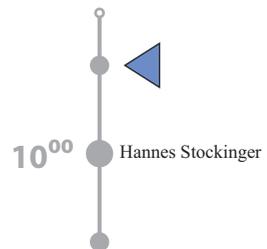
Leo A.B. Joosten

Radboudumc Nijmegen The Netherlands

The inability of innate immunity to build an immunological memory, considered one of the main characteristic differentiating it from adaptive immunity, has been recently challenged by studies in plants, invertebrates, and mammals. The concept of trained immunity has been described and the implications of this phenomenon on inflammatory diseases is not known yet. Monocyte reprogramming is the basis of trained immunity which leads to a state of activation of the monocytes. The innate memory of monocytes, macrophages or NK-cells will have also an impact on adaptive immune responses.

Here we discuss innate memory of monocytes and the mechanisms of that leads to reprogramming of the innate immune cells. Besides the well-known protective effects of trained immunity on infectious diseases, the role of monocyte reprogramming on chronic inflammation is not elucidated yet. The consequences for inflammatory diseases as atherosclerosis, rheumatoid arthritis or gout will be discussed.

Day 4



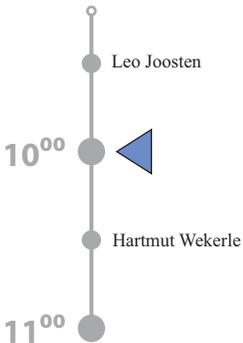
NOVEL MACROPHAGE SUBSETS WITH POTENTIAL IMPLICATION IN INFLAMMATORY DISEASES

Hannes Stockinger and Anna Ohradanova-Repic

Molecular Immunology Unit, Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

Macrophages are strategically located within the tissues where they play a crucial role in immune surveillance. In response to tissue damage or invading pathogens, macrophages effectively ingest and eliminate the source of danger and by concomitant release of proinflammatory cytokines they attract and activate other immune cells including macrophage precursors and T cells. However, exacerbated macrophage responses underlie the pathology of several inflammatory diseases including rheumatoid arthritis. A subset of macrophages found within the affected joints was characterised by expression of folate receptor β (FR β). In order to study the function of FR β -positive macrophages within the diseased tissue in more detail, we prepared *in vitro*-differentiated macrophages from peripheral blood monocytes. Our analyses, comprising a comprehensive gene expression profiling, revealed that FR β is strongly upregulated in several subsets of macrophages that can be distinguished by other markers as well as by functional properties. While FR β -positive subsets share high phagocytic potential, they display marked differences in their capacity to stimulate T cells. Our results revealed an important aspect of macrophage plasticity and potentiating a transition to an immunosuppressive phenotype *in vivo* could lead to a novel way of treatment for inflammatory diseases.

Day 4



IGNITION OF BRAIN AUTOIMMUNE DISEASES IN THE GUT

Hartmut Wekerle

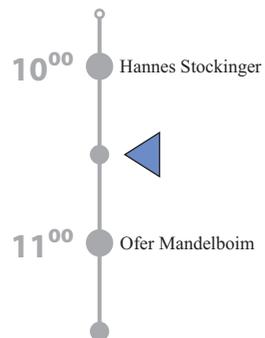
Max Planck Institute for Neurobiology, Munich, Germany

Over millions of years of evolution, the human organism has adopted microbial societies that colonize specific outer inner surfaces. Rather than menacing our body, these microbiota crucially contribute to our wellbeing. Thus, our microbial gut flora, one of the densest bacterial habitats known, contributes to the essential digestion of diets, produces essential nutrient factors, protects against invading pathogens and warrants proper immune function within the gut associated lymphatic tissues. Beyond this local functions, intestinal microbiota act systemically, on the immune, endocrine and nervous systems.

Unfortunately, the beneficial interactions of body and microbiota can go awry. We found that under particular conditions, gut microbes activate autoimmune T lymphocytes to attack the body's own nervous tissues. Studying an animal model of human multiple sclerosis (MS), we detail innate and adaptive immune mechanisms that underlie these triggering events. In addition, we have clinical trials to learn, whether similar activation leads to the development of human MS. First emerging results will be presented.

Supported by Hertie-Foundation, Max-Planck Society, DFG (Koselleck Award, SFB218), KKNMS, and SyNergy.

Day 4



RECOGNITION OF BACTERIA BY NK CELLS

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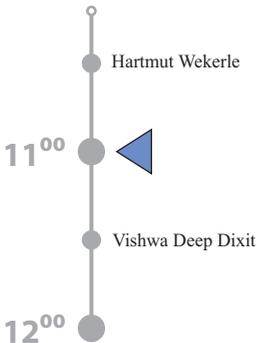
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Bacteria, such as *Fusobacterium nucleatum*, are present in the tumor microenvironment. However, the immunological consequences of intra-tumoral bacteria remain unclear. Here, we have shown that natural killer (NK) cell killing of various tumors is inhibited in the presence of various *F. nucleatum* strains. Our data support that this *F. nucleatum*-mediated inhibition is mediated by human, but not by mouse TIGIT, an inhibitory receptor present on all human NK cells and on various T cells. Using a library of *F. nucleatum* mutants, we found that the Fap2 protein of *F. nucleatum* directly interacted with TIGIT, leading to the inhibition of NK cell cytotoxicity.

We have further demonstrated that tumor-infiltrating lymphocytes expressed TIGIT and that T cell activities were also inhibited by *F. nucleatum* via Fap2. Our results identify a bacterium-dependent, tumor immune evasion mechanism in which tumors exploit the Fap2 protein of *F. nucleatum* to inhibit immune cell activity via TIGIT.

Day 4



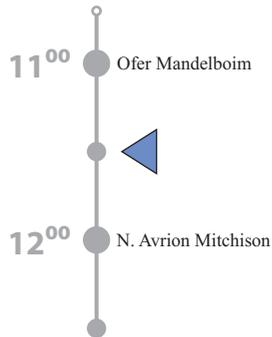
Vishwa Deep Dixit,

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Age is the greatest risk factor for chronic diseases. Data from several clinical studies suggest an association between increase in pro-inflammatory markers and emergence of age-related degenerative changes. However, it remains unclear whether downregulating inflammation, a critical protective response for host-defense and tissue remodeling, represents a viable therapeutic approach during aging process. Here we detail a mechanism by which the Nlrp3 inflammasome controls systemic low-grade age related "sterile" inflammation in both periphery and brain independently of the noncanonical caspase-11 inflammasome. The Nod-like receptor (NLR) family of innate immune cell sensors, such as the nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3) inflammasome controls the caspase-1 activation in myeloid-lineage cells in several organs during aging. The NLRP3 inflammasome is especially relevant to aging as it can get activated in response to structurally diverse damage-associated molecular patterns (DAMPs) such as extracellular ATP, excess glucose, ceramides, amyloids, urate and cholesterol crystals, all of which increase with age. Ablation of Nlrp3 inflammasome protected mice from age-related increases in the innate immune activation, alterations in CNS transcriptome, and astrogliosis. Consistent with the hypothesis that systemic low-grade inflammation promotes age-related degenerative changes, the deficient Nlrp3 inflammasome-mediated caspase-1 activity improved glycemic control and attenuated bone loss and thymic demise. Notably, IL-1 mediated only Nlrp3 inflammasome-dependent improvement in cognitive function and motor performance in aged mice. In addition, ablation of Nlrp3 inflammasome activation within thymic macrophages prevented age-related thymic atrophy and resulted in preservation of T cell receptor repertoire diversity in aged mice. These studies reveal Nlrp3 inflammasome as an upstream target that controls age-related inflammation and offer an innovative therapeutic strategy to lower Nlrp3 activity to delay multiple age-related chronic diseases and enhance healthspan.

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Day 4



IL-33-dependent immunosuppressive Treg responses to liver damage during MCMV infection

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Regulatory T cells (Treg) are critical for preventing autoimmunity mediated by self-reactive T cells, but also in modulating immune responses to infections and therefore controlling balance between activation and tolerance. Murine cytomegalovirus (MCMV) is a herpesvirus with pathogenic potential that can induce high levels of viral replication and modulate antiviral responses. Thus, early immune mechanisms are essential in controlling virus replication and protecting the host from virus-induced pathology. Studies on Treg cells reveal their role in suppressing early NK and T cell responses activated by MCMV in infection. However, absence of Treg cells does not influence viral clearance. Here we show that Treg cells are indispensable for preventing liver pathology induced by MCMV infection. In addition, their suppressive capacity is dependent on tissue alarmin, IL-33, which promotes Treg recruitment and function in liver of MCMV infected mice. Moreover, mice lacking IL-33 receptor showed higher death rate mediated by stronger liver pathology after infection with high viral dose. This is a consequence of decreased infiltration of Treg cells and lower expression of anti-inflammatory IL-10 and TGF- β , compared to control mice. In the light of recent discovery of IL-33's importance for accumulation and maintenance of Treg cells in acute colitis model, our results suggest protective and homeostatic role of IL-33 signaling in inflammation induced by MCMV infection.

Diagnostic utility of soluble interleukin-2 receptor in children with suspected hemophagocytic lymphohistiocytosis

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The levels of soluble interleukin-2 receptor (sIL-2R) in the plasma reflect the activation of the adaptive immune system and are extremely elevated in hemophagocytic lymphohistiocytosis (HLH). Cutoff value of sIL-2R according to the HLH-2004 criteria, devised by The Histiocyte Society, is 2400 U/ml. Other criteria include fever, hepatosplenomegaly, bi- or pancytopenia, hypoferritinemia, hypertriglyceridemia and/or hypofibrinogenemia, reduced NK cell activity and hemophagocytosis. Five of the eight criteria are necessary for the diagnosis of HLH. Measurement of sIL-2R levels by enzyme-linked immunosorbent assay (ELISA) is routinely performed in the Laboratory for Immunology of the University Children's Hospital in Belgrade since August 2008. Up to March 2015, we have performed this test for 41 child suspected to suffer from HLH. Twenty-six children (63,4%) were found to have HLH based on the HLH-2004 criteria, while in 15 (36,6%) HLH was not confirmed. Of 26 diagnosed children, sIL-2R was above the cutoff value in 21, yielding sensitivity of 80,8%. Only two of the 15 children who did not turn out to have HLH had sIL-2R above the cutoff. The specificity of sIL-2R measurement in diagnosing HLH was therefore 13/15 (86,7%). Given that different HLH-2004 criteria are often not fulfilled at the same time and that ELISA test can be performed rapidly and efficiently, the presented results warrant consideration of routine use of sIL-2R measurements in laboratories of tertiary care medical institutions, particularly if combined with meticulous patient selection, in order to reduce time to diagnosis and thus enable timely treatment of HLH.

Deletion of CMV inhibitor of PVR (CD155) results in enhanced virus susceptibility to innate immune control and dramatic attenuation in vivo

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Poliovirus receptor (PVR), or CD155, is highly conserved and ubiquitously expressed molecule which plays a role in immune response to tumors and viruses. It is recognized by activating (DNAM-1 and Tactile) and inhibitory (TIGIT) receptors expressed on variety of immune cells. Human cytomegalovirus (HCMV) protein UL141 downmodulates the expression of PVR, suggesting its role in immunosurveillance of CMV infection (Tomasec et al., Nat Immunol. 2005). Here we show that murine CMV (MCMV) also downregulates the surface expression of PVR and further, we characterized the viral protein involved. This protein is encoded by m20 gene region which displays a highly complex transcriptional profile: five 3'-co-terminal highly-overlapping transcripts are transcribed from this gene region and each has the potential to encode different proteins. Indeed, we have detected two different proteins with different temporal kinetics and likely different functions. Furthermore, we demonstrated the biological significance of PVR regulation on viral pathogenesis and virus susceptibility to various immune response mechanisms. Our results indicate that MCMV affects the maturation of PVR, rather than directly removing it from the surface of infected cells. Since PVR can ligate both inhibitory and activating immune receptors, the consequence of its viral downregulation in immune response was hardly predictable. Yet, deletion of MCMV inhibitor of PVR resulted in dramatic virus attenuation which could only partially be reversed by depletion of NK cells, suggesting the impact of other immune control mechanisms, in addition to NK cells.

The Hepatitis C Virus Contributes to the Aggravation of the Immunosuppressive Environment by Increasing the Suppressive Activity of Natural Regulatory T cells and Inducing the Emergence of Type 1 Regulatory T Cells

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Background & Aims: HCV infection is characterized by a high risk of chronicity. We have shown in a previous work that natural and induced human regulatory T cells (nTreg and Tr1) play an important role in the progression of hepatitis C to hepatocellular carcinoma and are associated with the severity of viral recurrence after liver transplantation. However, nothing is known concerning the specific impact of HCV on these two regulatory T cell sub-populations. Our hypothesis is that HCV may promote the recruitment of regulatory T cells in the infected liver, and alter their phenotype and suppressive activity for the progression of liver pathogenesis.

Methods: Treg cells were isolated from the blood of healthy donors and infected in vitro with HCV. The impact of HCV infection on nTreg and Tr1 phenotype and function has been assessed.

Results: We showed that Treg infection with HCV significantly increases the expression of viral receptors and that the viral proteins are internalized into Treg. We also showed that viral infection raises the Treg anergy and promotes the recruitment of infected Treg cells by HCV-infected hepatocytes and primary intrahepatic fibroblasts. In addition, HCV infection induced a significant increase in the expression of markers associated with Treg cells, thus potentiating their "suppressive phenotype." These results are correlated with the functional analysis of infected Treg cells, showing (i) a significant increase in the expression of markers associated with their suppressive activity, (ii) a significant increase in the secretion of immunosuppressive cytokines and (iii) an increase in immunosuppressive function. Finally, we have shown that HCV promotes conversion of conventional T lymphocytes into induced T regulatory type 1 cells.

Conclusion: This study shows for the first time that HCV can be able to be internalized into human Treg cells and could promote Treg recruitment into the infected liver. This may contribute to explain the mechanisms by which HCV escapes the immune system and promotes the progression of hepatitis C to cirrhosis and HCC.

Ocular conjunctiva as a mucosal immunization route: humoral and cellular immune responses against *Escherichia Coli Nissle 1917* bacterial ghosts

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Mucosal delivery of vaccines has the potential to eliminate the requirement for needles and induce strong immunity against pathogens. However, most subunit protein antigens are poor immunogens and there is an urgent need for the development of new and improved routes of immunization, as well as safe and efficacious delivery systems and adjuvants. In this study we characterized humoral and cellular immune responses after conjunctival immunization with corpuscular *Escherichia coli* Bacterial Ghosts (EcN BGs) in BALB/c mice and evaluated tolerability at the ocular surface after conjunctival application. BALB/c mice were immunized via the conjunctiva or subcutaneously with 12,5 µg EcN BGs per mouse. Three immunizations were performed at 2-week intervals and immune responses were evaluated 2 weeks after the last immunization. There was no pathology at the ocular surface of mice immunized with EcN BGs via the conjunctiva. EcN BGs specific secretory IgA levels in tears was significantly higher in the group immunized conjunctivally compared to subcutaneously immunized mice. Furthermore, immunization with EcN BGs via the conjunctiva promoted the establishment of a EcN BGs-specific Th1/Th17 immune response. Increased levels of IgA and Th1-biased immune response without any resultant pathology indicate that EcN BGs applied via the conjunctiva merit further investigations as adjuvant carriers in conjunctival vaccine development.

The Protective Potential of an individual Anti-Tetanus Monoclonal Antibody is improved if used in a combination with another MAb able to bind Tetanus Toxin simultaneously

Ana Filipovic

Institute of Virology, Vaccine and Sera "Torlak" Belgrade

Monoclonal antibodies (MAbs) are considered for a long time as a reagent of choice for prevention of tetanus as they can be produced in large scale *in vitro* and their quality is consistent. In this work we explored whether the MAbs mixture would confer better protectiveness than each MAb alone. The protective potential of anti-TTn MAbs, MAb 51 and MAb71, was evaluated in murine model system. MAbs were applied separately (10 µg of MAb in PBS, i.p) or as equimolar mixture (5 µg of each MAb in PBS, i.p), simultaneously or 2h, 6h and 10h upon i.p. challenge with lethal dose of TTn. Mice were monitored on a daily basis for two weeks for symptoms of tetanus disease (max. pathology score 5). MAb51 and MAb71 can bind TTn simultaneously without any steric hindrance (index of aditivity 89 ± 7). When applied separately, simultaneously with TTn challenge, MAb51 confer full protection (survival rate 100%, mean daily pathology score 2.10.18, no signs of pathology 7 days upon treatment) while survival rate with MAb71-treated mice was 60% (mean daily pathology score 3.80.18, 30% of alive mice had slight symptoms of tetanus two weeks upon a challenge). Clinical picture of mice treated in the same way with MAb51/MAb71 mixture was significantly better than with MAb51-treated mice (no pathology with 80% mice, pathology score 1). Separately or in equimolar mixture, MAbs also provided protection when they were applied early after TTn challenge (up to 10h, pathology score 1). In all cases the timing of MAb(s) administration did not significantly influence survival rate but the intensity of pathology and recovery time (positive correlation). Obtained results show that pooling of MAbs that are able to bind TTn simultaneously can improve their individual protective potentials.

Deletion of Galectin 3 Enhances Primary Biliary Cirrhosis in Mice by Enhanced Apoptosis of Biliary Epithelial Cells and Release of Autoantigens

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Galectin-3 (Gal-3) is expressed in various cell types and is involved in the pathogenesis of many chronic inflammatory diseases. Increased expression of Gal-3 in epithelial cells protects them from apoptosis. Apoptotic death of biliary epithelial cells (BECs) has important role in the pathogenesis of primary biliary cirrhosis (PBC). PBC is the liver-specific autoimmune disease characterized by a multilineage response against PDC-E2. When BECs undergo apoptosis the major mitochondrial autoantigen, PDC-E2, remains immunologically intact and is expressed at the apical surface of the small bile duct cells, perpetuating autoimmune response. The aim of this study was to analyze the role of Gal-3 in PBC pathogenesis. Female C57BL/6 wild type and Gal-3 KO mice were immunized with 2OA-BSA and disease was evaluated by histological examinations and measurement of the serum levels of PDC-E2 specific antibodies. Immunophenotyping of lymphocytes and dendritic cells was done by flow cytometry. Expression of Gal-3 and cytokeratin in BECs was detected by immunohistochemistry while apoptosis of BECs was analyzed by TUNEL assay and Annexin V staining. We report here that deletion of Gal-3 molecule amplifies PBC induced by 2OA-BSA mice in C57BL/6 mice on histopathological findings and serum analysis. Periportal infiltrations, bile duct damage, and fibrosis in livers of Gal-3 KO mice were more pronounced with granuloma formation observed only in Gal-3 KO mice. Four week after immunization, serum anti- PDC-E2 IgA antibody was significantly increased in Gal-3 KO mice compared to WT mice. Liver infiltrates of Gal-3 KO mice contained higher number of IFN- γ positive CD8+ lymphocytes. Enhanced PBC in Gal-3 KO mice is accompanied with reduced apoptosis of BECs. Besides BECs of Gal-3 KO mice had inherently higher response to apoptotic stimuli. There were more proinflammatory dendritic cells in the livers of Gal-3 KO 2OA-BSA immunized mice. Accelerated 2OA-BSA induced PBC in Gal-3 KO mice appears to be the result of enhanced release of autoantigen and consecutive stimulation of inflammatory antigen presenting and effector cells.

Key words: PBC, 2OA-BSA, Gal-3, BECs, apoptosis

This work was supported by the Ministry of Science, Republic of Serbia, grants ON175069, ON175071 and ON175103 Faculty of Medical Sciences, University of Kragujevac, Serbia grants MP 01/14 and 02/14.

Genetic deletion of Galectin-3 attenuate dextran sodium sulphate colitis in mice

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Introduction. Galectin-3 (Gal-3) is an endogenous lectin with a broad spectrum of immunoregulatory effects: it plays an important role in autoimmune/inflammatory and malignant diseases, but the precise role of Gal-3 in pathogenesis of ulcerative colitis is still unknown.

Methods. We used a model of dextran sulphate sodium (DSS)-induced colitis, that has a high degree of uniformity and reproducibility to human colitis, to test susceptibility of wild-type C57BL/6 and Gal-3-deficient mice (Gal-3^{-/-}) to this disease. DSS (3%, molecular weight 40kDa) was dissolved in water and given to C57BL/6 and Gal-3^{-/-} mice in place of normal drinking water (ad libitum) for 7 days. Disease Activity Index (DAI: weight loss, stool consistency, visible blood in feces), was used to assess the clinical signs of colitis. The cellular make up of colon and phenotype of colon-infiltrated immune cells were determined by flow cytometry.

Results. Genetic deletion of Gal-3 significantly reduce the damage of colon tissue of DSS-treated mice. Level of pro-inflammatory cytokines (IL-1 β , TNF- α и IL-6) were significantly lower in sera and colons of DSS-treated Gal-3^{-/-} mice when compared to WT DSS-treated mice. The total number of CD11c⁺ inflammatory dendritic cells (DC) which expressed CD80 and I-A and produce pro-inflammatory cytokines (TNF- α and IL-6) as well as TNF- α and IL-1 β producing CD45⁺CD11c⁺Ly6G⁺ neutrophils were significantly lower in colons of Gal-3^{-/-} DSS-treated mice. In addition, the total number of inflammatory colonic (F4/80⁺CD11b⁺SiglecF⁻, F4/80⁺CD11b⁺I-A⁺, IL-1, IL-6 and IL-12 producing) macrophages were significantly lower in Gal-3^{-/-} mice compared with WT DSS-treated mice. On contrary, there was significantly higher number of IL-10 producing regulatory DC and alternatively activated M2 macrophages in colon tissue of Gal3^{-/-} DSS-treated mice. In vitro lipopolysaccharide (LPS) and DSS-stimulated peritoneal macrophages isolated from untreated Gal-3^{-/-} mice produce lower amounts of TNF- α and IL-1 β when compared to WT cells. Adoptive transfer of WT macrophages managed to significantly enhance the severity of DSS-induced colitis of Gal-3^{-/-} mice. Antibiotic treatment did not affect differences between DSS-treated WT and Gal-3^{-/-} mice.

Conclusion. Gal-3 expression promotes acute DSS-induced colitis. This effect is due to its pro-inflammatory role in particular on peritoneal macrophages rather than its role as a receptor for pathogens.

Keywords. DSS, colitis, Gal-3

This study was financed by Grant of European Crohn's and Colitis Organisation (ECCO) and by Serbian Ministry of Science and Education (projects no.175069 and 175103)

Mesenchymal stem cells attenuate acute liver injury mediated by NKT cells

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The effects of mesenchymal stem cells (MSC) on phenotype and function of natural killer T (NKT) cells, major effector cells in acute liver injury, is not fully understood. We used two well-established experimental models of NKT-cell mediated acute liver pathology: Concanavalin A (Con A)-induced hepatitis (Con A; 12 mg/kg i.v) and α -galactosylceramide (α -GalCer; 50 μ g/kg i.v)-induced liver injury to evaluate effects of MSC on liver damage and NKT cell functions.

MSC (single iv. injection, 500 000/mouse, given immediately after Con A or α -GalCer) significantly attenuate Con A and α -GalCer mediated liver damage, as demonstrated by histopathological analysis and liver enzyme tests. MSC-treatment attenuates influx of inflammatory NKT cells in the liver (TNF- α -, IFN- γ -, T-bet+ CD4+ and CD1d tetramer+ as well as Gata-3+, IL-4-producing NKT cells) and down-regulates inflammatory cytokines (TNF- α , IFN- γ , and IL-4) in the sera of Con A and α -GalCer-treated C57Bl/6 mice. Serum levels of anti-inflammatory IL-10 and percentage of IL-10-producing NKT cells in the liver were significantly higher in MSC-treated mice. MSC did not significantly affect phenotype of macrophages and dendritic cells, suggesting that MSC modulate production of cytokines directly in liver NKT cells. Significantly lower amounts of inflammatory cytokines (TNF- α , IFN- γ , IL-4), and higher amounts of immunosuppressive IL-10 were noticed in supernatants of in vitro α -GalCer (100 ng/mL)-stimulated liver NKT cells cultured with MSC in transwell systems when compared to α -GalCer-stimulated liver NKT cells which were cultured alone. MSC treatment attenuate expression of FASL, CD107 and TRAIL, receptors known to be responsible for NKT cell mediated apoptosis and cytotoxicity. Accordingly, MSC treatment significantly reduced cytotoxic potential of liver NKT cells. The results obtained by xCELLigence system for monitoring real-time cytotoxicity showed that NKT cells isolated from MSCs+ α -GalCer-treated mice were significantly less cytotoxic against HEPG2 hepatocyte cells then NKT cells isolated from mice treated with α -GalCer-only. Human MSC managed to significantly attenuate production of inflammatory cytokines in α -GalCer-stimulated peripheral blood mononuclear cells and attenuate their cytotoxicity against HEPG2 cells. In conclusion, MSC protect from acute liver injury by attenuating cytotoxicity and capacity of liver NKT cells to produce inflammatory cytokines.

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Ethyl Acetate Extract of *Origanum Vulgare* Exerts Immunomodulatory Effect *In Vitro* and Reduces Hyperglycemia in Mice

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Type 1 diabetes (T1D) is autoimmune inflammatory disease with hallmark of pancreatic beta cell destruction *via* various proinflammatory mediators. Focus of T1D treatment is shifting towards establishing novel therapeutic approaches which could be more efficient than standard insulin replacement. *Origanum vulgare*, besides being a spice, is also known as a source of natural antioxidants and exerts anti-fungal, anti-bacterial and anti-inflammatory effects. The main aim of this study was to determine effects of ethyl acetate extract of *Origanum vulgare* (EAO) on immunomodulation and cellular viability, as well as its effect on diabetes development. EAO extract was prepared from Oregano leaves by polar extraction method. *In vitro* effects of EAO were determined by measuring proliferation (MTT test) and cytokine production (ELISA) of murine lymph node and peritoneal cells, and nitric oxide (NO) production of peritoneal cells (Griess reaction). To evaluate the effect of EAO on diabetes development, the extract was administered intraperitoneally to C57BL/6 mice that were subjected to T1D induction by multiple low doses of streptozotocin (MLDS). Our results indicate that 48h treatment with EAO did not alter basic or concanavalin A-induced proliferation of lymph node cells, as well as IL-4 secretion, while reducing IFN- γ and IL-17 secretion. EAO reduced IL-1 β secretion of peritoneal cells, while secretion of TNF remained unchanged. Also, EAO did not affect NO production or viability of peritoneal cells. Finally, 10-day of EAO treatment significantly reduced the diabetes incidence and the level of hyperglycemia in MLDS-treated mice. In conclusion, EAO alters secretory patterns of immune cells *in vitro* by reducing proinflammatory Th1 and Th17 response, and protects mice from developing hyperglycemia *in vivo*. Observed immunomodulatory and anti-diabetogenic effects of EAO are being further analyzed for potential treatment of diabetes.

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Carbon Monoxide-Releasing Molecule (CORM)-A1 Potentiates Regulatory Arm of the Immune Response and Exerts Protective/Regenerative Effects on Beta Cells

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Type 1 diabetes (T1D) is an autoimmune disease characterized by insulinitis and islet beta cell loss. Thus, an effective therapy requires beta cell preservation and immune suppression. Using mouse model of T1D induced by multiple low doses of streptozotocin (MLDS), we have recently reported that carbon monoxide-releasing molecule (CORM)-A1 can achieve both goals efficiently by acting on both: the islet beta cells and immune system. CORM-A1 therapy exerted an anti-inflammatory effect and produced a more favorable cytokine profile by shifting the M1/Th1/Th17 balance towards a M2/Th2 response, while directly interfered with beta cell apoptosis through the reduction of cytochrome c and caspase 3 levels. In the present work we tested the hypothesis that CORM-A1 therapy might influence regulatory arm of the immune response, as well as beta cell regeneration. We found that in CORM-A1-treated MLDS-induced mice the improvement of hyperglycemia was lost after depletion of cyclophosphamide-sensitive FoxP3+ Treg cells. Of note, the improvement was accompanied by decreased levels of IL-12, IL-2 and early activation marker CD25 in the spleen and pancreatic lymph nodes and increased TGF- β , resulting in reduced lymphocyte proliferation in both organs. In parallel, decreased transcript levels of IL-2, but increased mRNA expression of TGF- β was observed within pancreas, with concomitant local up-regulation of Ki-67 protein expression, suggesting proliferation of surviving beta cells. Taken together, our results indicate that CORM-A1 may attenuate autoimmune diabetes not only through immune cell resetting, but also by improving the survival/growth of insulin-producing pancreatic beta cells. These data shed new light on the mechanisms that underlie CORM-A1-mediated immunomodulation and provide insights into the role of CORM-A1 in regulating islet cell function and glucose homeostasis, which may find clinical application.

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Subchronic oral intake of low cadmium doses affects intestinal immune responses in rats

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Gastrointestinal (GI) tract is one of the main targets of cadmium (Cd), important food and drinking water contaminant. In this study, the effect of subchronic (30 days) oral (in drinking water) intake of environmentally relevant doses of cadmium (5 µg/ml and 50 µg/ml) on intestinal [(tissue of duodenum and mesenteric lymph node (mLN) cells)] was examined in Dark Agouti (DA) rats. Atomic absorption spectrophotometry (AAS) analysis revealed significant cadmium load in duodenum, which was associated with changes of both intestinal bacterial load and composition (Denaturing Gradient Gel Electrophoresis/DGGE). Shortening of villi, damage to epithelium, increases in goblet-like vacuoles and mononuclear cell infiltration in lamina propria were histologically evident at both cadmium doses, with massive necrosis at higher Cd dose (judging by High Mobility Group Box-1/HMGB-1 Western blot analysis). Increased levels of proinflammatory cytokines (IL-1β, IFN-γ, IL-17) were observed at both Cd doses (TNFα at higher dose solely). Cadmium administration affected draining lymph nodes as well, judging by signs of stress response (drop of cellular reduced glutathione/GSH at higher dose, rise of metallothionein/MT mRNA at both doses). Increased cellularity of mLN was observed at higher Cd dose, but with no changes in proliferative responses. Intake of both Cd doses resulted in higher (compared to controls) levels of IFN-γ and IL-17 mRNA as well as increased mLN cell responsiveness to ConA stimulation. Significant increases in numbers of CD68+ cells and stimulation of innate immune activities (numbers of dihydrorhodamine/DHR+ cells and intracellular myeloperoxidase/MPO+ cells, LPS-stimulated nitric oxide/NO production and ex vivo IL-1β expression) were observed at higher dose of cadmium. Proinflammatory effects of cadmium might have resulted from changes in gut microbiota and tissue damage. Interactions of this metal with intestinal immune system should be taken into account when assessing dietary cadmium as health risk factor.

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Characterization of single sorted ANTI-ADAMTS13 specific B cells from the spleen of acquired thrombotic thrombocytopenic purpura (aTTP) patients

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Introduction: Autoantibodies(Abs) that neutralize and/or accelerate the clearance of ADAMTS13 cause acquired Thrombotic Thrombocytopenic Purpura (aTTP). Since increasing evidence points at the spleen as a major reservoir for antigen specific memory B-cells, we investigated the splenic B-cell population and the frequency of ADAMTS13-specific B-cells as a prerequisite for understanding the humoral autoimmune response and for developing an optimized and specialised treatment.

Methods: Splenic mononuclear cells from 7 aTTP patients (2 treated with Rituximab) were analysed by flow cytometry. We calculated the frequencies of highly positive anti-ADAMTS13 B-cells among naïve, un-, switched memory B-cells and plasma cells. B-cells bearing anti-ADAMTS13 IgG were individually sorted based on binding to fluorescently labelled rADAMTS13. Gene transcripts of single cells were reverse-transcribed followed by nested PCR of IgG heavy/light chains. Gene analysis identifying the Variable domains (V) and V-(D)-J junctions was performed using IMIGT/V-QUEST tool.

Results: Anti-ADAMTS13 cells were detected in the spleen of all patients (average 0.057% among B-cells populations) with highest prevalence among plasma cells. The two Rituximab treated patients had decreased level of CD19/CD20 positive cells (1% and 20% of lymphocytes after T-cell/monocyte exclusion), compare to untreated patients (~63%). Splenic anti-ADAMTS13 specific B-cells of 4 aTTP patients revealed 149 antibodies sequences from which we analysed the CDR3 diversity. Most frequently used V-genes were of IGHV1-69, IGHV3-30, IGHV4-31 (20%, 12%, 7%).

Conclusion: Specific anti-ADAMTS13 B-cells were found in the spleen of all aTTP patients, in a range similar to ITP patients, including plasma cells known to be unaffected by Rituximab. Currently we clone selected single sorted monoclonal antibodies for functional tests to select the inhibitory Abs as tool to develop antigen-specific therapies for aTTP.

Level of regulatory cytokines correlate with the outcome of the inflammation in the process of PEX production

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Objective: The purpose of this study is to reveal the participation of different regulatory cytokines within the process of pseudoexfoliation (PEX).

Methods: Our study included 120 patients, referred to cataract surgery with early and late stage of pseudoexfoliation syndrome (XFS) or pseudoexfoliation glaucoma (XFG). Humour and serum levels of cytokines: TNF- α , IL-17, IL-6, TGF- β , PDGF, EGF, IGF IL-8 and ITAC were measured in a sample with high sensitivity enzyme-linked immunoabsorbent assay (ELISA) kit.

Results: Aqueous humour levels of proinflammatory cytokines TNF- α and IL-17 are increased in patients with the early and late stage of XFS and XFG, while IL-6 levels are increased in the early stage of XFS and XFG. Serum level of IL-6 showed a significantly increased level of IL-6 in patients with the early and late stage of XFS. Aqueous humour level of regulatory cytokine TGF- β is increased in the early stage of XFS. Early and late stage of XFS show increased level of PDGF. XFG patients have an increased level of EGF, IL-8, and ITAC; but IGF levels were detected only in XFG. Locally, profibrotic action was sustained by elevated TGF- β , followed by PDGF action in the late stage of XFS, and with the final action of EGF, IGF, IL-8 and ITAC in glaucoma. This process is supported by increased TGF- β in the serum within early stage of XFS, and enhanced PDGF, EGF and IL-8 within late stage, and IGF and ITAC in the glaucoma. Our results indicated that in the early and late stage of XFS and in XFG IL-17 and TNF- α play the main role in inflammation activation in the tissue; overlooked by IL-6 action in the early stage and in the phase of glaucoma. The final result of fibrous tissue deposition is the development of glaucoma without vasculogenesis (*de novo* blood vessel formation) and angiogenesis (new capillary branches from existing blood vessels) in the eye.

Conclusions: The purpose of this study is to indicate the significance of different cytokine actions in stress conditions, as well as to reveal their roles in different stadiums of PEX production and accumulation process. The mechanism of the development of XFS syndrome via cytokine formation will provide us new therapeutic insights for the treatment.

Key words: pseudoexfoliation, cytokines, fibrosis.

Standardized bovine colostrum attenuates development of experimental autoimmune diabetes

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Bovine colostrum is a rich source of immune components that play a role in conveying passive immunity to the offspring, protection and maturation of newborn's gastrointestinal tract and protective host immunity of the mammary gland itself. Colostrum has exerted positive effects in diseases affecting gastrointestinal tract, as well as type 2 diabetes. The aim of this study was to investigate therapeutic value of standardized bovine colostrum (SBC) in the model of type 1 diabetes (T1D). Autoimmune T1D was induced in C57BL/6 mice with multiple low doses of streptozotocin (MLDS, 5 x 40 mg/kg b.w./day). SBC was administered *per os* (8 g/kg b.w./day) for 10 consecutive days starting from the day of MLDS induction. The disease severity was evaluated by weekly measurement of blood glucose level and by histological analyses of the pancreas at the end of experiment (35th day). *Ex vivo* analysis of cytokine expression and production was measured on the 10th day in the spleen and pancreatic (PLN) and mesenteric lymph nodes (MLN). SBC administration to MLDS induced mice prevents diabetes development, indicated by euglycaemia and by the absence of insulinitis. In the MLN cells SBC disrupted harmful Th17 response, as judged by reduced expression and secretion of IL-17 compared to diabetic mice. Also, colostrum down-regulated two Th17 associated cytokines, IL-6 and IL-23. IFN- γ expression was down-regulated in MLN cells of SBG treated mice, while IL-4 secretion was up-regulated in comparison to MLDS diabetic group. Modulation of the immune response seen in MLN protrudes to the spleen and PLN, giving overall less infiltration of immune cells to the pancreas. SBC acts on immune cells and halts (auto)aggression towards pancreatic beta cells. Hence, this derivative could be tested in diabetes and other diseases with aberrant immune response.

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The Role of *Trichinella spiralis* Muscle Larvae Secretory Antigens in Shaping of Immune Response

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The helminth *Trichinella spiralis* communicates with the host immune system through excretory-secretory products (ES L1). ES L1 antigens drive immune response towards Th2 and regulatory type. However, there is no information about mechanisms by which ES L1 influences such polarization. Here, we investigated the impact of ES L1 and its components on immune response, Pattern Recognition Receptors (PRRs) involved in the interaction with these antigens and subsequently provoked signaling events in dendritic cells (DCs). ES L1 antigens significantly increased the production of IL-4 and IL-10, while they did not affect the levels of TGF- β and IFN- γ in vivo. Changes in the structure of ES L1 glycans greatly reduced the production of IL-4 indicating their importance for Th2 immune response. In vitro experiments with ES L1 and ES L1 components: 7C2C5Ag (containing 45, 49, and 53-kDa glycoproteins) and Tsp53 (recombinant p53) resulted in the same semimature DC phenotype accompanied by reduced production of IL-12p70 and elevated production of IL-10 and the capacity to promote Th2 and regulatory responses. Modification of ES L1 glycans (periodate treated ES L1 - pES L1) did not change DC phenotype but had an impact on cytokine production of DCs (reduced IL-12p70 and IL-10). DCs treated with pES L1 provoked low production of IL-10 and TGF- β in T cells, indicating the importance of intact glycans for Th2 and regulatory response induction. Considering investigation of PRRs involved in the interaction with ES L1 antigens, we have found that TLR2 is engaged by ES L1 and 7C2C5Ag, while TLR4 interacts only with ES L1. These interactions are impaired with changes in carbohydrate structure of ES L1. ES L1, 7C2C5Ag and Tsp53 transiently activated ERK MAP kinase, weakly activated p38 kinase and these activations were glycan dependent. This study indicates for the first time that some components of ES L1 (7C2C5Ag and Tsp53) possess immunomodulatory properties and that glycans on ES L1 are important for immune response polarization.

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Immunometabolic Differences in Prototypical Th1- And Th2-Type Mouse Strains in High-Fat Diet Induced Obesity

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Introduction & Aims: The inherent susceptibility to obesity-related metabolic disorders and type of immune/inflammatory response in metabolic tissues in mouse strains are markedly dependent on the genetic background which may affect the translation of experimental data to human pathology. Therefore, we have investigated the strain-dependent differences of visceral adipose tissue (VAT) and liver immunophenotype, liver steatosis, inflammation and fibrosis in experimental model of high-fat diet induced obesity in C57BL/6 and BALB/c, the prototypical Th1 and Th2 mouse strains.

Methods: Male 8-week old C57BL/6 and BALB/c mice were placed on HFD (60% kcal fat) or standard chow diet (10% kcal fat) for 24 weeks. We performed histological and liver and adipose tissue immunophenotypic analyses as well as expression of hepatic profibrogenic and lipid metabolism-related genes.

Results: After 24 weeks of dieting BALB/c mice exhibited higher weight gain on standard diet, while C57BL/6 mice exhibited higher weight gain on HFD. In comparison to BALB/c mice, the amount of visceral fat and fasting blood glucose levels was higher in C57BL/6 mice on both, chow and HFD. In contrast to BALB/c mice, HFD induced a significant increase of the amount of VAT and number of VAT associated CD3⁺CXCR3⁺ Th1 cells, dendritic cells (DCs) and F4/80⁺ macrophages in C57BL/6 mice. In livers, more numerous CD3⁺ and CD8⁺ T lymphocytes, myeloid DCs, proinflammatory macrophages (F4/80⁺CD11b⁺CD11⁺ and F4/80⁺IL-1β⁺) and CD11b⁺Ly6C^{high} monocytes and higher levels of IL-6, TNF-α and IFN-γ were detected in HFD-fed C57BL/6 mice than in diet-matched BALB/c mice. HFD-fed C57BL/6 mice had scarce liver steatosis in contrast to BALB/c mice which had marked hepatic steatosis and increased expression of genes related to lipid metabolism with higher serum levels of cholesterol and triglycerides and lower glycogen deposition in the liver. HFD induced prominent liver fibrosis in C57BL/6 mice while BALB/c mice developed scarce liver collagen deposition. The expression of mRNA for procollagen, profibrogenic IL-13 and TGF-β in liver and the levels of IL-33, IL-13 and TGF-β in sera and liver homogenates were higher in HFD-fed C57BL/6 mice compared to diet-matched BALB/c mice.

Conclusion: The obtained results indicate that Th1-type mice are susceptible to obesity, liver inflammation and fibrosis while Th2-type mice to liver steatosis in response to obesogenic HFD which is associated with differential phenotypes of immune cells in metabolic tissues. Immunometabolic differences in relation to Th1 and Th2 dominance may be relevant for studies of obesity-associated metabolic diseases in humans.

Galectin-3 Promotes Hepatic Inflammation and Fibrosis in Obesogenic Mouse Model of Nonalcoholic Steatohepatitis

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Introduction & Aims: Galectin-3 (Gal-3), a β -galactoside-binding lectin, is involved in the regulation of obesity, metaflammation, and type 2 diabetes, but its role in the pathogenesis of obesity-associated non-alcoholic steatohepatitis (NASH) is incompletely defined. In this study, we aimed to dissect the role of Gal-3 in liver inflammatory response and fibrosis, key parameters in the pathogenesis and progression of NASH, induced by obesogenic high-fat diet (HFD).

Methods: Gal-3-deficient (LGALS3^{-/-}) and wild-type (LGALS3^{+/+}) C57Bl/6 mice received HFD (60% kcal fat) or standard chow diet (10% kcal fat) for 24 weeks and metabolic parameters, gene expression and immunophenotypic analyses were performed.

Results: In comparison to WT mice, HFD-fed LGALS3^{-/-} mice developed increased obesity, type 2 diabetes and more pronounced liver steatosis which was accompanied by upregulation of hepatic FAS, PPAR- γ and Cd36 expression. However, ALT and AST levels, liver injury, inflammation and fibrosis scores, and hepatic procollagen and α -SMA mRNA expression were significantly increased in HFD-fed WT mice compared to diet-matched LGALS3^{-/-} mice. The more pronounced hepatic fibro-inflammatory response induced by obesogenic diet in WT mice was associated with increased myeloid DCs and proinflammatory monocytes/macrophages infiltrated into the livers, and higher hepatic CCL2, NLRP3 inflammasome and IL-1 β mRNA expression. Furthermore, the levels of profibrogenic IL-33 and IL-13 in liver homogenates and IL-33, ST2 and IL-13 mRNA expression in liver were markedly higher in WT than in LGALS3^{-/-} mice on HFD, while hepatic expression of TGF- β were similar. Moreover, in contrast to WT macrophages, *in vitro* stimulated LGALS3^{-/-} peritoneal macrophages with recombinant mouse IL-33 failed to upregulate ST2 expression and IL-13 production.

Conclusion: Gal-3 attenuates steatosis, but promotes liver injury, inflammation and fibrosis, thus participates in the progression of NASH induced by obesogenic diet in mice. Further, we show for the first time that Gal-3 plays an important regulatory role in the newly described profibrotic IL-33/ST2/IL-13 pathway.

Molecular genetic analysis of metastasis associated colon cancer 1 (macc1) and c-met expression in chronic myeloid leukemia

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Chronic myeloid leukemia (CML) is a hematopoietic neoplasm characterized by the Philadelphia chromosome and the related BCR-ABL1 oncoprotein. Several proangiogenic molecules have been implicated in disease acceleration, including the hepatocyte growth factor (HGF) and c-Met. Both c-Met and HGF have been shown to be deregulated and so correlated with poor prognosis in a number of major human cancers. The gene encoding the HGF receptor, c-Met, is a transcriptional target of MACC1. The newly identified proto-oncogene MACC1 (Metastasis-Associated Colon Cancer 1) gene stimulates proliferation, motility and invasion in colon cancer cells through up-regulation of c-Met. Once activated, c-Met can result in activation of several downstream signaling cascades (MAPK and PI3K/Akt pathways). The MACC1 was firstly identified to be over-expressed in primary and metastatic tumor tissue of colon cancer compared to healthy tissue. This study was directed to reveal the potential role of MACC1 in CML. Aim of this study was to investigate the level of mRNA expression of MACC1 and c-Met in patient with CML who was on treatment with imatinibmesilate (Gleevec). For this purpose, Real-Time PCR methodology was optimized and validated. Results are showing positive correlation between BCR-ABL and MACC1 expression, and between MACC1 and c-Met expression on transcript level. Additionally, results showed higher sensitivity of MACC1 in comparison to the BCR-ABL indicating so the potential role of MACC1 as novel biomarker in CML. To conclude, this pilot project could have an important impact on further studies and new perspectives about diagnostics as well as therapy for CML patients that could improve their life quality and survival.

Keywords: MACC1, c-Met, biomarkers, CML

Cytomegalovirus infection enhances development of diabetes mellitus type 2 in obesity

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Diabetes mellitus type 2 (DM2), main clinical complication of obesity, is characterized by chronic high blood glucose levels and insulin resistance, but also with chronic low-grade systemic inflammation that originates in the visceral adipose tissue (VAT) of obese individuals. As body weight increases, pro-inflammatory cells (macrophages, neutrophils, activated T cells, etc.) accumulate in VAT. These cells secrete pro-inflammatory cytokines (TNF α IL-1 β), which play a key role in the development of obesity-associated insulin resistance. Very little is known whether and how viral infections contribute to chronic VAT-inflammation and induction of DM2. Cytomegaloviruses (CMVs) are species-specific beta-herpesviruses which can infect a variety of cell types like fibroblasts, monocytes/macrophages, endothelial cells, dendritic cells, etc. Majority of human population (40 – 90%) is infected with CMV already during adolescence, and, after usually clinically inapparent acute infection in immunocompetent host life long latency is established. Infection of mice with mouse CMV (MCMV) represents a well accepted model for studying similar biological features of human CMV infection.

In this work we investigated how MCMV infection influences the development of a chronic systemic inflammation and insulin resistance under condition of high fat diet (HFD) – induced obesity. We observed that VAT can be efficiently infected with MCMV and the infection shows relatively fast clearance kinetics similar to the one observed in the liver. Infection of VAT is associated with a rapid accumulation of various immune cells. NK cells are activated on day 3 and their numbers rapidly increase until day 6 after infection. The augmentation of NK cell population is followed by a dramatic increase of CD4 and CD8 T cells. Very early, four weeks after induction of HFD and MCMV infection mice already develop insulin resistance and glucose intolerance. Moreover, upon establishment of MCMV latency (16 weeks p. i.), only in the group of MCMV and HFD treated mice we observed signs of glomerulopathy in juxtaglomerular cortex of the kidneys: enlarged glomeruli, mesangiosis, increased thickness of the filtration membrane and impaired podocytes.

Altogether, our results show that MCMV infection can cause significant enhancement of the VAT associated inflammatory process, insulin resistance and associated complications in obesity. However, the immunological mechanisms how MCMV infection influences these processes in VAT still need to be determined.

Impact of aging on calcium influx and potassium channel characteristics of T lymphocytes

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Adaptive immunity and T cell function are affected by aging. Calcium influx patterns, regulated by Kv1.3 and IKCa1 potassium channels, influence T cell activation. We aimed to compare calcium influx kinetics in CD8, Th1 and Th2 cells in human peripheral blood samples obtained from five different age groups (cord blood, 10-15 ys, 25-40 ys, 45-55 ys, 60-75 ys).

We measured calcium influx using flow cytometry in samples treated with or without specific inhibitors of Kv1.3 and IKCa1 channels (MGTX and TRAM, respectively).

Calcium influx was higher in Th1 cells of adults, however, its extent decreased again with aging. Importantly, these changes were not detected in Th2 cells, where the pattern of calcium influx kinetics is similar throughout all investigated age groups. MGTX had a more pronounced inhibitory effect on calcium influx in Th2 cells, while in Th1 cells the same was true for TRAM in the 25-40 ys and 45-55 ys groups. Calcium influx of CD8 cells were inhibited to a similar extent by both applied inhibitors in these groups, and had no effect in the elderly.

Altered lymphocyte potassium channel inhibitory patterns, regulators of calcium influx kinetics, might contribute to the development of age-related changes of T cell function.

Aging affects rat inflammatory peritoneal exudate composition and macrophage inflammatory mediator production in a strain-dependent manner

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The present study was designed to examine influence of aging on macrophage proinflammatory/anti-inflammatory capacity in rat model of thioglycollate-induced peritonitis. Peritoneal macrophages were isolated from young (3-months-old) and aged (18-months-old) Dark Agouti (DA) and Albino Oxford (AO) rats seven days post-injection of thioglycollate medium. Freshly isolated peritoneal exudate cells were examined for the expression of CD163, CCR7, CD14 and TLR4, whereas cytokine production (TNF- α , IL-6 and IL-10) and arginine metabolism end-products (NO and urea) were assayed *in vitro* under basal conditions and following stimulation with LPS. In DA rat inflammatory peritoneal exudate, aging diminished the frequency of cells with a “resolving macrophage” CD14+CD163+ phenotype. However, in AO rats, which exhibited stable frequency of CD14+CD163+ cells in inflammatory peritoneal exudate with aging, the proportion of CCR7-bearing peritoneal cells, presumably immigrating inflammatory monocytes, was diminished in aged animals. Under basal culture conditions, macrophages from aged rats of both strains released less amount of TNF- α , IL-6 and IL-10, but produced more urea than cells from young strain-matched rats. However, these changes were more pronounced in peritoneal macrophages from AO rats. Additionally, age-related decrease in the frequency of TLR4-expressing cells was observed among fresh peritoneal exudate cells from AO rats. Upon LPS stimulation, the production of prototypic inflammatory cytokines (TNF- α and IL-6) was diminished in macrophages from aged AO rats, whereas aging had the opposite effect on their production in DA rat macrophages. Moreover, aging increased NO production in LPS-stimulated macrophages from DA rats, whereas urea production was enhanced in macrophages from both strains, but this increase was strikingly more pronounced in macrophages from AO rats. Collectively, results suggest that aging affects inflammatory peritoneal exudate cellular composition and macrophage proinflammatory/immunomodulatory capacity in a strain-specific manner.

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Aging with MCMV maintains TCR repertoire diversity in late life

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Cytomegalovirus (CMV) is a ubiquitous persistent herpesvirus (worldwide infection rate 45-100%). CMV has coevolved with mammalian hosts for millennia. It has been postulated that lifelong persistent immune interactions between host and CMV may contribute to age-related immune senescence. We previously reported that mice aged with persistent murine CMV (MCMV) show impaired CD8 T cell function upon challenge with several microorganisms, including *Listeria monocytogenes* expressing the model OVA antigen (Lm-OVA) in late life. To further explore how lifelong persistent MCMV infection may influence aged immune responses, we challenged control adult and old mice, as well as lifelong MCMV-infected old mice (hereafter referred to as MCMV+) with Lm-OVA, and assessed the OVA-specific TCRb repertoire by single-cell sorting and TCRb CDR3 sequence analysis. We found that compared to old MCMV-negative animals, OVA-specific CD8 effector T cells generated in old MCMV+ mice contained diverse clonotypes exhibiting (i) non-canonical TCR genes; (ii) a loss of a conserved amino acid motif; (iii) reduced avidity for cognate antigen; and (iv) broader recognition of altered peptide ligands. Thus, lifelong MCMV infection appeared to maintain/broaden diversity within the CD8 T cell response to a new infection in late life, by recruitment of clonotypes with broad cross-reactive antigen recognition capacity. These results have profound implications for our understanding of naïve T cell maintenance over a lifespan, and suggest that our coevolution with CMV may include surprising, potentially positive impacts on adaptive immunity in late life.

Age-related effects on invariant NKT cells

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The immune system protects us against bacteria, viruses and other pathogens, and if compromised can result in susceptibility to infection. Immunosenescence refers to age-related immune impairments in immune function that may contribute to increased prevalence and severity of infectious disease in the elderly. Despite increasing understanding of molecular and cellular age-related immune alterations, knowledge is incomplete, particularly on dynamic and functional cellular changes.

Initial studies, using immunohistochemistry and flow cytometry, have defined key structural and cellular changes occurring at 3, 12 and 22 months of age and identified invariant NKT (iNKT) cells as a cell population of interest. iNKT cells are a subpopulation of T cell with an invariant TCR which recognises self and foreign lipids, for instance the glycolipid α -galactosylceramide (α -GalCer), presented on the CD1d molecule. iNKT cells play a role in the defence against a range of pathogens, including those that lack glycolipid, for example *Escherichia coli* and *Schistosomamansonii*[1], showing that iNKT cells can be activated in the absence of strong TCR signals. They have also proved to be protective or harmful in a range of autoimmune diseases and cancer.

In our aged model we observed a significant increase in the population size of iNKT cells, however no alterations in their distributions in spleen or inguinal lymph nodes. Using the surface markers CD44 and NK1.1, we can follow iNKT cell development in the thymus through stage 1 (CD44^{lo} NK1.1⁺), stage 2 (CD44^{hi} NK1.1⁺) and stage 3 (CD44^{hi} NK1.1⁻). Our data show a reduction in mature stage 3 iNKT cells corresponding with an increase in stage 2 in the thymus, spleen and liver. Current experiments will determine if age affects iNKT cell expression of activation markers and release of cytokine in response to *in vitro* stimulation

An improved understanding of how ageing affects functional activity in the immune system may reveal novel targets for intervention to alleviate age-related immune dysfunction and possibly lighten the medical burden of ageing.

1. Tupin, E., et al The unique role of natural killer T cells in the response to microorganisms. *Nature Reviews Microbiology* 5, 405-417.

Impaired CD4 and antibody responses in old mice increase *Chikungunya* virus disease severity and viral persistence

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Chikungunya virus (CHIKV) is a mosquito-borne alphavirus endemic to Africa and Asia, which causes sudden onset of fever, rash, and debilitating poly-arthritis in peripheral joints, which can persist for years, particularly in older individuals. The virus has been spreading around the world with >800,000 cases of autochthonous CHIKV infection in the Americas since its arrival in the western hemisphere in 2013 including the first confirmed transmissions of CHIKV in the U.S. In response to the increased geographic distribution of CHIKV and the likelihood that elderly immune-naïve populations may experience severe and life-threatening disease, we have developed a mouse model of age-related vulnerability to CHIKV infection. We demonstrate reduced ability of old mice to mount effective immune responses to CHIKV and control viremia, leading to increased disease severity and viral persistence in the joints. Ineffective immune responses were due, in part, to uncoordinated cytokine production. Specifically, CXCL9 and TGF β , were identified as key contributors to impaired CD4 and antibody responses against viral epitopes in old mice. These results provide a valuable tool for further mechanistic dissection of age-related vulnerability to CHIKV and point to possible targets in CHIKV disease treatment.

Dysregulated function of marginal zone B cells in aged mice

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As the average age of our population continues to climb the burden on the health system increases. This is due in part to the decreased ability of the aged immune system to deal with infection. Aging results in increased immunosenescence and along with this an increased susceptibility in aged individuals towards opportunistic pathogens and inflammatory conditions. In aged mice a disruption of the localization of immune cells within the spleen has been shown. Our laboratory is specifically interested in the disruption of the localisation of B cells and macrophages within the marginal zone (MZ) of the spleen as this has been demonstrated to be significantly altered in aged mice. First, an investigation into when the disruption of the splenic structure begins was performed. Spleens of mice from two months up to thirty months were analyzed via FACS and IHC. This analysis demonstrated that the number of B cells within the spleen increased with age. However, by 30 months there was a significant depletion in the number of B cells, predominately affecting the follicular B cell population as the representation of MZ B cells remained relatively intact. IHC analysis demonstrated an increasingly significant disturbance to the splenic structure with age. This disruption primarily resulted in an increased thickness of the marginal metallophilic macrophage and MZ B cell populations. Further analysis to assess the functioning of the B cell populations were performed via survival and chemotaxis assays. These assays unveiled an increased survival of aged MZ B cells. There also appeared to be an altered sensitivity of the aged MZ B cells towards S1P, an important chemoattractant, the implications of which will be discussed. These results demonstrate altered functioning of the MZ B cells with age, potentially altering the ability of cells to respond during an immune response.

Nitrosative events in asthma as a selective pulmonary disorder marker

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Allergic disorder pathogenesis is modulated by NO at the level of immune system. In the current study, we provided evidence of iNOS overexpression and NO overproduction under the chronic ovalbumin-induced asthma and considered the elaborated non-invasive approach to assess airway NO generation in asthmatic subjects.

Guinea pigs were randomly assigned to: intact group (*normal*); ovalbumin (OVA) sensitized animals (*OVA/0,9%NaCl*); OVA challenged group (*0,9%NaCl/OVA*); OVA-induced asthma group (*OVA/OVA*). Since IL-4 evokes switch for IgE, IgG1 and Th2 cell differentiation, sensitization to allergen (*OVA/0,9%NaCl*) was indicated with 4,5-fold growth of plasma IL-4 content compared with *normal* ($p(U)<0,05$). IL-13 values attained 1,5-fold increase in *OVA/OVA* and *0,9%NaCl/OVA* groups versus *normal* ($p(U)<0,05$). IL-13 lung level correlated with lung tissue histology, that defined mucus hypersecretion by goblet cells and mucous glands, basement membrane thickening in *0,9%NaCl/OVA*, and acute obstructive emphysema, sclerosis of interalveolar walls, fibrosis in *OVA/OVA*. The dynamics of NO metabolism under the chronic allergic asthma revealed, that iNOS lung content using antibodies increased markedly in 2,4- and 2,65-fold among *OVA/OVA* and *0,9%NaCl/OVA* groups respectively versus *normal* ($p(U)<0,05$). OVA multiple challenge procedures resulted in 2,4-fold and 2,3-fold rises of intracellular NO in the immune cells infiltrating airway of *OVA/OVA* and *0,9%NaCl/OVA* groups versus *OVA/0,9%NaCl* ($p(U)<0,05$).

Considering an important role of NO in the adaptive immune responses associated with allergic diseases, the experimental model of the device consisting of electrochemical gas sensor type NO₂-B4 (Alphasense) and Triton 6000U data acquisition system with measurement accuracy $\pm 10E-6$ V was developed. Because NO has a short half-life in an unbound state due to one unpaired electron, we assert that measuring NO₂ reflects the endogenous NO concentration produced in the lungs. Baseline sensor output signal and typical charts as a function of NO₂ concentration were recordered. The obtained data give an incentive for further studies.

IFN- γ and IL-17A drive distinct airway changes in a murine model of steroid-unresponsive severe asthma

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Background: Asthma is an inflammatory disease of the airways characterized by airway obstruction due to inappropriate immune responses against some environmental moieties like house dust mite (HDM) and pollens. Depending on the frequency and intensity of exacerbations experienced, asthma can be categorized as mild-moderate asthma (MMA) that is corticosteroid (CS)-responsive and severe asthma (SA) that is poorly managed by CS. The presence of neutrophils in asthma has been correlated with refractoriness to CS, fatal exacerbations and severity. To date, our understanding of the pathological mechanisms central to neutrophil-prominent SA is minimal primarily because of the lack of a suitable animal model, limiting the development and testing of new therapeutics.

Aim: The aim of this study was to elucidate the immune mechanisms underlying the difference in steroid-responsiveness in MMA and SA.

Method: Human asthma samples and a novel mouse model of SA were used to study cytokine responses, airway inflammation and hyperreactivity in MMA and SA.

Results: Unlike patients with milder disease, SA patients consistently have a mixed Th1/Th2/Th17 immune phenotype in their airways despite high dose CS treatment. The combination of an allergen, HDM, with agents that mimic infection induces a SA phenotype in mice. As in human severe asthmatics, the immune response in this mouse model elicits mixed granulocytic airway inflammation and airway hyperresponsiveness that are poorly responsive to CS. IFN- γ -deficient and IL-17A-deficient mice subjected to the SA model highlight the differential role played by each cytokine in modulating hallmark features of asthma such as airway inflammation, airway hyperreactivity and steroid responsiveness. Proteases and lipid mediators were identified as potential biomarkers of severe asthma using biological network analysis of RNA-Seq expression data.

Conclusion: The immune responses that contribute to the pathogenesis of severe and mild-moderate asthma are distinctly different.

Ragweed Allergic Subjects Have Decreased Serum Levels of the Chemokines CCL2, CCL3, CCL4 and CCL5 out of the Pollen Season Compared to the Healthy Individuals

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CC-chemokines are important mediators of the allergic responses and regulate the cell trafficking. The aim of this study was to examine the serum levels of CCL2/MCP-1, CCL3/MIP-1, CCL4/MIP-1 and CCL5/RANTES, and to determine whether there are differences between ragweed allergic subjects and healthy individuals out of the pollen season. Peripheral blood samples were collected from 14 subjects allergic to ragweed pollen and 12 healthy controls. Serum concentrations of chemokines/cytokines were measured by enzyme-linked immunosorbent assay. We observed significantly decreased concentrations of CCL2/MCP-1, CCL3/MIP-1, CCL4/MIP-1 and CCL5/RANTES in the sera of ragweed-allergic patients compared to the healthy individuals (32.2 vs 106.4 pg/ml, 89.5 vs 135.7 pg/ml, 63.4 vs 119.2 pg/ml and 11.2 vs 18.1 ng/ml, respectively, $p < 0.01$). In contrast to the CC-chemokines, the serum levels of IL-8/CXCL8 showed a significant increase ($p < 0.05$) in the allergic group compared to the non-allergic subjects. IL-4 levels were similar in both groups. In the sera of allergic patients, we have also detected significantly elevated levels of ragweed-specific IgE and IgG. However, decreased serum concentrations of the four CC-chemokines and elevated levels of IL-8 can be used as biomarkers for more accurate evaluation of the allergic status of patients with pollen allergy out of the season, to study the mechanisms for activation/inhibition of the subclinical allergic responses and for development of therapeutic strategies.

Adjuvanticity Of Lactobacilli In A Mouse Fes p1 Allergy Model

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Allergies of the respiratory tract represent a major health hazard to predisposed individuals. Species of the *Lactobacillus* genus have been examined extensively and oral/intranasal delivery of the bacteria was shown to be beneficial in different allergy models. We have chosen group I grass pollen allergen Fes p1 as an object of our study. Group I grass pollen allergens from different grass species show high sequence similarity, and high cross reactivity and is therefore a good target in an allergy model.

The aim of this study was to make a Fes p1 allergy model in Balb/c mice and to examine whether oral application of different species of *Lactobacillus* produces differences in antibody levels i.e. to test their adjuvanticity. Two chosen species were *L. plantarum* WCFS1, an extensively analyzed strain and *L. rhamnosus* LB64, a newly analyzed strain in this context.

Fes p1 allergen was isolated from pollen of *Festuca pratensis* with ion exchange chromatography, and 7 µg premixed with alum was injected i.p. into Balb/c mice, on days 0, and 7. *Lactobacilli* were given by oral gavage, and at the end of the experimental period antibody classes and subclasses were analyzed with ELISA. Peripheral blood smears were used to count eosinophil content.

Untreated animals had high Fes p1 specific IgE, and IgG1 levels, and eosinophilia. Oral application of either of the strains reduced peripheral blood eosinophilia. The administration of *L. plantarum* led to an increase in Fes p1 specific IgG2a, and *L. rhamnosus* increased both IgG2a, IgA, and IgE levels in mice.

The application of either of the tested *Lactobacillus* species had a beneficial effect in a Fes p1 mouse allergy model, with *L. rhamnosus* LB64 being superior because of its capacity to induce high serum IgA levels, which positively correlates to protective mucosal IgA.

Cell death of ED1⁺ cells in the central nervous system of Dark Agouti rats at the peak of experimental autoimmune encephalitis

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Infiltration of macrophages into the central nervous system (CNS), as well as activation of microglia is a hallmark of multiple sclerosis and its animal model - experimental autoimmune encephalomyelitis (EAE). Cell death in EAE has been demonstrated as an essential mechanism in the local regulation of the inflammatory reaction, but also as one of the major factors contributing to the destruction of the CNS tissue. Here, cell death of ED1⁺ cells (macrophages/microglia) in the spinal cord of EAE rats was investigated. Cell death in general was assessed using the TUNEL assay, while cleaved caspase-3 immunostaining was employed as the marker of "classical" apoptosis. Dark Agouti (DA) rats were immunized with spinal cord homogenate emulsified in complete Freund's adjuvant. Infiltrates of immune cells were detected both in white matter (WM) and grey matter (GM) of spinal cords in DA rats at the peak of EAE. ED1⁺, TUNEL⁺ and caspase-3⁺ cells were detected within, but also outside the infiltrates. While there were no differences in the proportion of TUNEL⁺ ED1⁺ cells between infiltrates and non-infiltrated areas in WM, there were more ED1⁺TUNEL⁺ cells in GM in infiltrates than in non-infiltrated areas. A similar distribution was observed for ED1⁺caspase-3⁺ cells. The observed discrepancy in distribution of dead ED1⁺ cells in infiltrates and non-infiltrated areas in GM and WM of spinal cord indicated that differential spatial regulation of macrophage/microglia cell death occurred in DA rats. These findings contribute to the understanding of pathogenesis of EAE in DA rats. It also opens new perspectives for a research aiming at more efficient treatment of multiple sclerosis.

Expression of miR146-a, an inflammation-associated microRNA, in Mesial Temporal Lobe Epilepsy

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Background: Neuroinflammation appears as an important epileptogenic mechanism. MicroRNAs (miRNA) are small non-coding RNA molecules that function as post-transcriptional regulators of gene expression. MicroRNAs control different biological processes including immune system homeostasis and function. Several evidences, both in patients and animal studies, have demonstrated an abnormal brain expression of miR-146a in Mesial Temporal Lobe Epilepsy. Knowing that miR expression is very stable in biological fluids such as plasma or serum our aim was to characterize miR146a expression in serum of MTLE patients.

Methods: Expression levels of miR146a and U6B small nuclear RNA gene (reference gene) were quantified by Real-Time PCR in serum of 14 MTLE patients all with Hippocampal Sclerosis (6F, 8M, mean age= 44.1±11.7 years, age of onset= 13.5±10.6 years, 7 with Febrile Seizures antecedents). A group of 10 healthy individuals was used as control. Relative expression values were calculated using the 2- $\Delta\Delta C_t$ method.

Results: We observed that expression of miR146a was 2 fold higher in MTLE-HS patients than in controls.

Conclusion: The results obtained in serum are in accordance with the results obtained from brain tissue of epileptic patients. This may confirm that miR-146a is a suitable biomarker of epileptogenesis. Additionally, it is thought that miR-146a has a role in fine-tuning the response to cytokines during epileptogenesis. Nevertheless its importance in epilepsy development it is yet not fully understood. The comprehension of this role may be relevant for the development of new therapeutic strategies.

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CMV infection in neonatal and adult mice induces susceptibility to EAE in resistant BALB/c mice

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In contrast to C57BL/6 mice BALB/c mice are relatively resistant to the induction of autoimmune encephalomyelitis evaluated by inflammation and demyelination in CNS after challenge with MOG35-55 peptide as encephalitogen. The aim of this study was to analyze the role of CMV infection in EAE pathogenesis. Female BALB/c mice were infected with murine cytomegalovirus before immunization with MOG35-55 and disease was evaluated by clinical and histological examinations. Immunophenotyping of CNS infiltrates was done by flow cytometry. Here we provide first evidence that both neonatal and adult infection with murine CMV (MCMV) abrogates this resistance. Infected BALB/c mice developed clinical and histological signs indistinguishable from those seen in susceptible C57BL/6 mice. MCMV infected and MOG35-55 immunized mice had massive infiltration in brain parenchyma, cerebellum and spinal cord. Further, analysis of infiltrating cells in infected mice revealed almost equal number of CD4⁺ and CD8⁺ infiltrating cells in CNS, similar with findings in multiple sclerosis. This study clearly indicates the influence of CMV in EAE pathogenesis and also reveals new experimental model more similar to MS. We also demonstrate that viral infection enhances the influx of MOG35-55 specific cells in CNS and favors pro-inflammatory type of APC in draining lymph nodes.

Key words: EAE, MCMV, BALB/c mice, CD8 lymphocyte

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New Strategies for Development of Antigen-specific T Regulatory Cells

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T regulatory cells (Treg)-based therapy of autoimmune diseases is rapidly developing. However, the low numbers of endogenous Tregs that can be isolated from an individual or laboratory animal have limited their study and potential therapeutic use. Therefore, various approaches are tested for *in vitro* Treg expansion. Antigen-specific Treg compared to polyclonal exert stronger suppressive effects in animal models of autoimmune diseases. During autoimmune process, the number of autoantigen-specific effector T cells is by far larger compared to autoantigen specific Tregs. Having in mind the plasticity of T cells, we hypothesized that it would be feasible to convert antigen-specific effector T cells isolated from animals with autoimmune disease to antigen-specific FoxP3⁺ Tregs. To this end several manipulations, including inhibition of Th17 cell differentiation, epigenetic modifications or redox modulations have been performed. To obtain autoantigen-specific T cells, NOD mice were immunized with Myelin Oligodendrocyte Glycoprotein (MOG) peptide emulsified in Complete Freund Adjuvant. Cells of draining lymph nodes and spleens were treated with MOG *in vitro* to reactivate MOG-specific effector T cells. After 96h of culture CD4⁺ cells were separated using magnetic beads (1,4% FoxP3⁺) and stimulated with pro-Treg cocktail (anti-CD3+anti-CD28 antibody+TGF- β +IL-2). After four days, the percentage of FoxP3⁺ cells reached 9,4% (as determined by flow cytometry). Simultaneous inhibition of Th17 cells with STAT3 inhibitor increased FoxP3⁺ population to 13,3%. On the other hand, promotion of histone acetylation by all trans retinoic acid and mytramincine (inhibitors of histone deacetylases) did not further increase the percentage of FoxP3⁺. Although simultaneous treatment with hydrogen peroxide (H₂O₂) had no effect on FoxP3⁺ cells, the administration of H₂O₂ 72h after stimulation with pro-Treg cocktail promoted generation of Treg by 5-fold. These results implicate that Treg population can be enriched from the pool of effector CD4⁺ T by various *in vitro* approaches.

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Anti-inflammatory effects of benfotiamine in LPS stimulated microglia: role of NF- κ B signaling

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Microglial cells are immune cells of the central nervous system (CNS) that play a major role in its surveillance. Changes in CNS homeostasis, invading pathogens or neuron impairment, lead to activation of microglial cells that quickly proliferate, acquire amoeboid morphology and produce toxic mediators such as nitric oxide (NO) and pro-inflammatory cytokines. These changes are regulated by transcription factors, most importantly NF- κ B. Although microglial activation is important for maintaining tissue homeostasis, excessive activation leads to chronic inflammation that can damage healthy neurons. Substances that suppress microglial activation are potential therapeutics for neurodegenerative diseases. Benfotiamine (S-benzoylthiamine-O-monophosphate) is a synthetic derivative of vitamin B1 that has anti-inflammatory properties. In this study we investigated anti-inflammatory properties of benfotiamine on activated microglia *in vitro*.

BV-2 microglia were pre-treated with benfotiamine, stimulated with LPS and their viability and morphology were evaluated. LPS induced prominent alterations in cell morphology, enlargement of cell bodies and spreading of multiple processes. Pre-treatment with benfotiamine before LPS stimulation suppressed these morphological changes. Additionally, benfotiamine diminished LPS induced NO production, without altering cell viability. Furthermore, benfotiamine decreased LPS induced production of pro-inflammatory cytokines TNF- α and IL-6, while increasing production of anti-inflammatory cytokine IL-10. Analysis of NF- κ B activation revealed that benfotiamine's effects were mediated by this transcription factor.

In conclusion, benfotiamine exerts anti-inflammatory properties in LPS activated microglia *in vitro* and should be further investigated as a potential therapeutic for neurodegenerative diseases.

Cellular and molecular correlates of sexual dimorphism in the clinical presentation of experimental autoimmune encephalomyelitis in rats

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Male Dark Agouti rats immunized for experimental autoimmune encephalomyelitis (EAE) exhibited more severe disease compared with their female counterparts. To elucidate the cellular and molecular mechanisms underlying this phenomenon, CD4⁺ T lymphocytes and antigen presenting cells from spinal cord and/or draining lymph node (DLN) were examined for activation molecule expression and cytokine profile. At the peak of EAE, consistent with the greater maximal neurological score in male rats, the number of re-activated CD4⁺ T-cells and proportion of highly activated microglial cells/macrophages (CD45^{high}) among CD11b⁺ cells (producing more TNF- α , IL-1b, IL-6 *in vitro*) in their spinal cord were greater. Furthermore, IL-17+/IFN- γ + T-cell ratio in male rat spinal cord was shifted towards the former on the account of the highly pathogenic IL-17+/IFN- γ + cells. This was in accordance with upregulated IL-23/p19, IL-1 β and IL-6, but downregulated TGF- β expression in male rat spinal cord mononuclear cells (MNC). In the inductive phase of disease, greater frequency of re-activated CD4⁺ and IL-17+ T-cells was also observed in male rat spinal cord. Consistently, greater frequency of CD45^{high} cells among male rat spinal cord CD11b⁺ cells and increased expression of cytokines favouring Th17 polarization in their spinal cord MNC were registered. On the contrary, in DLN from rats of the same sex lower frequency of activated cells within CD4⁺ T lymphocytes and cells expressing MHCII and CD40 within the CD11b⁺ population was found. Additionally, in male compared with female rats, the frequency of IL-17+ and IFN- γ + T-cells was reduced in DNL and DNL T-cells produced less IL-17 and IFN- γ upon MBP stimulation *in vitro*. Collectively, the results suggest that: i) DLN and spinal cord microenvironments differ in CD4⁺ T-cell activation and polarization capacity, and ii) the target organ microenvironment has important role in shaping a sexually dimorphic clinical outcome of EAE.

Longitudinal plasma lipid profile in multiple sclerosis patients during three years of treatment with interferon-beta

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Background: It has been recently suggested that interferon (IFN)- β , the first-line disease-modifying treatment in relapsing remitting (RR) multiple sclerosis (MS), might influence plasma lipid profile in treated patients. However, data on longitudinal plasma lipid profile in MS patients treated with IFN β are scarce and with conflicting results.

The aim of this study was to analyze longitudinal plasma levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and total triglycerides (TG) in RRMS patients treated with IFN β -1a or IFN β -1b (both subcutaneously) over 3 years and correlate those levels with clinical treatment response.

Materials and methods: We determined plasma TC, LDL-C, HDL-C and TG levels prior to IFN β -treatment (baseline) and at treatment months 6, 12, 24 and 36 by commercial methods. Neurological disability was scored by the Expanded Disability Status Scale (EDSS). Treatment response criteria were: no EDSS score increase ≥ 1 points sustained over ≥ 6 months and no relapse during 3 years of follow-up. Forty-five patients (n=45) were considered responders and 25 were found to be non-responders according to these clinical criteria.

Results: TC, LDL-C increased significantly at 6 months, but returned to baseline values at treatment month 12 and remained similar afterwards. By contrast, HDL-C decreased at treatment month 6, but continued to grow afterwards. TG levels peaked at month 6 and month 12 of treatment. At any study time point, analyzed plasma lipid profile parameters did not differ significantly between responders and non-responders and did not correlate with the EDSS score.

Conclusion: Our results further support a notion that IFN β treatment in MS patients might originate changes in plasma lipid profile. Further research is needed to investigate whether those changes might reflect changes in disease activity in patients with MS treated with IFN β .

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MicroRNA-155 Contributes to Re-activation of Encephalitogenic T Cells

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MicroRNAs (miR) are small non-coding RNAs that have a profound role in regulating immune response. It has been suggested that miR-155 significantly contributes to the pathogenesis of multiple sclerosis and its animal model experimental autoimmune encephalomyelitis (EAE). Here, the role of miR-155 in re-activation of encephalitogenic CD4⁺ T cells that are considered as the major pathogenic population in multiple sclerosis and EAE was investigated. Dark Agouti (DA) rats were immunized with myelin basic protein (MBP) emulsified in complete Freund's adjuvant. CD4⁺ T cells were purified from draining lymph node cells (DLNC) isolated on day 6 after the immunization (inductive phase of EAE) and from spinal cord immune cells (SCIC) isolated at the peak of EAE. CD4⁺ T cells obtained from SCIC (i.e. *in vivo* re-activated cells) had markedly higher expression of miR-155 in comparison to those purified from DLNC. Further, *in vitro* re-activation of CD4⁺ T cells obtained from DLNC with MBP also led to increase in miR-155 expression. In order to examine if the observed elevation of miR-155 expression during re-activation of encephalitogenic CD4⁺ T cells had a functional significance, DLNC, SCIC and CD4⁺ T cells purified from them were transfected with an inhibitor of miR-155 (anti-miR-155). As a consequence, expression of important CD4⁺ T cell effector cytokines IFN- γ and IL-17 was reduced. These results imply that miR-155 contributes to encephalitogenicity of CD4⁺ T cells in autoimmune neuroinflammation.

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IL-33/ST2 axis mediates resistance to EAE by promoting regulatory B and tolerogenic dendritic cells

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BALB/c mice are resistant to EAE induction with MOG₃₅₋₅₅. ST2 molecule is expressed on immune cells and its signaling is accompanied by accentuated ST2 response. We recently published (Milovanovic et al. PlosOne 2012) that deletion of ST2 molecule abrogates resistance to EAE, as evaluated by clinical and histological findings. Adoptive transfer of ST2 KO lymphocytes restimulated with MOG₃₅₋₅₅ induced clinical signs of the disease in ST2 KO and BALB/c mice. MOG₃₅₋₅₅ specific CD4⁺ lymphocytes of ST KO mice had higher expression of transcriptional factor T-bet, inflammatory cytokines IFN γ , IL-17, GM-CSF, TNF α and chemokine receptors important for migration to CNS (CCR6, CXCR5 and CXCR3). ST2 KO mice had higher incidence of activated antigen presenting cells, myeloid/regulatory dendritic cells (DC) ratio and percentages of myeloid cells containing inflammatory cytokines, IL-1, IL-12 and IL-6. ST KO DC induced higher *in vitro* proliferation of CD4⁺ cells and stimulated *in vitro* differentiation compared with DC isolated from BALB/c mice. Interestingly we also observed the increase of regulatory CD11b⁺CD5⁺ B lymphocytes, IL-10 producing B1 lymphocytes and decrease of IL-6 producing B cells in BALB/c mice after immunization with MOG₃₅₋₅₅ peptide. The opposite results were found in ST2 KO mice.

Results indicate that ST2 signaling influences the activity of regulatory B1 cells and differentiation of proinflammatory antigen presenting cells, consecutively affecting the differentiation of encephalitogenic T cells in the periphery.

Key words: EAE, ST2 molecule, dendritic cells, regulatory B cells, BALB/c mice

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T helper cells 1/17-related genes in interferon- β -treated relapsing-remitting multiple sclerosis patients – correlation to clinical response

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Interferon (IFN)- β , first-line treatment in relapsing remitting multiple sclerosis (RRMS), alters the expression of more than a hundred genes, but we still lack the explanation why many patients do not respond to therapy. In multiple sclerosis (MS), T helper cells (Th) 1 and Th17 are considered the orchestrators of the myelin destruction, thus we assessed expression of Th1/17-related genes in IFN- β -treated RRMS patients over 36 months.

RRMS patients (n=45) on IFN- β 1b therapy were considered responders (R, n=20), if without relapses or progression on EDSS during 3 year follow-up and the rest as non-responders (NR, n=25). Relative gene expression was determined in peripheral blood mononuclear cells by qPCR for marker of IFN- β biological response (MX1, OAS2), Th1/17 polarizing cytokines (IL-12/23p40, IL-12p35, IL-23p19), respective receptors (IL-12R β 1, IL-12R β 2, IL-23R), transcription factors (T-bet, RoR γ t) and effector cytokines (IFN- γ , IL-17A, IL-17F, GM-CSF) before start and at 6, 12, 24 and 36 months of IFN- β therapy.

All mRNA levels changed statistically significantly during the therapy, with, on average, similar values in R and NR. MX1 and OAS2 mRNA levels were up-regulated at every time point, and statistically highly correlated, in both groups. IL-12/23p40, IL-12p35, IL-23p19 and GM-CSF mRNA expression decreased, while IL-23R, RoR γ t and IFN- γ statistically significantly increased at 6 months. All mRNA levels reverted to baseline at 24 months. At 36 months, IL-12/23p40, IL-23p19 and T-bet mRNA levels decreased, IFN- γ and IL-12R β 1 levels were similar to 24 months ones, and the rest increased statistically significantly.

MX1 and OAS2 showed R and NR did not differ in IFN- β biological response. Although expression levels of Th1/17 axes' genes did not distinguish R and NR, we observed large inter-individual variations in temporal patterns of particular genes. Furthermore, overall changes of mRNA levels due to IFN- β treatment warrant further investigation.

Cytokine profiles in first episode psychosis and schizophrenia in relapse

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The field of “psychoneuroimmunology” has gained increasing interest and research work during the last 20 years. Several epidemiological studies demonstrate a co-occurrence of autoimmune diseases, chronic inflammatory conditions, and mental disorders. Since in many cases the elevated prevalence of autoimmune/chronic inflammatory diseases existed before the onset of psychosis, the hypothesis was put forward that the onset of psychosis could be induced by an inflammatory process elicited by the autoimmune reaction. The aim of our study was to analyze the serum concentrations of type-1, type-2, type-17 and regulatory cytokines in drug-naive patients with first episode psychosis and schizophrenia in relapse. We have demonstrated that type-1 and type-17 responses are blunted and type-2 overweight in schizophrenia. Our results also implicate anti-inflammatory response through TGF- β production. Analysis showed that TGF- β and IL-23 can be valuable markers for psychosis. The presence of enhanced anti-inflammatory/immunosuppressive activity in schizophrenia may be an attempt to counteract or limit ongoing pro-inflammatory processes and downregulating chronic inflammation. A chronic pro-inflammatory stage with a robust type-2 response, including high IL-6 levels, may also predominate in later stages. Our results suggest that immune pattern in schizophrenia can be similar with those in atopic disorders and this cytokine disbalance can be corrected with antipsychotics.

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Antigen-affinity mediates B cell survival through differential BAFF receptor signaling via the PI3K/Mcl-1 cascade

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Upon antigen encounter, the responsive B cell pool undergoes spectacular affinity increase through a stringent selection mechanism that eliminates cells with low B cell receptor (BCR) affinity. B cell selection in the Germinal Center (GC) has been well described, but the crucial phase of affinity-dependent selection before GC entry is relatively obscure. Previously, we showed that mice deficient for the pro-apoptotic protein Noxa have an antibody response of reduced affinity, due to increased survival of low-affinity clones that enter the GC. In this study, we investigated the mechanism behind early affinity-mediated B cell selection. We find that in the first days after activation, B cells depend on BAFF for survival and induce expression of the BAFF receptor in a BCR-affinity dependent fashion. In response to BAFF, PI3K signal intensity is larger in high-affinity compared to low-affinity B cells, resulting in increased levels of the pro-survival protein Mcl-1. Inhibition of PI3K following BAFF stimulation in activated B cells reduced Mcl-1 protein levels and negated survival differences between cells of high- and low affinity. Deficiency for the Mcl-1 antagonist Noxa reduced dependence on PI3K for survival in activated B cells. In the presence of redundant BAFF, or in absence of Noxa, immunization resulted in a relative increase of low-affinity B cells and an overall reduction of the total antigen-responsive B cell pool upon immunization. Our findings elucidate an important mechanism of antigen-affinity dependent selection in the earliest phases of B cell activation.

Inflammatory Microglial Activity is Inhibited by Short Term Exposure to Ethyl Pyruvate

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Ethyl pyruvate (EP) is a potent redox and anti-inflammatory agent that has recently been shown protective in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. Microglial cells basically have protective role in the central nervous system, but their inappropriate pro-inflammatory activity has been shown detrimental in various neurodegenerative disorders, including multiple sclerosis. It was previously shown that EP potently inhibits inflammatory activity in microglia. The aim of this study was to determine whether microglial inflammatory activity is sensitive to short term exposure to EP. As the result, short term treatment of BV2 microglial cells with EP inhibited production of pro-inflammatory cytokines (interleukin-6 and tumor necrosis factor) and nitric oxide in the cells. At the same time, activation of NFκB in BV2 cells was not affected by EP, implying that the observed effects of the agent on pro-inflammatory molecules was achieved in NFκB independent way. The observed immunomodulatory effects are important for understanding therapeutic potential of EP in treatment of neuroinflammatory disorders.

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The Potential Immunomodulatory Role of Cortisol on NKG2D Activating NK Cells Receptor in Ovarian Cancer

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Background: Tumour-infiltrating myeloid-derived suppressor cells (MDSC) or tumour-associated macrophages (TAM) which are abundant in ovarian cancer show a high expression of the enzyme 11Beta-Hydroxysteroid dehydrogenase I (11 β -HSD1) which able to convert inactive cortisone into active cortisol which has been detected in serum, ascitic fluid and tumour exudates from ovarian cancer patients. Considering that cortisol has a strong suppressive effect on immune cells, we investigated the effect of cortisol or TGF- β or both since they do not act separately of each other but modulate each other's activities on the activating NK cell receptor (NKG2D) expression. This shall now be investigated by pharmacological inhibitors of cortisol (RU486) and TGF- β (SD208).

Material and methods: Using immunohistochemistry, real-time PCR, luminescent immunoassays (LIA), Flow cytometry and immunofluorescent double staining.

Results: We found that 11b-HSD1 enzyme is highly expressed in human and murine ovarian cancer tissue via real-time PCR and immunohistochemistry. Luminescent immunoassays (LIA) showed elevated cortisol in serum, ascitic fluid and tumour exudates from ovarian cancer patients. Immunofluorescent double staining revealed a co-localization of 11b-HSD1 with CD14, CD68, and CD85, but not with EpCAM. Expression of 11 β -HSD1 can thus be attributed to TAM or MDSC. Expression of NKG2D on NK cells was analyzed by flow cytometry. The result indicated that NKG2D is downregulated by both cortisol and TGF- β 1. Cortisol and TGF- β 1 were further found to act synergistically in downregulating NKG2D. However, RU486 and SD208 were able to restore the activating NKG2D receptor expression on NK cells.

Key words: Ovarian Cancer, 11Beta-Hydroxysteroid dehydrogenase I, Cortisol, TGF- β , NKG2D; NK Cells.

IL-33 modulate innate immunity to tumours

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The role of IL-33/ST2 pathway in antitumor immunity is not elucidated. We have previously shown that ST2 deletion enhanced anti-tumor immune response in a murine model of metastatic 4T1 breast carcinoma which was associated with potentiated Th1/Th17 cell polarization and enhanced NK cell cytotoxic activity (Jovanovic I, et al. Eur J Immunol. 2011;41:1902–12). In this study we demonstrate time dependent increase of endogenous IL-33 at both the mRNA and protein levels in primary 4T1 tumors and metastatic lungs during breast cancer progression. Further, exogenous administration of recombinant murine IL-33 accelerated tumor growth and development of lung and liver metastases, which was associated with increased intratumoral accumulation of CD11b⁺Gr-1⁺ TGF-β1⁺ myeloid-derived suppressor cells (MDSCs) that expressed IL-13α1R, IL-13-producing Lin⁺Sca-1⁺ST2⁺ innate lymphoid cells (ILCs) and CD4⁺Foxp3⁺ST2⁺IL-10⁺ Tregs. Higher incidence of monocytic vs. granulocytic MDSCs and plasmacytoid vs. conventional dendritic cells (DCs) was present in mammary tumors of IL-33-treated mice. Intratumoral NKp46⁺NKG2D⁺ and NKp46⁺FasL⁺ cells were markedly reduced after IL-33 treatment, while increased frequencies of these tumoricidal natural killer (NK) cells were found within primary tumors in ST2-deficient mice compared to wild-type mice. Administered IL-33 promoted intratumoral cell proliferation and neovascularization, which was attenuated in the absence of ST2. Tumor-bearing mice given IL-33 had increased percentages of splenic MDSCs, Lin⁺Sca-1⁺ ILCs, IL-10-expressing CD11c⁺ DCs and alternatively activated M2 macrophages and higher circulating levels of IL-10 and IL-13. A significantly reduced NK cell, but not CD8⁺ T-cell cytotoxicity in IL-33-treated mice was observed and the mammary tumor progression was not affected by *in vivo* depletion of CD8⁺ T cells. We demonstrate a previously unrecognized role for IL-33 in promoting breast cancer progression through increased intratumoral accumulation of immunosuppressive cells and by diminishing innate antitumor immunity. Therefore, IL-33 may be considered as an important mediator in the regulation of breast cancer progression.

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Antimelanoma and chemosensitizing properties of hop plant derived compound isoxanthohumol

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It is well known that many synthesized and natural compounds from plants apart from anti-inflammatory properties possess strong anticancer activity. Since it has been recently demonstrated that beer and hop plant (*Humulus lupulus*) derived compound, isoxanthohumol (IXN), down regulates inflammation, the aim of this study was to investigate possible anticancer potential of IXN *in vitro* and *in vivo*.

Our results showed remarkable viability decrease of mouse (B16) melanoma cells in dose-dependent manner after the treatment with IXN. Oppositely, the autophagic process was not responsible for the decrease of cell viability. On the other hand, certain percentage of apoptotic and necrotic cells followed diminished division rate of survived melanoma cells. At the intracellular level, inhibited activity of well-known regulators of cell proliferation, p70-S6 Kinase and S6 ribosomal protein was determined. Microscopic observation of the melanoma cells exposed to IXN revealed remarkable morphological transformation synchronized by elevated tyrosinase activity, indicating enhancement in pigment synthesis as biochemical marker of differentiation toward melanocytes. In addition to observed direct tumoricidal effect, IXN potentiated the effectiveness of paclitaxel *in vitro*. Moreover, chemosensitization was confirmed in syngeneic melanoma model where mice were exposed to subtoxic doses of this chemotherapeutic.

Taken together, these results point out multiple benefits of IXN manifested through direct anticancer potential as well as remarkable capacity to sensitize melanoma cells to conventional therapy with paclitaxel.

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Systemic TNF ablation results in delayed tumor growth and reduced MDSC accumulation in transplantable tumor model

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Myeloid-derived suppressor cells (MDSC) represent a heterogeneous population that consists of immature myeloid cells (IMC). Under normal conditions, these cells differentiate into mature macrophages, granulocytes and dendritic cells. However, under pathological conditions such as inflammation, cancer or infection, there is an inhibitory effect on differentiation of IMC that leads to their expansion. MDSC have immune suppressive activity through upregulation of arginase 1 (Arg1) and inducible nitric oxide synthase (iNOS). These suppressive factors can enhance tumor growth by repressing T cell-mediated antitumor responses. Recently, it was shown that TNF-TNFRII axis sustains survival of MDSC through upregulation of cellular FLICE-inhibitory protein (c-FLIP) and inhibition of caspase-8 activity [1].

In our work we established an experimental model to study the effect of systemic TNF ablation on the tumor-induced expansion of MDSC in vivo. We used Etanercept, a soluble fusion protein of p75 TNF receptor and the constant end of IgG1 antibody, as an inhibitor of murine TNF in C57Bl/6 mice transplanted with MCA205 fibrosarcoma. Infliximab, a chimeric monoclonal antibody against human TNF, was used as control since it does not bind mTNF [2].

Mice were injected with 10µg/g of Etanercept or Infliximab every three days. Tumor cells were injected one week after the initiation of anti-TNF therapy. Tumor growth was monitored every 2–5 days. We also assessed the accumulation of MDSC in the peripheral blood by flow cytometry using epitope-specific antibodies against CD11b and Gr-1.

We show that systemic anti-TNF therapy results in delayed growth kinetics of MCA 205 fibrosarcoma. Mice injected with Etanercept have significantly reduced tumor volume and accumulate less MDSC in the peripheral blood three weeks after MCA tumor cell inoculation. Our data suggests that Etanercept-based therapy reduces solid tumor growth and tumor-induced accumulation of MDSC in MCA-205 fibrosarcoma tumor model.

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Decreased level of various peripheral blood lymphocyte signaling molecules in metastatic melanoma patients

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Background: As numerous signaling molecules regulate effector functions of peripheral blood lymphocytes (PBL) that have an important antitumor activity the aim of this study was to analyze their level in metastatic melanoma (MM) patients compared to healthy controls (HC).

Methods: Peripheral blood mononuclear cells (PBMC) of 33 MM and 26 HC were analyzed for the level of perforin, interferon-regulating transcription factor-1 (IRF-1), DAP10 and *Src homology 2* domain-containing tyrosine phosphatase-1 (SHP-1) by *reverse transcriptase* polymerase chain reaction (RT-PCR), level of *phosphorylated* signal transducers and activators of transcription (pSTATs), STAT-1, STAT-4 and STAT-5, by Western blot and IFN-gamma (IFN- γ) production by enzyme linked immunosorbent assay (ELISA). The expression of activating NKG2D and inhibitory killer immunoglobulin-like receptors (KIRs), CD158a, and CD158b, on PBL, CD3-CD56+ NK cells and CD3+CD8+ cytotoxic T cells (CTL), as well as the percentage of CD14+HLA-DR- cells in PBL were estimated by Flow cytometry.

Results: MM patients, compared to HC, had significantly lower level of cytotoxic molecule perforin and decreased IFN- γ production, as well as lower level of pSTAT-1, -4, -5 and IRF-1 signaling molecules in their PBMC. Furthermore, MM patients have decreased expression of activating NKG2D receptor on PBL and NK cells and low level of its DAP10 signaling molecule contrary to no changes in NKG2D expression on CTL and CD158a and CD158b KIR expression on all investigated cells. Increased percentage of immunosuppressive CD14+HLA-DR- myeloid-derived suppressor cells (MDSC) was estimated in MM patients.

Conclusions: The altered signaling molecules in peripheral blood lymphocytes of metastatic melanoma patients could represent biomarkers of impaired cytotoxic and immunoregulatory function of these cells indicating melanoma associated immunosuppression that may facilitate tumor progression. Furthermore, these molecules could be used as parameters for the evaluation of standard or developing immunotherapy in patients with advanced melanoma.

Decreased interferon γ production in CD3+ and CD3-CD56+ lymphocyte subsets in metastatic regional lymph nodes of melanoma patients

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As lymphogenic dissemination is very common in melanoma, regional lymph nodes (LN)s represent first immunological barriers to tumor invasion and play a complex role in antitumor immune defense. In this sense, the most prominent role is the presentation of tumor-derived antigens to naïve T cells and generation of cell-mediated adaptive immune response.

Since tumor micro-environment affects immune cell function in this study we have evaluated the ability of T cells and NK cells in metastatic (involved) and non-metastatic regional LNs to produce interferon γ (IFN γ), a pleiotropic cytokine that regulates adaptive antitumor immune response.

Our results show reduced IFN γ production in both T and NK lymphocyte subsets and decreased prevalence of T cells in metastatic regional LNs of melanoma patients. The decrease of IFN γ production in T cells was more pronounced with increased number of involved regional LNs indicating tumor-induced functional impairment of both T and NK cell lymphocyte subsets in involved regional LNs. Therefore, low IFN γ production in metastatic LNs may represent an obstacle in adaptive cell-mediated antitumor immune response and hence may enable tumor progression.

Tumor cell lines K562 and HEPG2 decrease human natural killer cell cytotoxicity and increase expression of inhibitory receptor TIGIT

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Natural Killer (NK) cells lyse tumor cells lacking MHC-I and expressing ligands for activating receptors, thereby participating in cancer immune surveillance. However, their function and phenotype change dramatically in the presence of tumors. The aim of the present study was to investigate changes of both receptor phenotype and cytotoxic activity of NK cells co-cultured with K562 and HepG2 cell lines in dual-chamber Transwell® plates. NK cells were obtained from peripheral blood of healthy donors by immunomagnetic separation. After co-culture with HepG2 or K562 in Transwells® for 48h, NK cells were assessed for cytotoxicity to K562 cells at a 2:1 effector:target ratio and flow cytometry analysis of phenotype. According to MTT assay, NK cell cytotoxicity decreased by $20.8 \pm 3.1\%$ ($p=0.007$) and $19.1 \pm 6.6\%$ ($p=0.038$) relative to controls after co-culture with HepG2 and K562 cells, respectively. No changes in the expression of perforin, granzyme B, or CD107a were observed. Changes in the expression of a majority (CD16, NKp30, NKp44, NKp46, CD69, DNAM-1, 2B4, NKG2A) of NK cell receptors did not reach statistical significance. TIGIT expression increased on NK cells after incubation with K562 ($12.0 \pm 5.7\%$, $p=0.015$) and HepG2 cells ($10.4 \pm 5.6\%$, $p=0.042$) compared to controls ($4.7 \pm 3.2\%$). In conclusion, the observed findings demonstrate that tumor cell derived soluble factors induce a decrease in NK cell cytotoxicity accompanied by an increase in inhibitory receptor TIGIT expression. It is of interest to determine whether this phenomenon is recapitulated in cancer patients and could represent a novel parameter in assessment of NK cell function.

Recombinantly produced Banana Lectin stimulates Humoral and Cellular Immune Response in the Colon

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Lectins are a broad group of bioactive molecules present in plant-based food in appreciable amount. Their biological functions are associated with the carbohydrate binding activities. They can bind the gut epithelium and, depending of their fine specificity, affect digestive tract functioning in a distinctive ways. We evaluated the influence of recombinantly produced banana lectin (rBanLec) on the immune response in the colon of BALB/c mice. Local immune response was monitored up to 72h upon a rectal administration of rBanLec (100 μ l of 0.1, 1 or 10 μ g/ml; equal to 0.5, 5 and 50 μ g/kg body weight, respectively). Immunohistochemical analysis has shown that rBanLec bound the mucosal surface of the colon and passed through the epithelial barrier. Skewing of immune response toward Th1/Th17 direction could be assigned as a general characteristic of rBanLec stimulation in the colon but qualitative and quantitative characteristics of the established cytokine network depended on the applied rBanLec concentration. rBanLec transitionally augmented secretion of IFN γ and IL-17A, which was counterbalanced by increase in IL-10 production. Significant rise in the secretion of sIgA and IgG2b indicated the local enhancement of TGF- β production as well. rBanLec promoted a transitional rise in local NO concentration and MPO activity that coincided with the peak in concentration of pro-inflammatory cytokines (48h upon the application).

Our results show that rBanLec, even in low dosage that could be taken in by everyday nutrition, acts as a polyclonal stimulator affecting (in)directly both humoral and cellular immune responses in the colon. Its ability to stimulate balanced pro-inflammatory immune response in the colon and to enhance the antimicrobial activity implies that rBanLec could be a valuable tool in prevention of a pathogen invasion via the colon.

Lack of association between TNF gene polymorphism and the acute kidney rejection

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Introduction: Acute and chronic rejections remain an important cause of graft loss after renal transplantation. The inflammatory microenvironment within the graft may play a role in the precipitation of rejection. Several lines of evidence have implicated TNF in the early alloimmune response to solid organ transplantation. Previous studies reported that tumor necrosis factor (TNF) promoter polymorphism (-308 G/A) has influence on the functional immune response of transplant recipient outcome.

Methods: One hundred fifty two patients who underwent renal transplantation were included in this study. The genotyping was based on the TaqMan method using commercially available probes. Genotype frequencies were assessed for Hardy–Weinberg equilibrium using a chi-squared goodness-of-fit test. The statistical difference in genotype distribution and allele frequencies in case subjects was analyzed using Pearson's chi-square or Fisher's exact tests.

Results: The genotype frequency in group of transplanted patients with and without acute graft rejection was AA 1.0%, GA 26.5%, GG 72.4% and AA 2.0%, GA 22.4%, GG 75.5% respectively. This study showed that TNF- α promoter polymorphism was not associated with the risk of acute rejection.

Conclusions: Our findings demonstrated no significant association between TNF- α gene polymorphisms and acute renal transplant rejection. However, due to conflicting results from this and other studies, a multi-centre collaborative study may be required to determine whether cytokine genotypes are significant, independent predictors of renal allograft rejection.

Vitamin D insufficiency affects IL-10 mediated regulation of cellular immunity

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Background: Vitamin D (vitD) has well-established immunomodulatory functions, at least in part associated with effector and regulatory immune cell cytokines. Although vitD deficiency in HIV+ subjects is well recognized, current evidence for its impact on cellular immunity is relatively weak.

Aim: To assess the effect of vitD insufficiency on the “ex vivo” cytokine profiles (IFN γ , TNF α , IL-4, IL-6, IL-10) and immune restoration of HIV+ patients subjected to antiretroviral therapy (ART).

Materials and methods: Serum vitD levels (ng/ml, VIDAS 25OHVitD BioMérieux ELISA), CD4 absolute count (CD4AC cells/mkl) and restoration rate (Δ CD4/months on ART), CD4/CD8 ratio (BDMultiSet, FACSCanto II), and viral load (VL, HIV-RNA copies/ml, COBASAmpliPrep/ TaqManPCR) were determined in 60 HIV+ART+ patients. For 30 of them, “ex vivo” cytokine concentrations (pg/ml) were measured in peripheral blood plasma by flow cytometry (CBA BD™).

Results: Patients fell into three groups according to vitD levels: **A**, deficient (<20ng/ml, n=23); **B**, insufficient (20-30ng/ml, n=35) and **C**, sufficient (>30ng/ml, n=11). While CD4AC, CD4/CD8 and VL did not differ between groups, a significantly lower CD4AC restoration rate was observed in group **A** (mean 6.4 vs.16.4/**B**), combined with decreased IL-10 level (mean 1.2 vs.1.6/**B**). After excluding patients with extremely low IFN γ expression and low CD4AC, considered as “exhausted”, decreased vitD was associated with higher levels of IL-6 (1.0/**A**, **B** vs.0.88/**C**); while vitD insufficiency was distinguished by increased IL-10 level (1.7 vs.1.3/**A**), and decreased mean IFN γ /IL-4 ratio (45 vs.71/**A**). For all comparisons p<0.05.

Conclusion: VitD insufficiency is a critical stage contributing to increased immune activation (IL-6), stimulated regulatory response (IL-10) and hence, suppression of Th1 response (IFN γ /IL-4). A hallmark of advanced vitD deficiency is the reduced regulatory potential (IL-10) bringing to further Th1 exhaustion and slower recovery of CD4 pool, regardless ART. Our data propose that vitD supplementation could improve the effect of ART when timely applied.

***In Vitro* Effects of Binuclear (η^6 -*p*-cymene) Ruthenium (II) Complex Containing Bridging *Bis* (nicotinate)-Polyethylene Glycol Ester Ligand on Differentiation Pathways of Murine Th Lymphocytes**

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T cell differentiation into distinct T-helper (Th) subpopulations is crucial in governing adaptive immune responses as well as some inflammatory and autoimmune disorders. In this study, the potential effect of the novel neutral binuclear ruthenium(II) complex [$\{\text{RuCl}_2(\eta^6\text{-}p\text{-cym})\}_2\text{-}\{(3\text{-py})\text{COO}(\text{CH}_2\text{CH}_2\text{O})_4\text{CO}(3\text{-py})\}$] (**4**) on Th cell differentiation in mouse splenocytes was evaluated *in vitro*. It did not affect mouse splenocyte viability (in concentration up to 50 μM), but significantly reduced secretion of representative Th1 cytokine, IFN- γ induced by T cell mitogen concanavalin A (ConA). Beside IFN- γ , **4** inhibited dose-dependently expression and production of representative Th17 cytokine, IL-17, in these cells. Otherwise, the production of anti-inflammatory cytokines IL-4 and IL-10 was up-regulated. Also, **4** significantly increased CD4⁺CD25⁺FoxP3⁺ Treg cell frequency in the ConA-activated splenocytes. Moreover, **4** reduced expression of Th1 transcription factors, T-bet and STAT1, as well as of Th17-related protein STAT3, while the expression of Th2-related transcription factor GATA3 remained stable. In conclusion, **4** modulates mouse immune system cell functions *in vitro* by inhibiting T cell differentiation towards pathogenic Th1/Th17 phenotype and inducing a regulatory phenotype characterized by IL-10 and IL-4 production. The ability of **4** to direct Th cell differentiation may provide novel therapeutic opportunities for immune-inflammatory and/or autoimmune disorders.

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TRP channels overexpression contributes to inflammasome activation in clavicular cortical hyperostosis?

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Background: Clavicular cortical hyperostosis (CCH) is a sterile inflammatory bone disorder of unknown etiology clinically characterized by pain and/or swelling of the clavicle. It has been regarded as a variant of chronic nonbacterial/recurrent multifocal osteomyelitis (CNO/CRMO) but due to lack of other inflammatory sites and recurrence it could also be regarded as a separate disease in the spectrum.

Objectives: Identification of specific gene expression patterns in CCH patients.

Methods: Total RNA was isolated from whole blood of 18 new-onset, untreated CCH patients and 8 healthy controls. DNA microarray gene expression was performed in 5 CCH and 4 control patients along with bioinformatical analysis of retrieved data. Carefully selected differentially expressed genes (TRPM2, TRPM3, TRPM7, CASP2, MEFV, STAT3, EIF5A, ERBB2, TLR4, NLRP3, CD24, MYST3) were analyzed by qRT-PCR in all participants of the study.

Results: Microarray results and bioinformatical analysis revealed 974 differentially expressed genes, while qRT-PCR analysis showed significantly higher expression of TRPM3 and TRPM7, and lower expression of ERBB2 (Graph 1).

Conclusions: TRPM3 and TRPM7 are transient receptor potential (TRP) proteins that act as multimodal sensor cation channels for a wide variety of stimuli, one of which is environmental temperature that in the case of CCH could be elicited by overuse of sterno-clavicular joint (SCJ) [1]. Altered TRP channel function can have strong effects on a variety of cellular and systemic processes, including the activation and the regulation of the inflammasomes, which are reported to be involved in CRMO pathogenesis [2,3]. ERBB2, third gene with significant expression change, belongs to a family of genes that encodes for cell surface growth factor receptors. ErbB activation promotes protective cellular outcomes during inflammation, hence lower expression of this gene could cause damage due to inflammation [4]. We hypothesize that CCH could be an autoinflammatory disease induced by SCJ overuse, TRP channel overexpression, inflammasome activation and reduced protection during inflammation.

Effects of royal jelly fatty acids on proliferation and cytokine production by human peripheral blood mononuclear cells

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Royal jelly has been widely used as healthy food in humans due to the presence of different biologically active components. Fatty acids, isolated from royal jelly, have recently been investigated as potentially immunomodulatory components. In this study, we investigated the immunomodulatory effects of two fatty acids isolated from RJ, 10-hydroxy-trans-2-decenoic acid (10-HDA) and 3,10-dihydroxy-decanoic acid (3,10-DDA), using a model of phytohaemagglutinin (PHA)-activated human peripheral blood mononuclear cells (PBMCs). We demonstrated that both fatty acids at higher concentrations (500 μ M) inhibited the proliferation of PBMCs and the process was followed by a decrease in the production of interleukin-2 (IL-2). 10-HDA at concentration of 500 μ M down-regulated the production of IL-1 β and tumor necrosis factor- α by stimulated PBMCs, whereas the same dose of 3,10-DDA had no effect on the levels of these cytokines. Regarding T-helper (Th) cytokine profile, higher concentration of 10-HDA, in contrast to the lower one (50 μ M), inhibited both Th1 and Th2 response, whereas Th17 response was not significantly modulated, as judged by the levels of interferon- γ , IL-5 and IL-17A in culture supernatants, respectively. Lower concentration of 3,10-DDA stimulated Th1 and Th17 responses and inhibited IL-10 production, whereas the higher dose augmented the Th2 response. In conclusion, our results showed a significant, dose-dependent, immunomodulatory effect of RJ fatty acids *in vitro*, which was also associated with their structure.

Compound A, a Selective Glucocorticoid Receptor Antagonist, has Immunomodulatory and Cytoprotective Effects In Vitro

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Autoimmune type 1 diabetes (T1D) is characterized by progressive destruction of insulin producing beta cells mediated by various pro-inflammatory mediators that leads to hyperglycemia. Chronic elevation of blood glucose impairs beta cell function and leads to beta cell death (glucotoxicity). Compound A (CpdA) is a selective glucocorticoid receptor antagonist that exerts anti-inflammatory and immunomodulatory activities. Since we have recently shown that CpdA protects C57BL/6 mice from developing of T1D induced by multiple low doses of streptozotocin, the aim of this study was to examine the direct *in vitro* effects of CpdA on multiple immune system components and glucotoxicity in beta cells. We cultured spleen cells (SC), lymph node cells (LNC) and peritoneal cells (PC) obtained from C57BL/6 mice in the presence or absence of CpdA (0.1 – 10 μ M) for viability assay (MTT test), or 10 μ M CpdA for cytokine production (ELISA); SC and LNC were stimulated or not with 1 μ g/ml concanavalin Concanavalin A for 48 h, and PC were stimulated or not with 5 μ g/ml Lipopolysaccharide (LPS) for 24 h. Also, we exposed the rat insulinoma INS-1 cells to high glucose (20 mM) in the presence or absence of CpdA (10 μ M) and determined cell survival by Real Time Cell Analysis. CpdA treatment significantly reduced the secretion of IL-1 β , TNF, IL-6 and IL-10 by PC. In SC and LNC CpdA reduced IFN- γ and IL-17 and up-regulated IL-4 secretion. Moreover, CpdA preserved INS-1 cells from high glucose induced toxicity. In conclusion, our results indicate that CpdA has direct immunomodulatory effects on activated macrophages and lymphocytes *in vitro* and also protects beta cells from glucotoxicity.

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Focused ion-beam scanning electron microscopy and particle induced X-ray emission as novel methods for the analysis of gold nanoparticles internalization by human dendritic cells

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Immunological effects of gold nanoparticles (GNPs) greatly depend on their size, mechanisms and levels of their internalization by dendritic cells (DCs), key antigen presenting cells and crucial regulators of T cells development. However, common methods for the internalization analyses often have limited spatial and depth resolution, approximative quantity detection and neglect the cell-to-cell variability in nanoparticles internalization. Therefore, we applied for the first time in such studies focused ion beam/scanning electron microscopy (FIB/SEM) and particle induced X-ray emission (PIXE) analysis, due to their great potential for 3D reconstruction of cell cross-sections at electron microscope scale and the precise intracellular quantification of elements, respectively. Confocal microscopy showed that GNPs are internalized by DCs predominantly via dynamin-dependent mechanisms, which was confirmed by FIB/SEM. However, cross-sectioning by FIB/SEM revealed that Dynasore-treated DCs contain higher amounts of smaller GNPs intracellularly compared to the larger ones, suggesting additional mechanisms of their internalization. Furthermore, FIB/SEM, similarly as transmission electron microscopy (TEM), revealed that in addition to endosomal localization of GNPs, smaller GNPs are more often found outside the endosomes. Analysis of GNPs internalization by light microscopy and flow cytometry indicated high variability in the capacity of DCs to internalize GNPs, and that higher percentage of DCs internalizes larger GNPs. Similarly, PIXE-based quantification by the thin sample approximation showed that the mass of GNPs internalized by DCs increase with GNPs size. However, when the mass of gold was recalculated to the number of GNPs per DCs, we observed an inverse proportion, i.e. the number of intracellular GNPs increase with their decreased size. These results suggest that FIB/SEM and PIXE facilitate and complement other methods for the analysis of nanoparticles internalization, but they also provide new information allowing one to avoid the misinterpretation of the results.

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Polymorphism of interleukin-23 receptor in Serbian patients with inflammatory bowel disease

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Inflammatory bowel disease (IBD) that includes Crohn's disease (CD) and ulcerative colitis (UC) is a group of disorders of unknown etiology, characterized by chronic inflammation of the gastrointestinal tract in the genetically predisposed individuals. The growing body of evidence supports a potential role of the pro-inflammatory cytokines like interleukin (IL)-23, a key modulator in differentiation of Th17 lymphocytes, in the pathogenesis of IBD. Also, genome wide association studies (GWAS) indicate that an uncommon coding variant of IL-23 receptor (rs11209026) confers strong protection against both types of IBD.

The aim of this study was to determine whether single-nucleotide polymorphism (SNP) of the gene encoding the IL-23 receptor is associated with IBD in Serbian population.

Genomic DNA samples from 206 IBD patients (106 CD, 100 UC) and 259 healthy volunteers were evaluated for IL-23R SNP (rs11209026) using TaqMan genotyping assay. Comparison between genotype and allele frequencies in different groups was performed using the Pearson chi-square test ($p < 0.05$ was considered statistically significant).

The frequency of the rs11209026 A allele was lower in CD (1%, $p < 0.01$) and UC patients (2%, $p < 0.05$), when compared with controls (6.4%). The frequencies of GG and GA genotypes were 98% and 2% in CD ($p < 0.01$) while 97% and 3% UC patients ($p < 0.05$).

This study supports the association of IL23R polymorphisms as potential protective factor against development of IBD in evaluated population and may represent a novel approach to therapy of Th17-mediated diseases.

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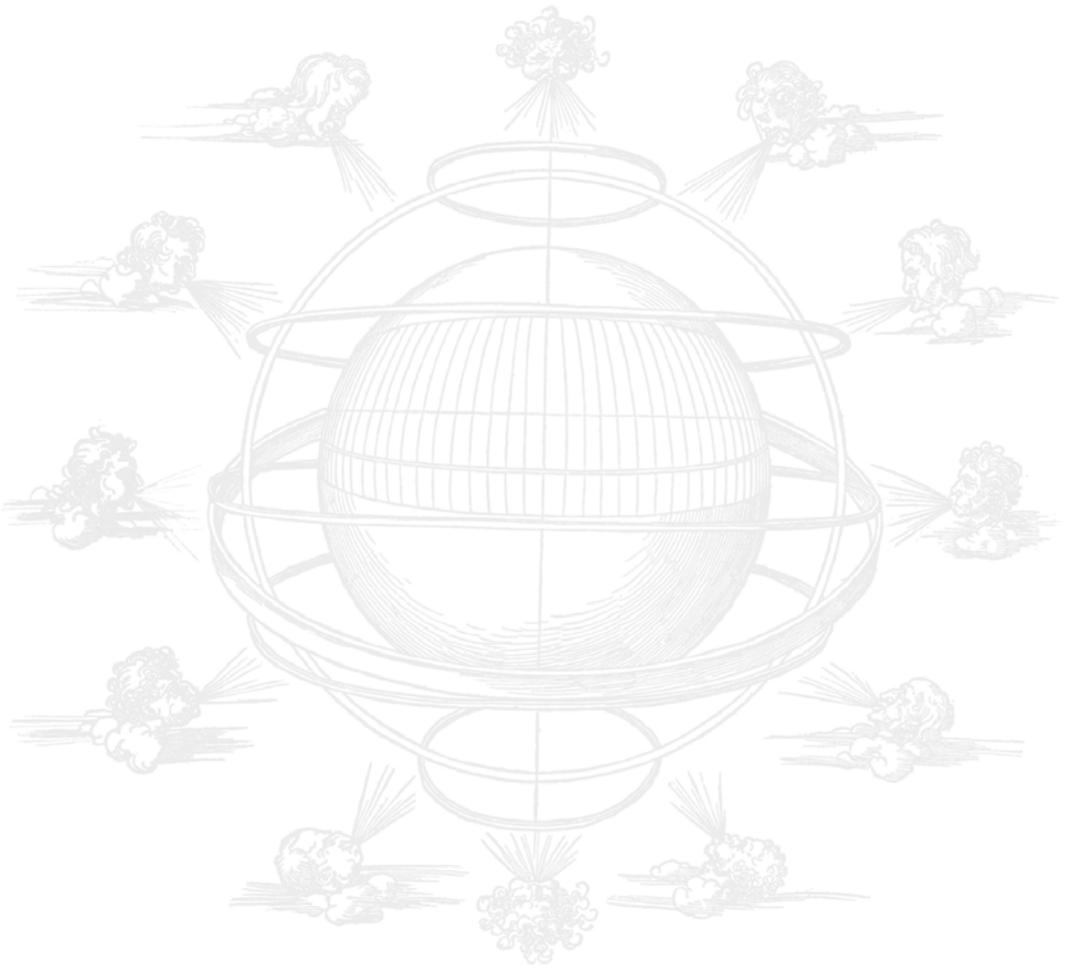
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In order to protect animal against invading microorganism host-defense peptides secreted by epithelial cells exhibit various antimicrobial and immunoregulatory effects. We have already provide evidence that peptides derived from frog skin may have additional effects on human pathogens and also in higher concentrations unwanted effects on human cells (i.e. platelets and erythrocytes aggregations). The potential immunomodulatory effects of frog skin peptides have been initially studied in vitro using lymphoid cells from the spleen and peritoneal cavity. In these studies we have shown that Plasticin-L1, Frenatin 2D, Pseudohymenochirin-1Pb and -2Pa, Frenatin-2.1S, -2.2S and -2.3S have immunostimulatory effects as evaluated by the production of proinflammatory cytokines such as TNF- α , IL-1 β , IL-12 and IL-23, while Esculentin-2CHa, Hymenochirin-1B, Tigerinin-1R, -1M and -1V preferentially stimulates production of IL-10 by peritoneal cells, alone or with concomitant stimulation with LPS. In order to test whether immunostimulatory effect of frenatin 2.1S may be reproduced in vivo, we analyzed the effects of intraperitoneal injections of this peptide on the cellular makeup in the peritoneum and spleen, 24 hours after injection, in both Th1 type C57BL/6 and Th2 type BALB/c mice. The data obtained strongly indicate that frenatin 2.1S enhances the activation state and homing capacity of Th1 type lymphocytes (CD3⁺CXCR3⁺ cells) and NKT cells in the peritoneal cavity of both C57BL/6 and BALB/c mice. Further we showed the activation of antigen presenting cells as well as high percentage of M1 macrophages. Injection of frenatin 2.1S in the presence or absence of LPS increased peritoneal B cells of B1a phenotype thus contributing to an inflammatory milieu. Frenatin 2.1S also facilitated accumulation of NK cells in peritoneal cavity, particularly CD69⁺ NK cells, indicating activation status of these cells. We suggest that immunostimulatory effect of frenatin 2.1S may be considered as therapeutic approach in disease states, such as cancer and infection, in which an enhanced inflammatory response may be beneficial.

Key words: antimicrobial peptides, peritoneal cells, Th1 lymphocytes, NK cells, macrophages

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