## 13th EFIS-EJI TATRA IMMUNOLOGY CONFERENCE

# *Molecular Determinants of T-Cell Immunity*


**June 09-13, 2018**
**Štrbské Pleso, Slovakia**

# PROGRAMME
# ABSTRACT BOOK


European Federation of Immunological Societies

# 13th EFIS-EJI TATRA IMMUNOLOGY CONFERENCE

# *Molecular Determinants of T-Cell Immunity*

## CONFERENCE VENUE

**Hotel Patria, Štrbské Pleso, High Tatra Mountains, Slovakia**

June 9-13, 2018

Organized by Czech, Slovak and British Societies of Immunology, Austrian Society for Allergology and Immunology, under the auspices of EFIS

**Organizing Committee:**
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H. Stockinger (Vienna)
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# PROGRAMME

## Saturday, 9th June

Arrival of participants

## Sunday, 10th June

8:30 - 8:45 Opening the Conference

**SESSION 1 Chairperson: Hannes Stockinger (Vienna)**

8:45 - 9:30 **Chiara Romagnani (Berlin)**: *Peptide-specific recognition by adaptive NK cells*

9:30 - 10:15 **Steffen Jung (Rehovot):** *Macrophage intricacies in gut and brain*

**10:15 - 10:30 Tea/Coffee break**

10:30 - 11:15 **Yvette van Kooyk (Amsterdam):** *Glycans in the control of resetting immune escape versus immunity*

11:15 - 12:00 **Rita Carsetti (Rome):** *Development, function and life history of innate and adaptive memory B cells*

**12:10 - 13:00 Lunch**

**SESSION 2 Chairperson: Juraj Ivanyi (London)**

16:30 - 17:15 **Ludger Klein (Munich):** *Central T cell tolerance: clonal deletion versus clonal diversion*

17:15 - 18:00 **Jakub Abramson (Rehovot):** *(Re)discovering the thymus through high-throughput single cell RNA sequencing*

18:00 - 18:45 **Kathryn J. Wood (Oxford):** *Immune regulation in transplantation - the potential of cell therapy*

**19:00 - 20:30 Dinner**

## Monday, 11th June

**SESSION 3** **Chairperson: Georg Wick (Innsbruck)**

8:45 - 9:30 **Antonella Viola (Padova):** *CXCL12/SDF-1 modulates the rheological properties of t nucleus of mouse neutrophils*

9:30 - 10:15 **Thomas Korn (Munich):** *Modes and outcome of IL-6 signaling into T cells*

**10:15 - 10:30 Tea/Coffee break**

10:30 - 11:15 **Michael Reth (Freiburg):** *From the cross-linking model to the dissociation-activation model*

11:15 - 12:00 **Andreas Radbruch (Berlin):** *The maintenance of immunological memory*

**12:10 - 13:00 Lunch**

**SESSION 4** **Chairperson: Hannes Stockinger (Vienna)**

Selected poster presentations (6 speakers, each 15 min. including discussion):

16:30 – 18:00

***Tomáš Brabec (Prague):*** *IL-17 drives Paneth cell-mediated control of segmented filamentous bacteria*

***Matej Fabišík (Prague):*** *Altered immune responses in mice with LST1 adaptor protein deficiency*

***Ekaterina O. Gubernatorova (Moscow):*** *IL-6 produced by macrophages and dendritic cells mediates distinct aspects of allergic airway inflammation*

***Rebeca C. Arroyo Hornero (Oxford):*** *A pro-inflammatory role for CD70 on human regulatory T cells*

***Dominika Polak (Vienna):*** *Neutrophils promote T-cell mediated inflammation in allergy*

***Margarida Souto-Carneiro (Heidelberg):*** *Blood $CD8^+$ T cells in rheumatoid arthritis rely on the Warburg effect to maintain their chronic inflammatory profile*

**18:30 - 19:30 Dinner**

**20:00 - 22:00 Poster session (with refreshments and wine)**

## Tuesday, 12th June

**SESSION 5** **Chairperson: Václav Hořejší (Prague)**

8:45 - 9:30 **Ondřej Štěpánek (Prague):** *Interactions with self-antigens generate functional diversity of T cells*

9:30 - 10:15 **Torsten Tonn (Dresden):** *Natural Killer cells as CAR drivers*

**10:15 - 10:30 Tea/Coffee break**

10:30 - 11:15 **Nadine Cerf-Bensussan (Paris*):*** *Host microbiota interactions across the immune system*

11:15 - 12:00 **Winfried Pickl (Vienna):** *Genetic restriction of allergen-presentation dictates allergic sensitization and disease in humanized mice*

**12:10 - 13:00 Lunch**

**SESSION 6** **Chairperson: Dominik Filipp (Prague)**

16:30 - 17:15 **Florian Kellner (Vienna):** *Showing strength: mechanical forces in T-cell antigen recognition*

17:15 - 18:00 **Ursula Wiedermann (Vienna):** *From one shot fits all to personalized vaccinology*

**18:10 - 18:45 Closing of the conference**

**19:30 - 23:30 Farewell Party**

## Wednesday, 13th June

Departure

# ABSTRACT BOOK

# SPEAKERS

**(RE)DISCOVERING THE THYMUS THROUGH HIGH-THROUGHPUT SINGLE CELL RNA SEQUENCING**

*Chamutal Bornstein[1,*], Shir Nevo[1,*], Amir Giladi[1,*], Noam Kadouri[1,*], Valérie Zimmermann[2], Marie Pouzolles[2], François Gerbe[2], Eyal David[1], Naomi Taylor[2], Philippe Jay[1], Ido Amit[1†], **Jakub Abramson[1†]***

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2. Université de Montpellier, F-34000 Montpellier, France.
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T cell development and selection is orchestrated in the thymus by a specialized niche of diverse stromal populations that are only partly characterized. Here, we combine single cell analysis and computational modeling, chromatin profiling, immunofluorescence, single molecule FISH, *in-vivo* fate-mapping and genetically manipulated mice to *de novo* characterize the entire stromal compartment of the thymus. We identified dozens of cell states within the thymic stroma, with epithelial cells (TEC) showing the highest degree of heterogeneity. Our analysis highlights four major medullary TEC (mTEC I-IV) populations, with distinct molecular functions, unique epigenetic landscapes and lineage regulators. Specifically, mTEC-IV constitutes a new and highly divergent TEC population, present in both mouse and human, that comprises 5-10% of the thymic epithelia, and bears strong molecular and morphological characteristics with IL25-producing intestinal tuft cells. Mice deficient in Pou2f3, a tuft cell master regulator, demonstrate a specific depletion of mTEC-IV, consequently resulting in perturbed homeostasis of various IL25R-expressing populations in the thymus, including an increased level of type-2 innate lymphoid cells. Overall, our study provides a comprehensive map of the thymic stroma and identifies a new tuft TEC population which is critical for shaping the thymus immune niche.

**DEVELOPMENT, FUNCTION AND LIFE HISTORY OF INNATE AND ADAPTIVE MEMORY B CELLS**

***Rita Carsetti***

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Immunological memory, with memory B cells, plasma cells and their antibodies, represents the adaptation of the human body to the environment. Immunological memory protects us from being re-infected by a previously encountered pathogen. Switched and IgM memory B cells execute different and non-interchangeable functions. We studied memory B cells in children of different ages, in peripheral blood and spleen and compared them with those of children born asplenic or unable to build germinal centers. We show that, whereas switched memory B cells are mostly generated in the germinal centers at all ages, IgM memory B cells can be distinct in three types with different developmental history. Innate IgM memory B cells, the largest pool in infants, are generated in the spleen by a germinal center-independent mechanism. With age, if the spleen is present and germinal centers are functional, innate IgM memory B cells are remodelled and accumulate somatic mutations. The third type of IgM memory B cell is a by-product of the germinal center reaction. Our data show that the B-cell memory developmental program is implemented during the first 5–6 years of life. In the adult, 50% of the B cells in the peripheral blood are memory B cells. Memory B cells play a fundamental role in pregnancy when they are used to produce antibodies transferred to the fetus through the placenta. After the delivery the maternal B-cell immune response generates memory B cells and plasma cells able to migrate to the breast and reverse secretory IgA in the milk. Understanding the actors, strategy and dynamics of human B cells memory is important for the study of immune deficiency, evaluation of vaccine responses, design of vaccination protocols and development of new vaccines for children, elderly individuals and pregnant women.

**HOST-MICROBIOTA INTERACTIONS ACROSS THE IMMUNE SYSTEM**

***Nadine Cerf-Bensussan***

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Over the past 15 years, considerable efforts have been invested worldwide in analysing the complex microbial community that settles in the distal part of the mammalian intestine and the highly dynamic dialogue in which it is engaged with its hosts. It is estimated that in each human adult, the intestine contains $10^{13}$ bacteria, distributed between approximately 1500 distinct species, which mainly belong to two phyla, the *Firmicutes* and the *Bacteroidetes*. In each individual, the bacterial metagenome is thought to encode approximately $3x10^6$ distinct genes, a sum that largely exceeds the 22 000 genes encoded by the human genome. Hence the suggestion that hosts and their microbiota have coevolved into superorganisms with interdependent metabolic pathways. Accordingly, it is increasingly clear that the microbiota is a key determinant in health and disease. The microbiota notably strongly influences host immune responses. While it is likely the massive microbial load present in he microbiota the intestinal lumen hhosts have likely evolved a spectrum of innate and adaptive immune mechanisms to cope with. This highly dynamic barrier is programmed ante-natally but fully develops only after birth in response to signals from the microbiota. To analyse the host-microbiota dialogue, we have chosen to address two main questions.

1- What are the respective roles of individual bacterium in driving the maturation of the gut barrier? Do they exert distinctive or redundant effects? Our studies in gnotobiotic mice have unexpectedly revealed that a restricted number of host-specific bacterial species, the prototype of which is Segmented Filamentous bacterium (SFB), are necessary for the complete maturation of the gut immune barrier. We will discuss how the ecological niche of SFB differs from that of other members of the microbiota, and enables SFB to deliver signals, which instruct the development of gut innate and adaptive immune responses and help establishing a

state of physiological inflammation, which protects the host against pathogens.

2- What are the key mechanisms evolved by the hosts, to cope with the microbiota? Our current work led in children affected by monogenic disorders causing early onset intestinal inflammation provides interesting insight on conserved genetic mechanisms to maintain mutualistic relationships with the microbiota.

**MACROPHAGE INTRICACIES IN GUT AND BRAIN**

***Steffen Jung***
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Macrophages are myeloid immune cells that are strategically positioned throughout the body tissues, where they ingest and degrade dead cells, debris and foreign material, and orchestrate inflammation and immune defense. Defying earlier notions, most tissue-resident macrophages are established prenatally. The respective cellular compartments thus develop locally, alongside with their host tissue and independent from each other through expansion of primordial macrophages that seed the tissue during embryogenesis. Maintenance of macrophage compartments relies on longevity and self-renewal and is in most tissues independent from ongoing hematopoiesis. Selected barrier tissues, but also the heart, display however substantial postnatal replacement of the embryonic populations by adult monocyte-derived cells, which seems linked to unique homeostatic challenges of these organs. Emerging evidence indicates that tissue macrophages are - aside from immune sentinels - integral components of their host tissue, which acquired also activities tailored to contribute to tissue homeostasis. These include specializations of the phagocytic machinery, provision of specific growth factors and morphological adaptations. Factors governing these local specializations are emerging and tissue specialization is also prominently reflected in discrete gene expression profiles and epigenetic signatures. Here I will discuss recent insights my laboratory gained through the comparative analysis of monocyte-derived gut macrophages and embryo-derived microglia cells in the brain.

Selected references

Varol, C., Mildner, A. & Jung, S. Macrophages: development and tissue specialization. Annu. Rev. Immunol. 33, 643–675 (2015).
Amit, I., Winter, D. R. & Jung, S. The role of the local environment and epigenetics in shaping macrophage identity and their effect on tissue homeostasis. Nat Immunol 17, 18–25 (2016).

**SHOWING STRENGTH: MECHANICAL FORCES IN T-CELL ANTIGEN RECOGNITION**

***Kellner F*[1]**, *Göhring J*[1,2], *Schrangl L*[2], *Platzer R*[1], *Schütz GJ*[2], *Huppa JB*[1]

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The recognition of antigenic peptide/MHC complexes (pMHC) on antigen presenting cells (APC) by T-cells is mediated by the T-cell receptor (TCR) and leads to the formation of an immunological synapse. This process is remarkably specific, sensitive and efficient but up to this date the underlying mechanisms are only poorly understood. There is mounting evidence that mechanical forces acting on the TCR are indeed instrumental in TCR-ligand discrimination and TCR-mediated signaling. To investigate this in sufficient detail, we will attach calibrated force sensors to pMHCs, which will be embedded either on a well-defined functionalized planar lipid bilayer system or on the surface of live APCs. Fluorescent dyes on both ends of the sensor will serve as donor and acceptor for Förster resonance energy transfer (FRET). We expect high FRET values when no tension is applied and the sensor is relaxed. However, cell-imposed forces on the TCR should stretch the sensor and reduce FRET due to larger distances between the FRET dye pair. We intend to perform FRET measurements in bulk to map forces within the immunological synapse and also to perform single molecule experiments to assess the true molecular force dynamics. To determine the role of TCR-imposed forces in T-cell triggering and ligand discrimination we will correlate them with the stimulatory potency of pMHCs and simultaneously imaged downstream signaling. In summary, we expect to visualize TCR-imposed forces most directly and establish them as a crucial factor in T-cell antigen recognition.

**CENTRAL T CELL TOLERANCE: CLONAL DELETION VERSUS CLONAL DIVERSION**

*Tobias Hassler, Maria Hinterberger, Emanuel Urmann, Christine Federle, Marc Jenkins, Dirk Busch,* ***Ludger Klein***

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Somewhat paradoxically, intrathymic encounter of ‚self' can elicit two diametrically different cell fate decisions of autoractive CD4 T cells: negative selection or re-programming into the regulatory T (Treg) cell lineage. The parameters specifying these opposing cell-fates remain poorly understood. Moreover, it is unclear whether and how within a polyclonal CD4 T cell repertoire, distinct TCRs with shared antigen-specificity are either lost or diverted into the Treg repertoire. I will discuss our attempts to visualize and characterize minute cohorts of antigen-specific cells in the absence or presence of myelin proteolipid protein (PLP), a disease-relevant CNS autoantigen, in order to (i) identify deleted or deviated TCR-entities by comparing ‘uncensored' and ‘censored' repertoires and (ii) to identify TCR intrinsic features that specify clonal deletion versus clonal deviation.

**MODES AND OUTCOME OF IL-6 SIGNALING INTO T CELLS**

*Sylvia Heink, Garima Garg,* ***Thomas Korn***

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Interleukin-6 (IL-6) has been identified as a non-redundant differentiation factor for TH17 cells. However, the cellular sources of IL-6 that are relevant for the differentiation of TH17 cells in vivo remain unclear. Here, we used a novel strategy of IL-6 conditional deletion of distinct IL-6-producing cell types to show that Sirpα+ dendritic cells (DC) were essential for the generation of pathogenic TH17 cells. During the process of cognate interaction, Sirpα+ DCs trans-presented IL-6 to T cells using their own IL-6Rα. While ambient IL-6 was sufficient to suppress the induction of the transcription factor Foxp3 in conventional T cells, IL-6 trans-presentation by DC-bound IL-6Rα (here defined as IL-6 cluster signaling) was required to prevent premature induction of IFN-γ in T cells and to generate pathogenic TH17 cells in vivo. Moreover, activated T cells as well as thymus derived Foxp3+ Tregs at the site of inflammation had shed their IL-6Rα and were thus unable to respond to soluble IL-6 but were still able to respond to IL-6 cluster signaling, and we investigated the effects of IL-6 cluster signaling on the identity and function of Foxp3+ Tregs in inflamed tissues. We found that IL-6 signaling into activated Tregs could in principle induce a loss of Foxp3 expression through induction of epigenetic modification. However, we discovered a counterregulatory mechanism that opposed the IL-6 driven attrition of Foxp3 expression in vivo and thus supported the maintenance of Treg identity in inflamed tissues. Together, these findings will guide therapeutic approaches targeting IL-6 in T cell mediated autoimmune diseases.

# GENETIC RESTRICTION OF ANTIGEN-PRESENTATION DICTATES ALLERGIC SENSITIZATION AND DISEASE IN HUMANIZED MICE

*Alina Neunkirchner [a,b], Bernhard Kratzer [a,b,]*, Cordula Köhler [a,b,]*, Ursula Smole [b], Lukas F. Mager [b], Klaus G. Schmetterer [b,c], Doris Trapin [b], Victoria Leb-Reichl [b], Edward Rosloniec [d,e,f], Ronald Naumann [g], Lukas Kenner [h,i,j], Beatrice Jahn-Schmid [k], Barbara Bohle,[a,k] , Rudolf Valenta [k], **Winfried F. Pickl** [a,b,] ***

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**Background:** Immunoglobulin(Ig)E-associated allergies result from misguided immune responses against innocuous antigens. $CD4^+$ T lymphocytes are critical for initiating and perpetuating that process, yet the crucial factors determining whether an individual becomes sensitized towards a given allergen remain largely unknown.
**Objective:** To determine the key factors for sensitization and allergy towards a given allergen.
**Methods:** We here created a novel human T cell receptor (TCR) and human leucocyte antigen(HLA)-DR1 (TCR-DR1) transgenic mouse model of asthma, based on the human-relevant major mugwort (*Artemisia vulgaris*) pollen allergen Art v 1 to examine the critical factors for sensitization and allergy upon natural allergen exposure via the airways in the absence of systemic priming and adjuvants.
**Results:** Acute allergen exposure led to IgE-independent airway hyperreactivity (AHR) and T helper(Th)2-prone lung inflammation in TCR-DR1, but not DR1, TCR or wildtype (WT) control mice, that

was alleviated by prophylactic interleukin(IL)-2-αIL-2 mAb complex-induced expansion of Tregs. Chronic allergen exposure sensitized one third of single DR1 transgenic mice, however, without impacting on lung function. Similar treatment led to AHR and Th2-driven lung pathology in >90% of TCR-DR1 mice. Prophylactic and therapeutic expansion of Tregs with IL-2-αIL-2 mAb complexes blocked the generation and boosting of allergen-specific IgE associated with chronic allergen exposure.

**Conclusions:** We identify genetic restriction of allergen presentation as primary factor dictating allergic sensitization and disease against the major pollen allergen from the weed mugwort, which frequently causes sensitization and disease in humans. Furthermore, we demonstrate the importance of the balance between allergen-specific T effector and Treg cells for modulating allergic immune responses.

**THE MAINTENANCE OF IMMUNOLOGICAL MEMORY**

***Andreas Radbruch***

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Conventional concepts of how immunological memory is maintained postulate that antigen-experienced memory B and T lymphocytes circulate through the body, in quest for their antigen, their numbers maintained by a balance of death and "homeostatic" proliferation. Plasma cells were believed to be shortlived and constantly generated again from memory B cells. This conventional picture of a circulating, restless memory has been challenged fundamentally recently, by the discovery of memory lymphocytes resting in the tissues, in particular in the bone marrow, and by the discovery of memory plasma cells providing "humoral memory". In 1997 we described longlived "memory" plasma cells, which are maintained in the bone marrow, to lesser extend in secondary lymphoid organs and also in inflamed tissues, as long as these are inflamed. Newly generated plasmablasts, the precursors of plasma cells, migrate to the bone marrow, guided by the CX-chemokine ligand 12 (CXCL12). In the bone marrow, they individually dock onto stromal cells which express CXCL12, and differentiate into memory plasma cells. These memory plasma cells rest in terms of proliferation and migration, while they vigorously secrete antibodies. Remarkably, one reticular stromal cell can only host one memory plasma cell. Stromal cells thus define the "capacity" of humoral memory as such. Once all niches are filled, one has to assume that new plasmablasts have to compete with preexisting memory plasma cells for niches. Two signals are required and sufficient to maintain memory plasma cells: cell contact to the stromal cell, probably mediated by integrins, induces PI3K signaling, and the cytokines BAFF or APRIL, ligands of the B cell maturation antigen (BCMA) of plasma cells, induce NFkB signaling. In synergy, both signaling pathways prevent apoptosis. Memory T and B lymphocytes provide a "reactive memory", adapting immunity to repeated, varied and enhanced challenges of pathogens of the environment, beyond protection by humoral immunity. While some of these memory lymphocytes are circulating through blood and secondary lymphoid organs, others are

residents of distinct tissues, in particular epidermis and bone marrow. These resident memory cells have a distinct repertoire. We have shown that bone marrow is the home of memory lymphocytes specific for systemic, bloodborne antigens. Other groups have discovered memory T cells resting in skin and lung, specific for epidermotropic pathogens. It is increasingly recognized that these resident memory lymphocytes have a lifestyle reminiscent of that of memory plasma cells. They are individually docking onto stromal cells, and PI3K signaling is required to keep them alive. They rest in terms of proliferation, activation and mobility, no "homeostatic" proliferation is detectable. In secondary immune reactions, resident memory T lymphocytes are mobilized to move to secondary lymphoid organs and coordinate the reaction there. Others are moving in the bone marrow to "immune niches" where they just proliferate, then return to their memory niches, enhancing the numbers of resident memory cells for future challenges. The emerging picture of a multilayered and redundant organization of immunological memory, and the maintenance of memory lymphocytes as resident and resting cells by mesenchymal stromal cells, offers entirely new perspectives for the intentional induction of immunity by vaccination, and the treatment of diseases based on a pathogenic immunological memory, like allergies and chronic inflammatory diseases.

**FROM THE CROSS-LINKING MODEL TO THE DISSOCIATION-ACTIVATION MODEL**

*Jianying Yang, Kathrin Kläsener, Melanie Sieder* ***Michael Reth***

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The clonal selection theory provides an explanation how an infection or a successful vaccination leads to antibody production and humoral immunity through the antigen-specific activation of B lymphocytes. The B cell antigen receptor (BCR) plays a central role in the clonal selection process that requires distinctly different conformations of the BCR on resting and activated B cells. The cross-linking model (CLM) of B cell activation suggest that the 100.000 BCR complexes on the B cell surface are randomly distributed monomers, and that it is the "cross-linking" of two BCR monomers that generates the activation signal. However, how the many BCR monomers stay signaling inert on the compartmentalized B cell surface where the BCR is densely clustered and how the many structurally different ligands including many monovalent antigens can place the BCR dimers in the productive conformation for signaling is poorly explained by the CLM. In 2000, my lab discovered an oligomeric structure of the BCR on resting B cells and we proposed in 2010 the dissociation activation model (DAM) as an alternative to CLM. According to DAM the oligomeric BCR has an autoinhibited conformation on resting B cells that is guarded by the cytoskeleton. Furthermore, the activation of the BCR by the many different ligands does not require the "cross-link" but rather the opening of the BCR. Using a Fab-based proximity ligation assay (Fab-PLA), we now can monitor the opening of the BCR and study the molecular requirement for this process. In addition, we are employing the CRISPR/Cas9 technique to find further elements involved in antigen sensing and B cell regulation.

**PEPTIDE-SPECIFIC RECOGNITION BY ADAPTIVE NK CELLS**

***Chiara Romagnani***

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Human Cytomegalovirus (HCMV) infection shapes the repertoire of human Natural Killer (NK) cells. HCMV infection is associated with the clonal-like expansion and persistence of an adaptive subset of NK cells expressing the activating receptor NKG2C and undergoing global epigenetic remodeling, similar to memory CD4+ Th1 and CD8+ cytotoxic T lymphocytes.
We have assessed the activation requirements of adaptive NKG2C+ NK cells and observed that NKG2C exhibits fine recognition of HCMV-encoded peptides. Engagement of NKG2C by peptides derived from different HCMV strains resulted in strikingly differential activation of effector functions and proliferation as well as in generation of adaptive signatures with different requirements for co-stimulation. We propose that peptide specificity is a hallmark of adaptive NK cells and that recognition of viral ligands from different HCMV strains together with pro-inflammatory signals controls the expansion of NKG2C+ NK cells in HCMV-infected individuals and promotes their phenotypic shift from conventional to adaptive cells.

**INTERACTIONS WITH SELF-ANTIGENS GENERATE FUNCTIONAL DIVERSITY OF T CELLS**

*Ales Drobek, Alena Moudra, Veronika Horkova, Michaela Pribikova, Martina Huranova, Peter Draber,* ***Ondrej Stepanek***

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Positive and negative selections of T cells establish a self-MHC-restricted and self-tolerant pool of mature T cells. The variability in the level of self-reactivity within the positive selection window is usually not considered as an important factor regulating the biology of mature T cells. However, increasing amount of recent evidence suggests that individual T-cell clones with a differential level of self-reactivity show distinct responses to their cognate foreign antigens.
We will present data showing that relatively highly self-reactive CD8+ T cells spontaneously form *virtual memory* T cells that exhibit qualitatively different types of responses from naïve T cells and *bona fide* memory T cells.
We developed a cell line model to characterize mouse TCRs in a system that completely lacks any interaction with the self-antigens. Using a set of 6 TCRs specific for a model antigen ovalbumin, we could find a correlation between a self-reactivity of a TCR and its intrinsic ability to initiate down-stream signaling when engaged with its cognate antigen.
We identified that the dynamic regulation of the stoichiometry of the interaction of CD4 and CD8 coreceptors with Lck shapes the self-reactivity of mature T cells. Because of increased stoichiometry of the CD8-Lck interaction during maturation, CD8+ T cells are on average more self-reactive than CD4+ T cells.

**NATURAL KILLER CELLS AS CAR DRIVERS**

***Torsten Tonn***

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Natural killer (NK) cells are increasingly considered as immunotherapeutic agents in particular in the fight against cancers. NK cell therapies are potentially broadly applicable and, different from their T cell counterparts, do not cause graft-versus-host disease. Efficacy and clinical in vitro or in vivo expansion of primary NK cells will however always remain variable due to individual differences of donors or patients. Long-term storage of clinical NK cell lots to allow repeated clinical applications remains an additional challenge. In contrast, the established and well-characterized cell line NK-92 can be easily and reproducibly expanded from a good manufacturing practice (GMP)-compliant cryopreserved master cell bank. Moreover, no cost-intensive cell purification methods are required. To date, NK-92 has been intensively studied. The cells displayed superior cytotoxicity against a number of tumor types tested, which was confirmed in preclinical mouse studies. Subsequent clinical testing demonstrated safety of NK-92 infusions even at high doses. Despite the phase I nature of the trials conducted so far, some efficacy was noted, particularly against lung tumors. Furthermore, to overcome tumor resistance and for specific targeting, NK-92 has been engineered to express a number of different chimeric antigen receptors (CARs), including targeting, for example, CD19 or CD20 (anti-B cell malignancies), CD38 (anti-myeloma) or human epidermal growth factor receptor 2 (HER2; ErbB2; anti-epithelial cancers). The concept of a NK cell line as an allogeneic cell therapeutic produced ‘off-the-shelf’ on demand holds great promise for the development of effective treatments.

# GLYCANS IN THE CONTROL OF RESETTING IMMUNE ESCAPE VERSUS IMMUNITY

***Yvette van Kooyk***

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Alterations in glycosylation is a hallmark in pathogen recognition by the host but also in cellular communication in inflammation and cancer within the host. Glycosylation is a post-translational event, regulated by up and down regulation of glycosylation genes, and communicates with the immune system through the recognition by glycan binding receptors such as C-type lectins and siglecs on immune cells. We have been studying how glycosylation of pathogens modulates the host immune response. Also our increased understanding how alterations in glycosylation within the host, often at microenvironmental level, regulate immune responses towards immunity or tolerance opens new venues for immune interference.

We identified new mechanisms of immune tolerance through the modification of glycosylation of tumours (melanoma and glioblastoma). In particular high sialylation of tumours results in the increase of FoxP3 CD4+ T cells (Treg) and lower frequencies of effector T cells (Teff) and NK cells at the tumour site. In contrast, low sialylation of tumours converts the frequencies Treg/Teff to favourable anti-tumour immunity. These sialic acids can be used for antigen specific immune tolerance when coupled to a specific antigens, such as OVA, or MOG.

On the other hand dendritic cell (DC) targeting glycans are identified to improve antigen responses and the induction of antigen specific CD4 and CD8 T cell responses. These are further studied in their improvement of nanovaccines for cancer immunotherapy. Our therapeutic vaccination studies in mice shown long term anti-melanoma immunity when T regulatory cells are temporarily reduced.

By studying posttranslational processes such as glycosylation, glycosciences, a novel language will be uncovered that regulate the communication between immune cells. Because this new language can be immune stimulating or inhibitory we implement our discoveries in the treatment of cancer and auto-immune diseases.

# CXCL12/SDF-1 MODULATES THE RHEOLOGICAL PROPERTIES OF THE NUCLEUS OF MOUSE NEUTROPHILS

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To reach the inflamed tissues leukocytes have to face several physical obstacles represented by the endothelial barrier and the dense fibrillar interstitial spaces. Although the cytoplasm can quickly change consistence and form to allow cells to interact with and penetrate the endothelium, the deformation of the nucleus, the largest and stiffest cellular organelle, represents the most challenging step during trans-migration. Since chemokines guide cell migration, it is likely that they may also modulate nuclear plasticity of migrating cells. Exploiting micro-fabricated devices to mimic confining environments, we show that CXCL12 enhances the nuclear pliability of mouse neutrophils, sustaining their transendothelial migration towards inflammatory stimuli. Intriguingly, investigating the signaling pathways connecting CXCL12 signals to the nuclear biomechanical properties of mouse neutrophils, we found that atypical receptors and unexpected protein kinases are involved in the modulation of nuclear deformability. On this basis, we propose that, in addition to mechanical signalling, chemical cues regulate the rheology of the leukocyte nuclei during their migration inside the tissues.

**FROM ONE SHOT FITS ALL TO PERSONALIZED VACCINOLOGY**

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Vaccines represent one of the greatest public health achievements of modern medicine. Vaccination programs during the last century have resulted in the global eradication of smallpox and the near eradication of polio, and exerted a dramatic impact on reducing the morbidity and mortality caused by infectious diseases, such as measles, rubella, or tetanus. Furthermore, vaccination— through the control of infectious diseases— has contributed to a striking increase in life expectancy in many countries. Ironically however, we nowadays face an increasing anti-vaccine movement in many industrialized countries which lead to re-emergence of many vaccine preventable diseases, propagated by too low vaccination coverage and lack of herd protection in the population. This public health challenge is accompanied by increasing demographic changes and the question how to vaccinate diverse populations, which relates to genetic diversity, nutritional status including rising obesity, increasing age, or an increasing incidence of chronic inflammatory disorders, and chronic infectious diseases. New concepts in vaccinology therefore focus on stratified/personalized vaccination programs which include changes in vaccine schedules, doses and routes of applications or the use of new adjuvants.
Within the last years we concentrated our research programs on the immunological characterization of non-responsiveness to routine vaccines in healthy as well as different risk groups. Non-responsiveness in healthy individuals occurs in 2-10% of vaccinees and is defined by a lack of sufficient protective immune-responses after primary or booster vaccination. We demonstrated that non-responsiveness differs according to the respective vaccine antigens and certain HLA subtypes. Immunosenescence is another risk factor for non-responsiveness to vaccination. With increasing age humoral and cellular responses are significantly lower, particularly to novel

vaccine antigens, than in younger vaccinees and thus associated with increased infection susceptibility. We recently described that primary vaccination led to humoral and cellular non/low responsivenss in up to 47% of the vaccinated elderly, which is associated with a higher frequency of regulatory T cells, late differentiated effector memory cells and an increased rate of CMV-seropositivity. Similar studies have been performed in allergic patients as well as obese individuals, since their immunological status is changed to that of health individuals and thus effectiveness of vaccination is often unclear. Along these lines, a multicentre study on vaccination of cancer patients is on the way to answer important questions, such as the influence of tumor entity, stage of diseases and chemotherapy on vaccine responsiveness.

Thus, based on the obvious need for significant and solid data on vaccine responsiveness in diseased subjects, studies making use of omics-technologies (vaccinomics, transcriptomics) are required to a greater extent to provide improved evidence for specific/stratified or personalized vaccination strategies as well as novel vaccines for a globally increasing number of different risk populations.

# IMMUNE REGULATION IN TRANSPLANTATION – THE POTENTIAL OF REGULATORY T CELL THERAPY

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Immune regulation is fundamental to any immune response to ensure that it is appropriate for the perceived threat to the host. Strategies for the induction of specific unresponsiveness to donor major and minor histocompatibility antigens currently under investigation in the clinic take advantage of two of the major mechanisms for the induction of tolerance to self antigens – deletion and immunoregulation/suppression.

We have demonstrated that human regulatory T cells (Treg) expanded *ex vivo* can protect human allografts (skin, islets and vessels) from rejection. Treg migrate to the allograft and function in situ. Donor alloantigen reactive Treg are more effective on a per cell basis than polyclonal Treg. Together with other leukocyte populations, including regulatory T cells, B cells and macrophages as well as myeloid derived suppressor cells and dendritic cells, Treg contribute to the regulation of immune responses *in vivo* after organ transplantation

The identification and characterisation of Treg that can control immune responsiveness to alloantigens has opened up exciting opportunities for new therapies in transplantation. Phase1/2a clinical trials are in progress – www.onestudy.org.

# POSTER PRESENTATIONS AND SHORT ORAL PRESENTATIONS

**BIOACTIVE PROTEIN SHOWS ANTI-INFLAMMATORYEFFECTS LOCALLY IN THE SMALL INTESTINE BUT NOT SYSTEMIC IN APOE-/- MICE**

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Proteins of marine origin have many health benefits, including lipid lowering and anti-inflammatory effects. A bioactive protein from Atlantic salmon has been generated using specific extraction methods. This study aimed to penetrate the effect of this bioactive protein on inflammation and atherosclerosis in a mouse atherosclerosis model, apoE-/-. Female apoE-/- mice were fed a high-fat control diet (20% w/w casein) or bioactive protein diet (3.0 % w/w bioactive protein and 17 %w/w casein) for 12 weeks. The cytokine gene expression in the mucosa (jejunum – proximal part of the small intestine) was analyzed. The diet containing bioactive protein significantly reduced cytokine levels in the jejunum mucosa area (TNFα and IL-1β) compared to control. Plasma cytokine levels were also measured by multiplex analysis, but no significant reduction in these parameters were observed. We also studied the atherosclerotic plaque area, which is influenced by inflammation and involves many immune modulating cells. After 12 weeks of high-fat feeding, mice fed the bioactive protein showed no change in atherosclerotic plaque area in the aortic arch and thorax compared to control (P=0.188). Overall, these data show that bioactive protein from Atlantic salmon was able to counteract inflammation locally in the jejunum mucosa area but not systemic inflammation, and did not influence atherosclerotic plaque development in apoE-mice.

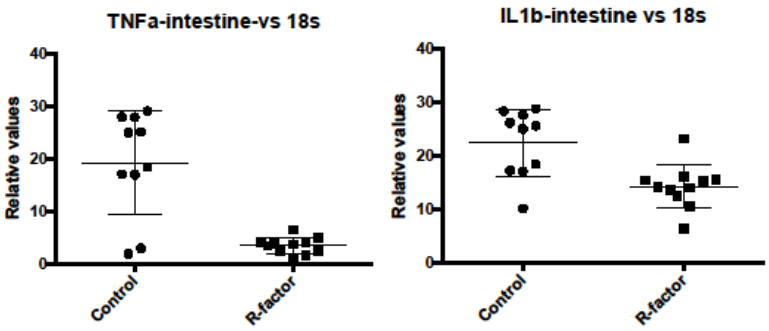


**Fig.** Expression of inflammatory markers in vivo in the proximal part (jejunum) of the small intestine

**INNATE LYMPHOID CELLS (ILCS) IN SKIN AND BLOOD**

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Innate lymphoid cells (ILCs) represent a relatively newly discovered cell type. They are defined as lineage-negative ($Lin^{-}$) cells of lymphoid morphology, which also express the α-chain of the IL-7 receptor (CD127). As opposed to T and B cells, ILCs lack recombination-activating genes (RAG) and, therefore, do not express antigen-specific receptors. The family of ILCs has been subdivided into 3 categories – ILC1s, ILC2s and ILC3s – according to their cytokine production and transcription factor profiles. In this regard, the ILC subsets represent innate counterparts of T helper 1 (Th1), Th2 and Th17 cells. In contrast to cells of adaptive immunity ILCs have the potential to rapidly react to injured or infected cells/tissues and promptly release soluble mediators aimed at neutralising/eliminating the pathogen. ILCs have been identified in many tissues including the skin. While, under homeostatic conditions, they are present in small amounts only, their numbers are substantially increased in inflamed tissues.

It is the purpose of my research project to investigate ILCs in normal and inflamed skin (e.g., in atopic dermatitis) and, for comparative reasons, in the peripheral blood. As can be deduced from experiments conducted so far, it appears that we can reliably detect and isolate them in/from both tissues. Having accomplished this, we are now in the position to carefully analyse and characterise these cells at a molecular, phenotypic and, most importantly, functional level. We are confident that the results of our studies will shed light on the role of ILCs not only in (cutaneous) host defence, but perhaps also in the development as well as maintenance of homeostasis of the host's outmost organ.

**INTERPLAY BETWEEN *T.SOLIUM* EXCRETORY SECRETORY PROTEIN AND HOST MIRNAS DECREASES PHAGOCYTOSIS IN HUMAN MACROPHAGES**

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*Taenia solium* larvae infects human central nervous system and causes Neurocysticercosis (NCC). NCC is among neglected tropical diseases and is a major cause of acquired epilepsy in the developing world. Pro-inflammatory cytokines are responsible for occurrence of symptoms in NCC. The parasite's secretome is the means of communication with the host. It consists of proteins essential for survival, differentiation, maturation and for masking host immune system. The *T. solium* excretory secretory (ES) proteins with its attributes makes up for an important target to understand the NCC pathogenesis. *T. solium* cysts were cultured *in vitro* in RPMI 1640 media at 5cysts/ml for 24 hrs. The cyst free media was collected, centrifuged (ES protein) and used for treatment of THP1, U937 and human macrophages isolated from healthy donors. Cells were stimulated for 24hrs with ES proteins (30µg/ml), LPS (100ng/ml), PBS. RNA, miRNA and cell soup were collected. Expressions of different miRNAs were quantified by qpcr, while cytokines were analysed by qpcr and ELISA, both. We found IL-1β, IL-6 and IL-10 cytokines were up regulated, while TNF-α and IL-4 were down regulated at transcription level, compared to PBS treated control. Interestingly IL-10 expression was comparable with LPS treated cells at RNA level but down regulated at protein level thus suggesting the role of miR-146 and miR-155 in post transcription regulation of this cytokine. We also observed down regulation of hsa-miR-21, hsa-miR-155 and upregulation of hsa-miR146, these promote Th-2 like response in THP-1, U937 and human macrophages by inhibiting the TNF-α expression as evident by the reduced potential of these cells to kill *Eschericia. coli*. Thus, making suitable niche for parasite's survival in human host with the help of ES protein. The *T. solium* secretome induces Th2 phrnomenon in macrophages which may promote parasite's survival and delay their recognition by host immune system.

**A PRO-INFLAMMATORY ROLE FOR CD70 ON HUMAN REGULATORY T CELLS**

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The CD27-CD70 costimulatory receptor-ligand pair belongs to the TNFR superfamily. Ligation of CD27 on T cells with CD70 on APCs promotes T cell effector function and survival, but impairs Th17 activity. CD70 is also expressed by T cells upon activation, although the role of T cell-expressed CD70 is unknown. We have previously shown that CD27 expression on human Tregs correlates closely with suppressive potency. In this study, we hypothesised that modulation of the CD27/CD70 pathway may allow for the generation of a purer and more potent Treg population.
$CD4^+CD127^{low/-}CD25^+$ Tregs were flow sorted from healthy donors PBMCs and expanded for 14 days *in vitro* via αCD3/αCD28 stimulation in the presence of IL-2. Tregs differentially altered CD27 and CD70 surface expression, resulting in two distinct subpopulations. Tregs were then flow sorted according to CD27 and CD70 and expanded for 14 days. Functional *in vivo* and *in vitro* assays revealed that suppressive activity was confined to $CD27^+CD70^-$ Tregs, while $CD27^-CD70^+$ Tregs promoted T cell proliferation and produced higher levels of IL-17A. Stimulation or blockade of CD27 signalling did not affect Treg suppressive activity. In contrast, blocking CD70 on Tregs significantly enhanced their suppressive potency, abolishing the pro-inflammatory effect of $CD70^+$Tregs. Hence, this study reveals for the first time that $CD70^+$Tregs provide stimulatory signals by ligating CD70 to CD27 on T cells.

**DISTINCT CYTOKINE PATTERNS MAY REGULATE THE SEVERITY OF NEONATAL ASPHYXIA**

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Neuroinflammation following perinatal asphyxia may have dual aspects being a hindrance, but also a necessity in the recovery of the CNS. We aimed to assess intracellular cytokine levels of T-lymphocytes and plasma cytokine levels in moderate and severe asphyxia in order to identify players of the inflammatory response that may influence patient outcome. Contrary to previous data, we described both plasma and intracellular levels of cytokines, enabling a much more precise and thorough characterization.

We analyzed data of 28 term neonates requiring moderate systemic hypothermia in a single-centre observational study. Neonates were divided into a moderate (n = 17) and a severe (n = 11) group based on neuroradiological and aEEG characteristics. Blood samples were collected at 6 h, at 24 h, 72 h, 1 week and 1 month of life. Whole blood samples were stimulated for 6 h, then intracellular cytokine levels were determined using flow cytometry. Cytokine plasma levels were measured using Bioplex immunoassays.

The prevalence and extravasation of IL-1b+ CD4 cells was higher in severe than in moderate asphyxia at 6 h. The prevalence of CD4+ IL-1β+ and CD4+ IL-1β+ CD49d+ cells at 6 h appears to be able to predict severity of the insult at an early stage in asphyxia. At 1 mo, intracellular levels of TNF-α were higher in the severe group. Plasma IL-6 levels were higher at 1 wk in the severe group and decreased by 1 mo in the moderate group. Intracellular levels of IL-6 peaked at 24 h in both groups. Intracellular TGF-β levels were increased from 24 h onwards in the moderate group only.

IL-1β and IL-6 appear to play a key role in the early events of the inflammatory response, while TNF-α seems to be responsible for prolonged neuroinflammation, potentially contributing to a worse outcome. TGF-β has a compensatory role in decreasing inflammation.

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# CD4 – Lck – α–ACTININ-1 – RACK1 AXIS IN PROXIMAL TCR SIGNALLING

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The initiation of T-cell receptor (TCR) signalling is critically dependent on the function of Lck kinase. We and others have previously suggested that TCR triggering requires the activation-induced redistribution of active Lck within the plasma membrane. In our comparative screen, we have identified RACK1 – the Receptor for Activated C-Kinase, as a Lck-interacting protein that is involved in the regulation of this redistribution. The formation of transient Lck-RACK1 complexes was detectable in primary $CD4^+$ T-cells with their maximum levels detected 5-10 seconds after TCR-CD4 co-aggregation. Consistent with its regulatory role, Lck redistribution was impaired in primary $CD4^+$ T-cells where RACK1 protein levels were diminished by its transcript knock-down.

Using the mass spectrometry approach, we have identified several other components of Lck-RACK1 complexes and determined the kinetics of their interaction with RACK1. We have focused on the cytoskeletal protein α-actinin-1 which exhibited transient complex formation with RACK1, thus acting as a linker between Lck-RACK1 complex and cytoskeletal network.

In agreement with our biochemical data, various types of advanced microscopic examinations of primary $CD4^+$ T-cells and Jurkat cell line mutants expressing fluorescent protein-tagged Lck, RACK1 and α-actinin-1, revealed that upon TCR triggering, these proteins briskly and transiently co-redistribute to the forming immunological synapse (IS). The 3D-high resolution live cell imaging microscopy confirmed that within less than one minute after the initial contact between a T-cell and antigen presenting cell, these three proteins are synchronously relocated to the outer ring of IS. Then, whereas Lck and α-actinin-1 remains there, RACK1, in next few minutes, moves back and evenly distributes within the cytoplasm of a T-cell.

Together, these results describe RACK1 and α-actinin-1 as relevant intracellular signalling components involved in the regulation of TCR-induced Lck redistribution within plasma membrane by linking CD4-Lck complex to the cytoskeletal network.

# TOLL-LIKE RECEPTOR 2 TRACKS THE EMERGENCE OF HEMATOPOIETIC PROGENITORS AND STEM CELLS IN PRE-CIRCULATION EMBRYO

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Toll-like receptors (TLRs) play a central role in host cell recognition and defense responses to pathogens. Moreover, pathogen sensing of bone marrow hematopoietic stem cells and progenitors (HSPCs) via TLRs nudges the fate of these cells towards enhanced myelopoiesis, enabling rapid replenishment of immune effector cells. Despite the critical role of TLRs in adult hematopoietic cells, the functional expression of TLRs in embryonic hematopoietic progenitors has not been addressed. We show here that TLRs are initially detected on short-lived maternal myeloid cells, which are gradually replaced by myeloid cells of embryonic origin. Interestingly, embryonic erythro-myeloid progenitors, which appear at day 7.5-8.5 of mouse embryogenesis (E7.5-E8.5), also express TLRs, and respond to TLR2 triggering by an enhanced proliferation and myeloid differentiation rate in a MyD88 adaptor protein dependent manner. Using *in vivo* fate mapping, we show that the *Tlr2* locus is already active in E7.75 yolk sac-derived hematopoietic precursors and at E8.5 in progenitors which contribute to adult long-term HSCs. Our results thus demonstrate that *Tlr2* locus is active in yolk sac derived erythro-myeloid progenitors as well as precursors of adult hematopoietic stem cells and that TLR2 triggering endows these cells with the ability to boost the production of myeloid cells, revealing the so far unrecognized activation of pattern recognition transcription program in developing embryonic hematopoietic cells.

**CHANGES IN THE SKIN MICROBIOME DURING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION**

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Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative therapy for hematologic malignancies, but its success remains limited due to severe side-effects, such as infections and graft-versus-host disease (GVHD). Recent studies suggest that dysbiosis of intestinal microbes is associated with an increased risk of GVHD and poor outcome after HSCT, while the role of the cutaneous microbiome in this setting remains elusive. To investigate this further, we obtained patient material (peripheral blood, skin scales, stool and skin biopsies) at 5 time points before myeloablative conditioning and up to one year after HSCT (n= 20). The cutaneous and intestinal microbial communities are analyzed with 16S ribosomal RNA sequencing. Interactions of bacteria with immune cells are investigated using multiple staining approaches, such as gram stains, immunofluorescence and fluorescence in situ hybridization (FISH). Bacterial numbers/mm2 as well as distance calculations from CD45+- and HLADR+- cells are assessed via the StrataQuest Analysis Software (TissueGnostics GmbH). Of the 20 patients included in this study, 8 developed acute or chronic GVHD. We successfully established an extractionprotocol for microbial DNA from stool and skin scales and could visualize bacteria by gram stains and 16S rRNA FISH (EUB338 probe) in the epidermis and dermis of skin sections. FISH stainings revealed a decrease in bacterial numbers/mm² skin in the epidermis as well as the upper (500µm) and lower (500µm) dermis at day 0 and day 14 after transplantation (n=2). In this new and ongoing project, we aim to build individual risk profiles for patients based on their skin and gut microbiome and further explore the interaction between the immune system and the residing microbiome in this unique cohort.

# ASSOCIATION BETWEEN TUMOR NECROSIS FACTOR ALPHA-308 G/A POLYMORPHISM AND MULTIPLE SCLEROSIS IN ALGERIAN POPULATION

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Tumor necrosis factor alpha (TNFα), proinflammatory cytokine, has been considered the prototypic cytopathogenic cytokine in multiple sclerosis (MS). A bi-allelic single nucleotide substitution of G (TNFA1 allele) with A (TNFA2 allele) polymorphism at −308 nucleotides upstream from the transcription initiation site in the TNF-α promoters associated with elevated TNF-α levels and MS susceptibilities. We investigated the association between TNFα -308G>A (rs 1800629) polymorphism and susceptibility to multiple sclerosis development, also, the association between this polymorphism, intrathecal secretion of IgG and HLA-DRB1*15 allele.

The study included 56 Algerian patients with defined MS, who were recruited to the neurology department of Mustapha Pacha University Hospital (Algiers). The control population was composed of 34 healthy subjects; all these subjects are free from any inflammatory or autoimmune pathology. Single Nucleotide Polymorphism (SNP) analysis was performed using a real-time PCR (taqman technology).

Comparison of genotypic, allelic and phenotypic frequencies between patients and controls found no statistically significant difference. Our results remain statistically insignificant after stratification of patients according to: sex, age of onset of illness, clinical form of MS and intrathecal synthesis. However, our study showed a significant difference between HLA DRB1*15 patients and non-HLA DRB1*15 patients: the frequency of 308 GG genotype was significantly higher in DRB1*15 patients (89% vs 65%, P = 0.049): the frequency of the G allele is significantly higher in DRB1*15 patients versus non-DRB1*15 patients (95% vs. 77%, P = 0.02), while the A allele is significantly higher in non-DRB1*15 patients versus DRB1*15 patients (23% vs. 5%, P = 0.02). The GG genotype and the G allele are significantly associated with the DRB1*15 allele in our MS patients.

The results of our study did not reveal an association between the -308 G> A polymorphism of TNFα and MS, but it seems that the G allele redisposes to MS in the subgroup of HLA DRB1*15 patients and the A allele predisposes to MS in the subgroup of non-DRB1*15 HLA patients.

**IMMUNE-CELLS DERIVED MIRNAS CONTRIBUTION IN DOXORUBICINE RESISTANCE OF WALKER-256 CARCINOSARCOMA**

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Tumor microenvironment is involved in drug resistance formation. Particularly, immune cells (tumor associated macrophages, fibroblasts etc.) can express exosomes, containing miRNAs, that affect the neighbor tumor cells and contributes to their drug resistance. In *in vivo* experiments we explored the relationship of miR-155 and miR-320a expression and resistance to doxorubicin. It is known, that this miRNAs are expressed mainly by immune cells in tumor tissue. In our investigation high levels of miR-155 and low levels of miR-320a correlated with the later stages of tumor growth of Walker-256 carcinosarcoma. Also, tumor and blood samples from animals with doxorubicine resistant strain were characterized with pronounced expression of miR-155, comparatively with sensitive strain ($p < 0,05$).

Supported by
NAS of Ukraine Scientific Project (2015-2019) No 2.2.5.395/1 "Investigation of cancer-associated miRNAs as extratumor predictive breast cancer markers"

**DIFFERENT ROLES OF SRC FAMILY KINASES IN INITIATION OF TCR AND BCR SIGNALING**

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Although, antigen specific signalling has been studied for several decades our understanding of the process is still limited. In our study, we focused on the roles of Src family kinases (SFK) in initiation of BCR and TCR signalling. Why BCR signalling is resistant to inhibition of SFK compare to TCR signalling which is very sensitive to it? Could be BCR signalling initiated by other kinases than SFK? Is that kinase Syk as it was previously suggested? Using Zap-70 deficient T cell line, we have shown that Syk and Zap-70 have comparable ability to initiate TCR signalling, although Syk significantly increased resistance of TCR signalling to inhibition of SFK. Moreover, B cells transduced with fusion protein composed of zeta chain of TCR receptor were sensitive to SFK inhibition when stimulated through TCR but resistant when stimulated through BCR receptor. Consistently, T cells transduced with BCR receptor were slightly more resistant to SFK inhibition. Moreover, BCR signalling in T cells were delayed after SFK inhibition, similarly as it happens in B cells. Altogether, our data suggest that TCR signalling operate in a short time window in which is decided whether T cell will or will not be activated, which is most likely sensed by activity of SFK. In contrast to TCR signalling, BCR signalling is rather less controlled and it is set up toward signal transmission as we showed by comparing activity of Syk and Zap-70 kinase or by differences between TCR and BCR signalling complexes. The resistance of BCR signalling to inhibition of SFK is based on our data rather effect of incomplete inhibition of SFK and an ability of BCR signalling complex to start signalling with minimal activity of SFK, rather than ability of Syk kinase to initiate signalling independently of SFK but additional experiments are still necessary to confirm this hypothesis.

**IL-17 DRIVES PANETH CELL-MEDIATED CONTROL OF SEGMENTED FILAMENTOUS BACTERIA**

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Paneth cells (PCs) are critical players in a complex network that maintains the homeostasis of intestinal immune system. On one hand, they protect the host from intestinal pathogens, however they also shape the composition of the intestinal microbiome. The main effector molecules of PCs are enteric α-defensins (EDs). Their proper production and secretion is a tightly controlled process. While the innate mechanisms involved in this regulation have been well described, the knowledge concerning the contribution of the adaptive immune system, particularly T cells, is sparse. Recently it was shown that IL-17 receptor (IL-17R) signaling in the intestinal epithelium controls the expression of EDs. This mechanism seems to be crucial to limit the number of segmented filamentous bacteria (SFB), intestinal commensal strain with the profound capability to induce Th17 responses. However, from previous studies it is unclear if IL-17 acts directly on PCs. Therefore, we analyzed the expression pattern of IL-17R in the small intestine epithelium by flow cytometry and found that PCs are the major population expressing this receptor. We also confirmed that this receptor is functional *in vitro*. Notably, injection of IL-17 into mice resulted in the increased presence of PC lysozyme in the intestinal lumen, suggesting increased secretion of PC products. Conversely, IL17 administered mice had significantly lower number of SFB in their intestine. Furthermore, our data suggests that intestinal T cells may be the source of IL-17 that stimulates PCs. To confirm this scenario, we are using approaches which combine the adoptive T cell transfer system with the genetic ablation of IL-17R. Together, our results reveal a new mechanism regulating intestinal homeostasis via IL17 axis acting through a feedback loop that involves T cells, PCs and microbiota.

**SUPPRESSION OF AUTOREACTIVE T AND B LYMPHOCYTES BY ANTIBODY THERAPY IN MRL/LPR MURINE MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS**

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Systemic lupus erythematosus is an autoimmune syndrome characterized by the development of autoantibodies to a wide range of antigens and multiple organ involvement. Together with B cells, respective self-reactive T cells have an important contribution in disease progression as being responsible for inflammatory cytokines secretion, B cell activation, and promoting amplification of the autoimmune and inflammatory response.

Annexin A1 is expressed by many cell types and binds to phospholipids in a $Ca^2$+ dependent manner. Abnormal expression of annexin A1 was found on activated B and T cells in both murine and human autoimmunity suggesting its potential role as a therapeutic target.
Groups of lupus-prone MRL/lpr mice were treated with the anti-annexin A1 monoclonal antibody and the disease activity and survival of the animals were following up. ELISA assays, RT-PCR, Histological and Immunofluorescence kidney analyses were used to determine the levels of cytokines, anti-dsDNA antibodies and kidney injuries. The administration of this monoclonal antibody to lupus-prone MRL/lpr mice resulted in suppression of IgG anti-dsDNA antibody production and of proteinuria, modulated cytokines secretion, decreased disease activity and prolonged survival compared to the control group.

**COOPERATIVE ANTIGEN TRANSFER FROM MEDULLARY THYMIC EPITHELIAL CELLS TARGETS A NOVEL SUBSET OF DISTINCT THYMIC ANTIGEN-PRESENTING CELLS**

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The multifactorial mechanism of central tolerance, which operates in the thymus, markedly reduces the repertoire of self-reactive T cells and thereby protects against the development of autoimmune disease. The key players in these mechanisms are medullary Thymic Epithelial Cells (mTECs) which are capable of producing and presenting thousands of tissue restricted antigens to developing T cells in order to either eliminate self-reactive clones or convert them into regulatory T cells (Tregs). Importantly, it has been shown that unidirectional cooperative antigen transfer (CAT) from mTECs to thymic dendritic cells (DCs) is essential for efficient deletional tolerance and selection of Tregs. Interestingly, in addition to DCs, the thymus harbours several other subtypes of antigen presenting cells (APCs) whose potential involvement in CAT and in mechanisms of central tolerance has not yet been assessed. Here, we present evidence for the transfer of model neoantigen from mTECs to several distinct subsets of thymic APCs. Notably, using the Foxn1-Cre-Rosa26-tdTomato mouse model, where the fluorescent tdTomato protein is expressed in the thymus exclusively by thymic epithelial cells, including mTECs, we demonstrate that apart from DCs, also granulocytes, monocytes and thymic macrophages are capable of acquiring mTEC antigen and participate in CAT. Furthermore, using a signaling reporter T cell line, we show that these distinct subsets of APCs are able to present antigens to T cells and thus potentially participate in thymic negative selection. These results provide new insight extending the concept of CAT from mTECs to various subsets of thymic APCs and show that antigen presentation in the context of thymic selection processes is likely much more complex than originally proposed. Functional importance of these newly identified APCs in T cell selection remains to be determined.

**COMPLEMENT MASP-1 SYNERGIZES WITH OTHER INFLAMMATORY FACTORS IN ENDOTHELIAL CELLS**

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The mannan-binding lectin associated serine protease 1 (MASP-1) is a key protease of the complement lectin pathway and it can be activated by apoptotic and necrotic cells as well as microbial macromolecules. Previously we demonstrated that MASP-1 is able to stimulate human umbilical vein endothelial cells (HUVECs) to acquire a pro-inflammatory phenotype. During inflammatory conditions there are several factors, which simultaneously affect the endothelial cells, therefore we aimed to explore, whether MASP-1 is able to cooperate with other known pro-inflammatory factors (histamine, lipopolysaccharide (LPS), IFNgamma, bradykinin and lipoteichoic acids (LTA)).

Confluent HUVEC culture was used as a model and we treated the cells with suboptimal dose of pro-inflammatory factors. We assessed the adhesion molecules with cellular ELISA and immunofluorescence microscopy, and the chemokines with sandwich ELISA. To investigate the Ca2+ influx, pCREB and NFkappaB activation we used fluorescence microscopy.

MASP-1 increased the Ca2+ influx, the NFkappaB and pCREB activation, increased the expression of IL-8 and E-selectin, but had no effect on ICAM-1 and VCAM-1 expression. LPS affected the E-selectin, ICAM-1 and VCAM-1 expression and the pCREB and NFkappaB activation, but did not influence the Ca2+ influx. Bradykinin and histamine were able to increase the Ca2+ influx, and histamine also induced IL-8 secretion. LTA and IFNgamma did not provoke any of these responses in HUVECs.

LPS synergized with MASP-1 in the upregulated expression of IL-8 and E-selectin. Histamine, IFNgamma and LTA showed synergistic effect with MASP-1 in the IL-8 induction, whereas LTA and

bradykinin synergized with MASP-1 in VCAM-1 expression modification.
In the case of ICAM-1 expression we could not find synergism between MASP-1 and the investigated pro-inflammatory mediators.
MASP-1 was able to cooperate with pro-inflammatory factors in a differentiated way depending on the molecules induced. The most prominent synergism was found in the case of IL-8 expression, a highly important neutrophil chemoattractant molecule, and this coincides with our previous findings, where MASP-1 induced endothelial cells significantly increased the neutrophil chemoattraction. The diverse synergism of MASP-1 and other pro-inflammatory factors may contribute in the formation of the most appropriate immune response against different pathogens.

Supported by Hungarian National Research Grant NKFI K115623.

# NEW PIPELINE FOR PRODUCTION OF ANTI-OSPA SINGLE-DOMAIN ANTIBODIES FOR IMMUNOTHERAPY

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Single-domain antibodies (sdAbs) recombinant fragments of Heavy-chain antibodies of *Camelidae* have been used successfully in cases where classic antibodies failed, such as inhibition of specific enzyme functions. Borrelial surface protein OspA, is vital in translocation of *Borrelia* to CNS, which makes it perfect candidate for development of immunotherapy against neuroborrelliosis. The aim of this study was to establish a pipeline for *in vitro* production of anti-OspA sdAb. Recombinant OspA (of neuroinvasive *Borrelia bavariensis* SKT-7.1) was used as antigen in *in vitro* immunization of B-cells isolated from peripheral blood of healthy *Vicugna pacos*. Isolated B-cells were incubated with OspA for 3 days, mRNA was extracted, reverse-transcribed into cDNA, and sequences encoding sdAb were amplified. These sequences were cloned into psex81 phagemid vector and electroporated into *E. coli*. Transformed *E. coli* were infected by hyper-phage M13K07ΔpIII in order to create phage clones that expressed sdAbs on surface. Phage clones were incubated with immobilized OspA to select OspA specific sdAb. Two phage clones were selected further for quantitative phage ELISA to assess sensitivity and specificity to antigen. Plastware and non-related antigen SAA (*Salmo Salar*) were used to assess non-specificity in ELISA. Both phage clones showed significant ($P>0.05$) affinity to OspA, however one of the clones showed non-specific affinity to plastware as well as to SAA. Clone showing specific affinity to OspA was produced as soluble form (in cytoplasm of *E. coli*) and its binding strength was quantified by Bio-layer interferometry (1.55 μM). Pipeline described in this study is faster and cheaper alternative to current methods used in production of sdAbs, which may accelerate discovery of new immunotherapies for infectious diseases. Furthemore, isolated sdAb may be suitable for subsequent applications in immunotherapy against neuroborrelliosis, however, for therapeutic use it may be necessary to increase affinity of sdAbs up to nanomolar range.

**Acknowledgement**: Work was supported by APVV-14-0218 and VEGA 1/0258/15

**IMPORTANCE OF ADENYLATE CYCLASE ISOFORMS IN REGULATION OF T-LYMPHOCYTES FUNCTIONS**

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Cyclic AMP (cAMP) is an important intracellular second messenger, which is produced by adenylate cyclases (ACs). Ten mammalian AC isoforms identified up to date differ in their tissue distribution and biochemical regulation. The only known specific activator of membrane AC isoforms 1 to 8 is labdane diterpene forskolin. cAMP regulates both innate and adaptive immune cell activities as a potent immunosupressor. In leukocytes, two dominant AC isoforms (AC7 and AC9) are suggested. Our study is aimed to clarify importance of different AC isoforms in regulation of function of selected subpopulation of T-lymphocytes and to elucidate possibilities how to specifically modulate production of cAMP in these cell types. Subpopulations of helper ($CD4^+$) and cytotoxic ($CD8^+$) T cells were sorted and stimulated by selected activators. Formation of cAMP was measured by homogenous time-resolved fluorescence resonance energy transfer (TR-FRET) at different time points together with cell proliferation, cytokine production and immunophenotypic characterisation of lymphocyte activation state. Further, analogues of forskolin were screened using unique assay covering all membrane AC isoforms. Obtained data suggest changes in AC isoform expressions during activation of T-lymphocytes and potential of some forskolin analogues to specifically modulate particular AC isoforms. AC isoform selective modulation may represent a new therapeutic approach for the treatment of T-lymphocytes related pathological processes.

**5-FLUOROISOCYTOSINE DEAMINASE – A NEW PROMISSING TOOL FOR THE PRODRUG CANCER THERAPY**

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We have developed bacterial deaminase based system which converts nontoxic prodrug into toxic drug *in situ*. As a substrate we used 5-fluoroisocytosine which was shown to be not toxic at all to different cell cultures *in vitro* and in *in vivo* tests on animals. Our isolated deaminase is able to convert this compound into well know anticancer drug *5-fluorouracil*. Deaminase gene was introduced into plasmid pTO/Vcz-IG and further transfected into different carrier cells. *In vitro* experiments have shown that this plasmid system is efficient in killing cancer cells. Moreover, immunosuppressive murine mesenchymal stem cells (MSC), transfected with this plasmid, were shown to convert non toxic *5-fluoroisocytosine* into toxic *5-fluorouracil in situ* and to improve mice survival in tumor model *in vivo*. In a glioblastoma murine model utilizing GL261 cell line, survival results let us presume that prodrug based 5-isocytosine system is 10% to 20% more efficient than standard 5-fluorouracil based treatment. After 40 days of treatment, keeping in mind the poor performance of the control group (10% survival), 5-isocytosine + transfected MSC treated group shows that our deaminase based treatment technology supports over 50% of mice survival. On the other hand, standard 5-fluorouracil-only treated group animals survive merely in 30-40%.

**REMODELING OF EXTRACELLULAR MATRIX OF THE LAMINA PROPRIA IN THE UNINVOLVED HUMAN RECTAL MUCOSA 10 AND 20 CM AWAY FROM THE MALIGNANT TUMOR**

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Despite the mounting evidence about the role of tumor stroma in the genesis and progression of the malignant disease, there are a very few data regarding the uninvolved stromal tissue in the remote surrounding of the tumor. The aim of our study was to investigate the organization of extracellular matrix (ECM) components in the lamina propria of the rectal mucosa surrounding the malignant tumor and quantitate them using an objective morphometric approach. Tissue samples of rectal mucosa 10 cm and 20 cm away from the malignant tumor were endoscopically collected from 30 patients with adenocarcinoma located in the sigmoid colon. The samples of rectal mucosa from 30 healthy persons were used as control. Gomoris's silver impregnation technique and Masson's trichrome staining were used to identify the reticular and collagen fibers. Also, the nonlinear laser-scanning microscope (NLM) was used for second harmonic generation (SHG) imaging of collagen fiber on fixation- and label-free samples. For visualising hyaluronic acid, immunohistochemical staining with HABP antibody was used. The presence of reticular, collagen fibers and hyaluronic acid were determined in the Icy software using Color Picker Threshold Plugin. The diameter of spaces between reticular fibers and chains of hyaluronic acid were measured using plugin BoneJ within Fiji software. There were significant reduction of reticular, collagen fibers and hyaluronic acid 10 cm and 20 cm away from the malignant tumor, compared with healthy lamina propra. Also, the diameter of spaces between reticular fibers and HABP were significantly larger in comparison with healthy lamina propra. In conclusion, there is profound remodeling of extracellular matrix of the lamina propra in the uninvolved human rectal mucosa 10 cm and 20 cm away from the malignant tumor.

**REMODELING OF EXTRACELLULAR MATRIX OF THE LAMINA PROPRIA IN THE UNINVOLVED HUMAN RECTAL**

**MOLECULAR MECHANISMS GUIDING IL-17 SIGNALING RESPONSES.**

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The immune response against invading pathogens often requires an orchestrated crosstalk between adaptive and innate immune system in order to mount an appropriate response. One such mechanism is the production of IL-17A by Th17 cells. Stimulation of nonhematopoietic cells with IL-17A leads to the production of a variety of pro-inflammatory cytokines, such as CXCL1, CXCL2, CCL2, IL-6, or TNF. This in turn promotes the recruitment of neutrophils and macrophages to the site of inflammation and strong inflammatory response. Inability to signal via IL-17 receptor leads to severe susceptibility to Candida infections. On the other hand, aberrant IL-17 signaling is involved in numerous immune-mediated disorders, such as psoriasis, rheumatoid arthritis, inflammatory bowel disease, or multiple sclerosis and IL-17 neutralizing antibodies are approved for treatment of psoriasis and psoriatic arthritis. Although IL-17A is a critical mediator of a variety of diseases, the molecular mechanism guiding IL-17 signaling is still not very well understood. Here, we aimed to study the composition of endogenous IL-17A receptor-signaling complex by mass spectrometry in order to identify novel components of the IL-17A receptor and to understand the mechanism of regulation of IL-17A signaling. Our discovery of surprising regulatory loop guiding the signaling outcomes of IL17A will be discussed.

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# COGNATE INTERACTION WITH CD4$^+$ T CELLS INSTRUCTS M2-LIKE MACROPHAGES TO AQUIRE M1-LIKE PHENOTYPE

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The immunosuppressive tumor micro milieu established by immune cells such as regulatory T cells (Tregs) and tumor associated macrophages (TAMs) still forms a major obstacle for successful immunotherapies. TAMs can be attracted from the circulation by chemokines secreted by the growing tumor. Depending on the activating stimuli, these cells can develop into different subsets including classically (M1-like) or alternatively (M2-like) activated macrophages with distinct gene and protein expression patterns. Here, we show that immunosuppressive M2-like macrophages can be repolarized into pro-inflammatory M1-like macrophages when co-cultured with CD4$^+$ T cells. Peptide loaded M2-like macrophages co-cultured with CD4$^+$ T cells showed a strong upregulation of typical M1-like markers. Gene expression analysis revealed an increased expression of M1-like associated genes and a decreased expression of M2-like associated genes. Additionally, phagocytic activity of co-cultured macrophages was diminished. Taken together, our results show that M2-like polarized macrophages can be repolarized into M1-like macrophages through direct interaction with CD4$^+$ T cells. In ongoing *in vivo* experiments we are using the murine B16F10-OVA tumor model to study the effects of adoptively transferred ovalbumin specific CD4$^+$ T cells on the polarization of TAMs. Tumor antigen specific CD4$^+$ T cells might polarize TAMs into immunostimulatory M1, thereby neutralizing the immunosuppressive micro-milieu and facilitate cytotoxic T lymphocyte mediated tumor rejection.

**DECIDUAL MICROENVIRONMENTS FROM ABORTION PRONE MICE SHOW THE STIMULATORY EFFECTS ON DENDRITIC CELLS MATURATION STATE AND FUNCTION**

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Dendritic cells (DCs) represent a system of antigen presenting cells with a dual property of triggering an immune response or promote cell tolerance, depending on the cellular and molecular context of their microenvironment. This study was done to investigate the effects of decidual cell from normal pregnant and abortion prone mice on DCs maturation and function.

DCs were differentiated from mouse bone marrow cells in the presence of DC differentiation cytokines, GM-CSF and IL-4. The DCs were then co-cultured with decidual cells from normal pregnant and abortion prone mice and their immunophentype and pinocytotic activity were evaluated using the flow cytometric analysis. The obtained DCs were also pulsed with paternal antigens and used for immunization studies. The antigen specific T cell responses were determined in T cells derived from lymph node of immunized animals.

Our finding revealed that treatment of dendritic cells with decidual cells from abortion prone mice significantly increased the MHC-II, CD86 and CD40 expression by DCs compared to DCs treated with decidual cells from normal pregnant mice. A remarkable reduction in the endocytic capacity were also observed in iDCs that were co-cultured with decidua from abortion prone mice. Meanwhile, the ability of DCs to induce lymphocyte proliferation significantly increased following their treatment with decidua of abortion prone mice.

We concluded that in decidua of abortion prone mice, an immunopotentiating microenvironment dominancy could be observed which can modulate the DCs phenotype and functions, leading to triggering of an undesirable immune response that is associated with fetal rejection.

# ALTERED IMMUNE RESPONSES IN MICE WITH LST1 ADAPTOR PROTEIN DEFICIENCY

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Transmembrane adaptor protein LST1 is expressed in leukocytes of the myeloid lineage, most abundantly in macrophages, while it is absent in T and B cells. Previous study has revealed mild effects of LST1 deficiency on the outcome of influenza infection in mice. However, except for this specific case its overall function at the organismal level is still to be determined. At the molecular level, LST1 was shown to interact with cytoskeleton regulating proteins and to promote the formation of tunneling nanotubes. It also contains an ITIM motif in its intracellular tail, which was shown to bind phosphatases SHP-1 and SHP-2 in monocytes. To study the physiological function of LST1 we have performed a thorough analysis of LST1-deficient mice. At steady state these mice displayed no apparent phenotype, with the exception of slight changes in the trabecular bone structure detectable only in male animals. However, these alterations did not have any effect on their survival and overall health. On the other hand, when we challenged LST1-deficient mice with several pro-inflammatory stimuli some aspects of their responses were altered. In response to LPS, we observed slight reduction in percentages of splenic dendritic cells and macrophages. In addition, IP injection of viral mimetic PolyI:C (synthetic dsRNA analog) resulted in significant reduction in splenic $CD8^+$ T cell percentages. However, the most striking differences were observed when we induced acute colitis in these mice by DSS (dextran sodium sulphate), as a model of disease, where myeloid cells are heavily involved. We found significantly better course of acute colitis in LST1-deficient animals in all observed parameters – body weight, colon length, occult bleeding and stool consistency. This was accompanied by alterations in splenic monocyte populations. Interestingly, we also saw the same significant decrease in CD8+ splenic T cells as after polyI:C injection. Collectively our data suggest that LST1 is not required for leukocyte development and immune system homeostasis, but it is involved in the regulation of several types of immune responses.

# LAT TRANSMEMBRANE DOMAIN IN THE PLASMA MEMBRANE OF T CELLS

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LAT is transmembrane adaptor protein essential for T cell development and function. It is phosphorylated by ZAP70 protein tyrosine kinase upon T cell receptor activation and recruits multiple adaptor proteins to form a multisubunit signalling complex. It contains a very short extracellular region, transmembrane domain (TMD) and tyrosine-rich cytoplasmic tail. LAT is palmitoylated on two conserved cysteines (amino acids 26 and 29). Furthermore, TMD of LAT contains two proline amino acids (position 8 and 17) function of which is still unknown. In agreement with the literature about other proteins, our MD simulations of LAT TMD demonstrate that the presence of prolines disrupts α-helical structure and causes so-called proline-kinks in its structure. Expression of mutants with prolines changed to alanines or in combination with mutants lacking the palmitoylation sites suggest their importance for sorting of LAT to the plasma membrane and their impact on T cell signalling.
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# IL-6 PRODUCED BY MACROPHAGES AND DENDRITIC CELLS MEDIATES DISTINCT ASPECTS OF ALLERGIC AIRWAY INFLAMMATION

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Asthma is a common inflammatory disease of the airway, which is caused by a combination of genetic and environmental factors, and is characterized by airflow obstruction, wheezing, eosinophilia and neutrophilia of lungs and sputum. IL-6 is implicated in the regulation of both protective and pathogenic responses in the lung, however, its role in allergic lung inflammation and critical IL-6 producing cellular sources remain unclear. To elucidate critical IL-6-producing cells in pathogenesis of allergic airway inflammation, we generated mice with specific inactivation of IL-6 in CD11c-expressing dendritic cells (DCs) or lysozyme-expressing myeloid cells (predominantly macrophages and neutrophils) and subjected them to intranasal administration of HDM (house dust mite) extract for 5 days a week with sensitization treatment one week prior to the main course. We show that complete genetic inactivation of IL-6 or pharmacological inhibition using blocking antibody ameliorated the disease with significant decrease in eosinophilia of the lungs. Moreover, we demonstrate that IL-6 from both CD11c-expressing DCs cells and lysozyme-expressing myeloid cells distinctly contributes to the disease. Specific deletion of IL-6 in dendritic cells reduced key indicators of allergic inflammation including lymphocyte infiltration, eosinophil and Th2 cell accumulation in the lungs, and expression of asthma-associated inflammatory mediators but no effect on IgE levels. In contrast, macrophage-derived IL-6 deficiency exhibited attenuated IgE production. These results suggest that IL-6 plays a pathogenic role in HDM-induced asthma model and that both lung macrophages and dendritic cells provide the considerable source of IL-6 and distinctly contribute to the disease. The evaluation of relative contribution of IL-6 from dendritic cells and macrophages to the disease is underway.

The work was supported by the Russian Science Foundation, grant No. 14-25-00160.

# THE DISTURBANCES OF THE PROTEIN SYSTEMS CONTROLLING NEURON – MICROGLIA INTERACTIONS IN THE FRONTAL CORTEX OF YOUNG OFFSPRING RATS – THE COMPARATIVE STUDY IN LPS- AND POLY I:C-INDUCED SCHIZOPHRENIA MODELS.

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**Introduction:** Animal models of maternal infections with bacterial and viral agents support a uniform conclusion that schizophrenia-related changes in the offspring are related to maternal immune activation (MIA) during pregnancy. Also, prenatal insults disturb natural neurodevelopmental processes and change normal neuron – microglia communication, what suggests a potential contribution of this system to the pathogenesis of schizophrenia. **Aim:** The study was designed to compare the impact of MIA on the levels of CX3CL1, CD200 and their receptors (CX3CR1 and CD200R, respectively) in the frontal cortex (FCx) of young offspring rats in two neurodevelopmental animal models of schizophrenia – based on the administration of lipopolysaccharide (LPS), and polyinosinic:polycytidylic acid (Poly I:C). **Materials & methods:** In the 1st model, pregnant rats were injected subcutaneously with LPS (2 mg/kg) every second day from the 7th day of pregnancy until the delivery. The 2nd model was based on the intravenous administration of Poly I:C to pregnant rats at GD15. The 7-day-old offspring male rats from both models were sacrificed and frontal cortices were dissected. The levels of the above-mentioned proteins in FCx were measured using ELISA kits. **Results:** The obtained data demonstrated that the young offspring rats after prenatal exposure to LPS exhibit elevated concentrations of cortical CX3CL1 and CD200 but a reduced level of CD200R (CX3CR1 was not affected). For the model based on Poly I:C administration, the biochemical study showed the significantly higher level of CX3CL1. No changes in the concentrations of CX3CR1, CD200 and CD200R in FCx were observed in this model. **Conclusions:** Our data indicated that the type of prenatal insult, used in the mothers, determine changes in the neuron – microglia controlling protein systems. Therefore, our comparative study clearly supports the need for further

**SYNERGISTIC EFFECT OF MSCS AND IMMUNOSUPPRESSIVE DRUGS ON AN INFLAMMATORY RESPONSE**

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Mesenchymal stem cells (MSCs) possess significant immunomodulatory, anti-apoptotic and cytoprotective capabilities. It was shown in several studies that the treatment of graft recipients with MSCs together with immunosuppressive drugs prolongs the graft survival but the exact mechanism of this synergy has not yet been described. In our study, we determined the therapeutic effect of MSCs in mouse model of allogenic cell transplantation. Application of MSCs into cyclosporine A (CsA) treated recipients significantly increased the survival of PKH-26 labelled transplanted allogeneic cells and moderated the reduction of T cells in draining lymph nodes caused by CsA. Study of the distinct T cell subpopulations showed a significant decrease in proportion of Th1 ($CD4^+Tbet^+$) and Th17 ($CD4^+ROR\gamma t^+$) cells, increase in Th2 ($CD4^+GATA^+$), whereas Treg ($CD4^+FoxP3^+$) population remain unchanged when allogeneic cell recipients were treated with CsA together with MSCs in comparison with recipients treated only with CsA. These changes were accompanied with lower production of Th1/Th17 related cytokines IFNγ and IL-17 and on contrary, higher production of anti-inflammatory cytokine IL-10.

Our results indicate that MSCs promote the immunosuppressive effect of CsA and at the same time attenuate its adverse effects. Understanding these interactions of MSCs and immunosuppressive agents is essential for selection of an appropriate therapy and the achievement of desired therapeutic effect in clinic.

**EXPRESSION OF ENDOGENOUS INTERLEUKIN-36 ACTIVITY**

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Interleukin-36 cytokines belong to the IL-1 family. IL-36 cytokines comprise three agonists, IL-36α, IL-36β, IL-36γ, and antagonist IL-36Ra. IL-36 cytokines use receptor IL-36R (IL-1Rrp2), and the IL-1 receptor co-receptor IL-1RacP. A cytoplasmic complex is formed between the receptors and Myd88, activating NF-kB and MAP kinases. IL-36 appears to be an important mediator for inflammation and immunity, particularly in the skin and other epithelia. We intend to identify processes that lie between the activation of the IL-36 genes and activation of the cytokines through post translational proteolysis. Our initial studies are focused on finding a simple system for observing IL-36 gene activation.

Previous work showed that IL-36β and IL-36γ strongly expressed by stimulated keratinocytes (KC) *in vivo*, while IL-36α has been identified as a product of monocytes and has been identified in the cell line THP-1. We used rhIL-1α, rhN$^6$-IL-36α and rhTNF at close to saturating concentrations to activate the IL-36 genes. IL-36 mRNAs were detected in both HaCaT (an untransformed human KC line) and HT-29 (a human colorectal carcinoma/epithelial cell line). Analysis by RT-PCR and RT-qPCR showed that IL-36β, IL-36γ, IL-36R and IL-36Ra mRNA were expressed in the KC line HaCaT in monolayer, while IL-36α was barely detectable. Cell de-differentiation and differentiation of HaCaT in monolayer by $Ca^{2+}$ modulation had little effect on the inducibility of IL-36β or IL-36γ. IL-36β, IL-36γ and IL-36R mRNA were also expressed by HT-29 but the standardised levels of IL-36β and IL-36γ were an order of magnitude lower compared with HaCaT. In HaCaT, the inducer cytokines were effective in the order TNF>IL-1>IL-36. In HT29 IL-36 was more effective as an inducer than IL-1. HaCaT also responded to inducers of IL-8/CXCL8 secretion in the order TNF>IL-1>IL-36. In HT-29, IL-8 secretion was very effectively induced by rhIL-36α. In monolayer epithelial cell line culture, expression levels of IL-36β and IL-36γ seem to be intrinsically cell line dependent. Maximum expression in HaCaT was an order of

magnitude stronger than in HT-29. In HaCaT, dependence of IL-36 expression on even gross changes in cell morphology was weak. In both HaCaT and HT-29, there was a correlation between the proportionate increase in IL-8 secretion (as a surrogate for NFκB activity) and the expression of IL-36 genes, which may reflect relative expression of cell surface receptors rather than the intrinsic responsiveness of the IL-36 genes to signaling by specific activators.

# AUTOIMMUNITY AGAINST ENTERIC A-DEFENSINS AS A POSSIBLE TRIGGER OF CROHN'S DISEASE

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The incidence of inflammatory bowel diseases (IBD) is gradually increasing in the western world. Crohn's disease (CD), one of the major subsets of IBD, is associated with the decreased expression of enteric α-defensins (ED), secretory products of Paneth's cells (PC). In the healthy individuals, EDs can shape the composition of the intestinal microbiota. Consistent with this notion, CD patients display an altered microbial composition. We have recently shown that the disruption of ED expression within the thymus leads to improper T cell selection and escape of the autoreactive T cells to the periphery, where they then infiltrate the intestine and cause a drop in PC numbers. Based on this set of published data, we currently investigate if the loss of thymic central tolerance to EDs can potentially act as one of the triggers for CD. Our main approach is the analysis of clinical samples collected from paediatric CD patients, namely blood sera, stool samples and ileum biopsies. Our preliminary observations confirmed the expected changes in microbial composition. Correlating these results with the presence of ED-specific auto-antibodies, diminishment of Paneth cell number and the presence of T cell infiltrates in the intestine, will provide a better strategy to stratify patients and understand the role of immune tolerance to EDs in the development of IBD.

**IMMUNE STATUS OF HEALTHY APOCRINE GLAND-RICH SKIN REGIONS AND ITS ALTERATIONS IN HIDRADENITIS SUPPURATIVA**

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Microbial community is highly diverse on the skin surface and can change in response to environmental challenges. Its mutual relationship with the host and its key role in tissue homeostasis have also been reported. Previously, our workgroup described a non-inflammatory Th17/Treg milieu in sebaceous skin region which can be connected to the different microbiome and chemical milieu of this area. Since these factors also differ in apocrine gland-rich (AGR) areas, we aimed to compare the immune milieu of healthy sebaceous gland-poor (SGP) and AGR skin regions and to study its changes in Hidradenitis Suppurativa (HS), an inflammatory disease characteristically localized on AGR areas. Cytokines, cell surface markers, activation markers and transcription factors of keratinocytes, dendritic cells (DC) and T cells were detected by immunohistochemistry and qPCR in biopsies from healthy AGR and SGP skin and from HS lesional skin. In AGR skin, higher non-inflammatory TSLP expression, DC appearance without prominent activation and T cell presence with IL-17/IL-10 cytokine milieu were detected compared to SGP skin. The level of these parameters further increased in HS with the presence of activated DCs and a prominent influx of IL-17+ and IFN-γ+ cells. qPCR analyses of the aforementioned factors showed a similar pattern. Similarly to sebaceous region, AGR skin areas represent distinct immune milieu from SGP regions, which can be the result of the different microbiome and chemical milieu. These changes may be connected to the maturation of apocrine glands and change in microbiome during puberty, but it needs further investigation.

**MEMBRANE PROTEIN ANTIBODY DISCOVERY AND OPTIMIZATION USING WHOLE CELL SELECTIONS AND INFORMATICS**

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Identification of antibodies that target highly-integrated membrane proteins remains a key challenge for therapeutic antibody development. No single discovery technology has shown clear superiority, with murine immunization representing the most commonly deployed approach. *In vitro* technologies, however, have unique advantages, such as the routine ability to identify triple species cross-reactive (i.e., human, cyno, and mouse) antibodies that enable pre-clinical animal modeling and rapid optimization, including tuning of affinity and developability. While a multitude of formats may be employed to represent the target, native antigenicity is typically dependent on presentation in a lipid membrane environment, usually a primary or recombinant mammalian cell line that express the target of interest.

However, the use of whole cells as selection reagent format leads to co-selection of non-target specific background-binding antibodies. To address this issue, next generation sequencing (NGS) may be employed for deconvolution of target-specific binders from non-target binders in the enriched binder output. Integration of NGS into antibody discovery has also proven to be an effective method for the identification and recovery of rare specific clones that would not have been observed by Sanger sequencing. Here, we report outcomes of two antibody selection efforts targeting a class A 7-transmembrane G-Protein-Coupled-Receptor (GPCR) and a tetraspanin membrane protein. In all, these efforts exemplify the successful use of Adimab's fully-synthetic human antibody libraries presented on yeast, whole cell selection, NGS, and high-throughput cell binding.

# NANOTHERAPEUTICS FOR TREATMENT OF CHEMOTHERAPY-RESISTANT TUMORS

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Polymeric nanotherapeutics have been used as targerted delivery systems of anti-cancer drugs. Amphiphilic diblock copolymer conjugates based on hydrophilic *N*-(2-hydroxypropyl)methacrylamide (HPMA) and hydrophobic poly(propylene-oxid) (PPO) were evaluated as an effective system enabling prolonged circulation of the cytostatic drug (Doxorubicin, Dox) in blood, its high accumulation, and controlled release in target tumor tissue. In addition, the PPO was able to inhibit P-glycoprotein (P-gp) of which upregulation is the common mechanism of tumor multidrug resistance (MDR). To increase the inhibitory activity of the diblock copolymer, biodegradable disulfidic bond was incorporated between the hydrophobic and hydrophilic blocks. The resulting biodegradable HPMA-SS-PPO carrier showed higher ability to inhibit MDR in tumor cells *in vitro* compared to HPMA-PPO copolymer. Therefore, the HPMA-SS-PPO-Dox conjugate proved better cytotoxic effect in Dox-resistant P388/MDR cancer cell line overexpressing P-pg. Importantly, the diblock-Dox conjugate demonstrated high therapeutic efficacy in vivo in the CT26 colon carcinoma model with congenital P-gp expression. Inclusion of additional amount of the HPMA-PPO copolymer carrier further increased the therapeutic effect of the diblock-Dox conjugate. Four of total eight experimental mice were completely cured compared to one of eight completely cured mice in the group treated by diblock-Dox alone.

**SCREENING OF OUTER MEMBRANE PROTEINS OF *Neisseria meningitidis* FOR INTERACTION WITH HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS**

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*Neisseria meningitidis* is a Gram-negative bacterium responsible of life-threatening diseases such as meningitidis and fatal sepsis worldwide. During the pathogenesis meningococci are able to express and bind their ligands to receptors of brain microvascular endothelial cells (BMECs) and trigger cell responses allowing the crossing of the blood brain barrier. The aim of this study was confirmation of selected neisserial surface exposed proteins for their adhesion to the human BMECs receptors. Biotinylated neisserial whole cell lysate was incubated with human BMECs. Unbound proteins were washed and bound proteins were recovered with Nutravidin beads and identified with SWATH MS. With the detailed bioinformatic tools we selected 5 (adhesin MafA (Q9JS44), putative adhesin and invasion protein (Q9JXK7), putative lipoprotein (Q7DDH4), outer membrane lipoprotein (Q7DD63) and major outer membrane protein P.IB (P30690)) out of 41 identified candidates and recombinantly prepared for validation of interaction. The interaction was judged by using ELISA and immunocytochemistry. In ELISA proteins of BMECs were incubated with recombinantly prepared neisserial proteins and detected with anti-HIS Nickel probe. Both, immunocytochemistry and ELISA confirmed the binding of selected proteins to receptors of human BMEC, although interaction of lipoprotein was not statistically significant in ELISA. These interacting proteins can be ligand candidates, triggering various cellular processes leading to induction of pathological processes in the host cells.

Acknowledgement: This work was supported by APVV-14-0218 and VEGA 1/0258/15.

**IMMUNOTOPOGRAPHICAL DIFFERENCES OF HUMAN SKIN**

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The immunological barrier of the healthy skin is considered to be unified on the whole body surface – however, recent indirect findings have challenged this dogma since microbial and chemical milieu (e.g. sebum, sweat, pH) exhibit remarkable differences on topographically distinct skin areas. Therefore, in the present study, we performed whole transcriptomic and subsequent pathway analyses to assess differences between sebaceous gland rich (SGR) and sebaceous gland poor (SGP) regions. Here, we provide the first evidence that different skin regions exhibit a characteristic innate and adaptive immune and barrier milieu as we could detect significantly increased chemokine (CCL2, 3, 19, 20, 23, 24) and antimicrobial peptide (S100A7, A8, A9, lipocalin, β-defensin-2) expression, altered barrier (keratin 17, 79) functions and a non-inflammatory Th17/IL-17 dominance in SGR skin compared to SGP. Regarding pro-inflammatory molecules (IL-1α, IL-6, IL-8, IL-33, TNF-α), similarly low levels were detected in both regions. Our data may explain the characteristic topographical localization of some immune-mediated and autoimmune skin disorders and we also propose that the term „healthy skin control sample", widely used in experimental Dermatology, should only be accepted if researchers carefully specify the exact region of the healthy skin (along with the site of the diseased sample).

# INVESTIGATING THE EFFECT OF OMEGA-3 (DHA) AND OMEG-6 (LA) FATTY ACIDS ON THE EXPRESSION OF MICA IMMUNOSTIMULATORY MOLECULE, METASTASIS-RELATED GENES, AND THEIR CONTROLLING MICRORNAS IN METASTATIC BREAST AND GASTRIC CANCER CELL LINES

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Molecular signatures of useful effects of dietary supplements in prevention, controlling and treatment of cancer have not been clearly understood. MICA is expressed upon cellular stress and also in cell malignancies. This molecule is recognized by NKG2D receptor on NK cell, leads to direct killing of MICA-expressing cells by NK cells. Tumor cells can evade from NK-mediated killing in part by down-regulation of the expression of MICA. In parallel, metastasis is one of the most important steps in cancer progression. Several proteins are involved in this process e.g. Matrix metalloproteinases (MMPs) and Talin-2. MicroRNAs have been interested recently as major players in cancer development and progression by acting as tumor-suppressor or -inducer factors. In this study, we aimed to investigate the effects of DHA and LA on the expression of MICA, MMP-2 and Talin-2 genes, and their regulating miRNAs in metastatic breast (MDA-MB-231) and gastric (MKN-45) cell lines. Cells were cultured and treated with 100 uM DHA and 50 uM LA, alone or in combination with each other and also Paclitaxel (3uM) and Docetaxel (18.5 uM) for 24 hours. Alterations in the expression of molecular targets were analyzed by quantitative real-time PCR. Results showed significant down-regulation of MICA and its regulating mir-20a in all treated MDA-MB-231 cells ($p<0.0001$). Treated MKN-45 cells also showed such pattern of expression, except of LA-treated cells. DHA and its combination with Paclitaxel/Docetaxel showed significant down-regulation of MMP-2 ($p<0.05$) in two cell lines. Also, mir-194 showed significant increased expression in all treated cells ($p<0.001$). Collectively, our results showed that DHA and LA both have no beneficial effect to increase the expression level of MICA molecule on cancer cells. However, beneficial anti-metastatic effect of DHA alone or in combination with chemotherapeutic agents was shown either by down-regulation of metastasis-related genes or up-regulation of tumor-suppressor miRNAs.

**CLINICAL USEFULNESS OF AUTOANTIBODIES TO M-TYPE PHOSPHOLIPASE A2 RECEPTOR (PLA2R) FOR DIAGNOSTIC AND MONITORING DISEASE ACTIVITY IN IDIOPATHIC MEMBRANOUS NEPHROPATHY**

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**Objectives** PLA2R is the major target antigen in adult idiopathic membranous nephropathy (iMN). This study aimed to assess the prevalence and specificity of these antibodies (Abs) in a cohort of Algerian patients with iMN and to correlate this Abs with clinical parameters reflecting the disease activity.

**Patients and methods** We measured anti-PLA2R Abs using an immuno-enzymatic assay in the serum of 40 patients with iMN, 09 with secondary MN and 10 with other forms of primary glomerular diseases.

Anti-PLA2R Abs levels were correlated with proteinuria, serum albumin and serum creatinine in patients with iMN. In 6 anti-PLA2R positive iMN patients, Abs levels were assessed at various stages of clinical disease (active, remission) and correlated with disease activity.

**Results** Anti-PLA2R Abs were detected in 57,5% of patients with iMN, but not in those with secondary MN or other forms of primary glomerular diseases. In 24 patients with iMN, proteinuria was >3g/24h at the time of PLA2R Abs measurement. 23 (91,66%) of them were positive for PLA2R Abs, while in 14 patients the proteinuria was < 3g/24h. Among them, only 1 patient was positive for Abs. In patients with iMN, the Abs levels correlated positively with proteinuria and negatively with serum albumin. No correlation was found between Abs and serum creatinine. During the clinical course of the 6 anti-PLA2R positive patients, Abs levels correlated with clinical status, which were high at the initial phase of active disease and decreased during remission.

**Conclusions** anti-PLA2R Abs is a sensitive and specific test for iMN. Abs levels correlate with clinical disease activity, there measurement may provide a tool for monitoring disease activity.

**THE GUT-LUNG AXIS: A TWO–WAY ROAD?**

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It has been shown that host-microbe interactions shape immune responses and their impact is not limited to the local environment. Indeed, changes in the gut microbiome are associated with allergic diseases in the lung, suggesting a vital cross-talk through the gut-lung axis. Here we raised the question, whether this axis is bi-directional.

We tested the impact of ovalbumin (OVA)-induced allergic airway inflammation and orally induced specific tolerance on the composition and function of the intestinal microbiome. First, we established the corresponding mouse models. Next, Fourier-transform infrared spectroscopy (FT-IR) and Illumina MiSeq sequencing of 16S rRNA gene amplicons were used to perform microbial community analysis. Finally, metabolites in feces and serum were analyzed with mass spectrometry (MS).

Compared to allergic mice, orally-tolerized mice exhibited reduced methacholine-induced airway hyperresponsiveness, eosinophilia and decreased levels of OVA-specific Th2 cytokines in the re-stimulated lungs, mediastinal lymph nodes and splenocytes. Cecal samples analyzed with FT-IR indicated shifts in the microbial communities of allergic and tolerized mice compared to naïve controls. Preliminary analysis of 16S rRNA gene sequence data indicated changes in α-diversity and relative abundances of the families *Prevotellaceae, Rikenellaceae* and *Defferibacteraceae*. MS revealed a strong decrease of L-carnitine and its alkylated forms in sensitized mice compared to controls. Next, we aim to evaluate whether

allergic airway disease or tolerance induction can be transferred by the intestinal microbiome to germ-free mice.
The precise characterization of the gut-lung axis, the understanding of the communication between the different mucosal compartments and its impact on shaping the immune system might pave the way to novel intervention strategies for allergic diseases.

**MOLECULAR IMAGING OF THE ANTIGEN RECOGNITION DYNAMICS IN CD8+ CYTOTOXIC T-CELLS**

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Cytolytic T-cells (CTLs) can detect with their low affinity T-cell antigen receptors (TCRs) the presence of even a single antigenic peptide-loaded MHC molecule I (pMHCI) among thousands of structurally related yet non-stimulatory pMHCs (Purbhoo et al. 2004). How they achieve this is not clear but appears to depend at least in part on the special binding conditions within the special constraints of the immunological synapse, the area of contact between a T-cell and an antigen presenting cell. Here receptors and their ligands are not only pre-oriented, but they are often enriched in specific membrane domains and also subjected to cellular forces. To relate these cell biological parameters to T-cell antigen sensitivity in a more comprehensive manner we are monitoring TCR-pMHC binding in nascent synapses with the use of molecular imaging modalities. We confront TCR transgenic CTLs with a glass-supported lipid bilayer (SLB) functionalized with pMHCI, adhesion and costimulatory molecules. This allows us to conduct (single molecule) measurements in noiseattenuated Total Internal Reflection (TIRF) mode, to control for ligand composition and density to quantitate their specific influence on TCR-pMHCI binding and TCR-proximal downstream signaling. We also plan to assess the role of CD8 co-receptor engagement with the use of pMHCI mutants, which are deficient in CD8 binding. In this fashion we expect to gain novel insights into cell biological and molecular processes underlying the phenomenal sensitivity of CTLs towards antigen.

Supported by FWF, CCHD

**ROLE OF SRC FAMILY KINASES ON NEUTROPHIL ACTIVATION AND EFFECTOR FUNCTIONS**

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Neutrophils represent a key component of the innate immune system. They are among the first cells migrating into inflamed tissue and phagocytizing infection agents in order to eliminate infection spreading. Killing of ingested pathogens usually takes place in phagosomes, which fuse with neutrophilic granules containing various serine proteases, myeloperoxidase, lysozyme and many other components toxic to microbes. Src-Family Kinases (SFKs) transduce signals downstream of various immunoreceptors to enable cell activation and thus execution of their effector functions. The activity of SFKs is negatively regulated by phosphorylation of their inhibitory tyrosines by Csk kinase. This effect can be potentiated by artificially targeting Csk to the plasma membrane, resulting in strong inhibition of SFK activity. We decided to use constructs coding for membrane targeted Csk and constructs enriching Csk in granule membranes to investigate a new roles of SFKs in neutrophil activation and effector functions (eg. ROS production, NETosis and bacteria killing). For targeting Csk to the plasma membrane, we generated constructs with Csk fused to transmembrane adaptor proteins LAT and SCIMP or their membrane-anchoring sequences, and fluorescent proteins for verification of membrane localization. We also made similar constructs with Csk fused to PSTPIP2, an adaptor protein containing F-bar domain, which could be recruited to the membrane upon activation. All the constructs were made also in inducible forms in order to avoid effects of SFKs inhibition on neutrophil differentiation and survival. We have developed the method of expressing these constructs in primary murine neutrophils and in a pilot study determined the localization of fluorescent PSTPIP2 construct in these cells. Studies of the remaining constructs are currently ongoing.

# CHARACTERIZATION OF EXOSOMES IN THE DEVELOPMENT OF NOVEL ANTI-CANCER ADVANCED THERAPY MEDICINE PRODUCTS

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**Background**. The immune system is critical in fighting cancer. Dendritic cells (DCs) are the major antigen-presenting cells and play a central role in cancer immunity. But tumors have means of suppressing *in vivo* DCs function. Exosomes are extracellular vesicles acting as mediators in cell-to-cell communication and influencing biological functions in the recipient cells. However, biological roles of tumor secreted exosomes on DCs remain largely unexplored. We hypothesize that tumor-derived exosomes might transfer tumor information which could be recognized *in vitro* by DCs to induce antitumor responses after transplantation. **Aim** of this study was to investigate DC activation properties of exosomes secreted by different tumor cell lines. **Methods and materials**. Exosomes were isolated from murine tumor cell lines B16, LLC and GL261 by ultracentrifugation or sequential filtration methods. Exosomes were identified by flow cytometry and dynamic light scattering analysis. Immature DCs were prepared from murine bone marrow cells. Maturation of DCs pulsed with LPS and tumor cell line secreted exosomes were evaluated by flow cytometry. **Results**. Both isolation protocols yield a population of nanoparticles up to 300 nm in size. Extracellular vesicles affect expression of DC activation markers in a dose-dependent manner. **Conclusions**. Sequential filtration is quick, simple and effective method for isolation of exosomes with the capacity to induce non-inhibitory DCs, thus opening new ways for the development of more efficient anti-cancer advanced therapy medicinal products.

**LACTOFERRIN IS A NATURAL INHIBITOR OF PLASMINOGEN ACTIVATION**

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The serine protease plasmin is harnessed in a wide variety of physiological processes and its activity is tightly regulated. Uncontrolled plasmin contributes to unwanted cell migration in tumor progression. Some virulent bacterial species, e.g. Streptococci or Borrelia, bind human plasminogen and hijack the host's plasminogen system to penetrate tissue barriers. Here, we show that lactoferrin, an iron-binding milk glycoprotein, blocks plasminogen activation on the cell surface through direct binding to plasminogen. We mapped the mutual binding sites into the amino-terminal region of lactoferrin, encompassed in to lactoferricin, and kringle 5 of plasminogen, respectively. Finally, lactoferrin blocks tumor cell invasion in vitro and also plasminogen activation driven by Borrelia. Our results not only explain many of diverse biological properties of lactoferrin, but also identify a novel tool for therapeutic interventions to prevent both invasive malignant cells and virulent bacteria from penetrating host tissues.

*This work was supported by the Austrian Science Fund (grants P19014-B13, P22908 and I00300), the Science and Technology Assistance Agency of the Slovak Republic (APVV-16-0452), the Slovak Grant Agency VEGA (2/0020/17), the European Union Seventh Framework Program (FP7/2007-2013) under grant agreement NMP4-LA-2009-228827 NANOFOL, and the EU structural funds ITMS 26240220008. The research received also funding from the European Union's Horizon 2020 Research and Innovation Program under the grant agreement No 683356 (FOLSMART).*

# XENOTRANSPLANTATION OF HUMAN CANCER CELLS IN ZEBRA FISH: A TOOL TO STUDY AN IN-VIVO METASTASIS PROCESS

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**Introduction**: Cancer is responsible for 13% of all death worldwide and metastases are incriminated in 90% of cancer deaths. The *Danio rerio* model known as zebrafish has a number of advantages and is nowadays a common scientific model used in the cancer field. Indeed, transparent zebrafish embryos do not develop a functional adaptive immune system until about 30 days post fertilization (dpf), making immunosuppression in young embryos unnecessary. However, their innate immunity is detectable and active at 1dpf.
**Objective**: The aim of our project is to develop a sustained zebrafish model for xenotransplantation of human cancer cells to visualize *in vivo* and in real time cancer cells metastasis and the contribution of the zebrafish innate immune system in this process.
**Material and methods:** Zebrafish transgenic lines allowing the visualization of macrophages, neutrophils and vasculature were used. Xenotransplantation of A431 GFP + and MDA-Mb231 GFP+ human cancer cells were done at 48 hours post fertilisation (hpf) in the duct of Cuvier or the brain ventricle. Then, we used a confocal microscope to enable a real-time monitoring of xenografted cancer cells fate.
**Results**: Preliminary results showed that cancer cell lines clinical behavior seems to be preserved in the zebrafish concerning their proliferation, migration and extravasation. Moreover, innate immune cells seem to play a crucial role in the different steps of metastasis process.
**Conclusion**: Deciphering metastasis mechanisms and defining precisely how innate immune cells contribute to this process would allow the discovery of effective anti-metastatic drugs.

**TDB MODULATES MICROGLIA-MEDIATED NEUROINFLAMMATION VIA MINCLE-INDEPENDENT PLC-Γ/PKC/ERK PATHWAY**

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Microglia, the resident macrophages of the central nervous system (CNS), plays an important role in pathogenesis of CNS-tuberculosis (CNS-TB). Trehalose-6,6'-dimycolate (TDM), the mycobacterial cord factor and its synthetic analogue trehalose-6,6'-dibehenate (TDB) is a new adjuvant currently in phase I clinical trials as a tuberculosis subunit vaccine. Both TDM and TDB can activate macrophages and dendritic cells through binding to C-type lectin receptor Mincle; however, it is still unknown their mechanism of actions in microglia and its relationship with neuroinflammation. In this article, we reported that TDB inhibits LPS-induced M1 microglia polarization and promotes M2 phenotype in primary microglia and BV-2 cells. Although TDB itself had no effects on IKK, p38, JNK activity and cytokine expressions. TDB activates ERK1/2 through a PLC-γ1/PKC signaling, in turn decreasing LPS-induced NF-κB nuclear translocation. Furthermore, TDB induces AMPK activation via PLC-γ1/calcium/CaMKKβ-dependent pathway, and thereby enhances M2 gene expressions. Interestingly, knocking out Mincle did not alter the anti-inflammatory and M2 polarization actions of TDB in microglia. In murine neuroinflammation model, TDB suppresses LPS-induced M1 microglia activation, sickness behaviour and promotes M2 microglia polarization in both WT and Mincle$^{-/-}$ mice. Taken together, our results suggested that TDB exhibit anti-inflammatory properties by regulating microglia M1/M2 polarization in neuroinflammatory conditions via Mincle-independent PLC-γ1 signaling.

**CAN DOUBLE SENSITIZATION INFLUENCE FOOD PREFERENCE?**

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Food allergy is an immune-mediated reaction to food antigens, which may be associated with IgE, IgG or a combination of the two. Considered a worldwide problem, the incidence of food allergy has increased significantly in recent decades. Within this context, the development of in vivo models ex vivo, in vitro and in silico etc. as well as the production of systems and instruments can contribute to immunology. Thus, our aim was to develop two models that allow different levels of analysis of food allergy - from in vivo to in silico - and a feeding system for use in biotechnological research. The study for the development of models and the system started with the induction of allergy to two concomitant seeds in mice, followed the submission of a challenging diet. During this period the consumption of the diet components and the weight evaluation were monitored. At the end of the experiment, fecal material and intestinal tissue samples were collected to determine the microbiota profile and intestinal inflammation. Double-sensitization resulted in smaller individual titers for each antigen than in mono-sensitized animals. When submitted to a challenging diet, the mono-sensitized individuals with an option of choice avoid ingesting the respective seed and, while the double-sensitized animals ate both seeds that were allergic. The intestinal alterations demonstrated a lower inflammatory intensity in the double-sensitized ones. The evaluated microbiota presented changes compatible with inflammatory processes of the gastrointestinal tract. This study allowed the final development of an animal model with multiple allergies and a dietary selection evaluation system, whose patent has already been submitted, both of which allow the evaluation of several parameters related to inflammation and allergy.

**STUDY OF INTERACTION BETWEEN HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS AND DOMAIN III OF ENVELOPE PROTEIN OF WEST NILE VIRUS**

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West Nile virus (WNV, genus *Flavivirus*) is a mosquito-borne neurotropic pathogen which causes severe damage of the central nervous system (CNS). How the WNV enters the CNS is not well understood. Domain III (DIII) of the structural envelope (E) protein, the most antigenic protein, is responsible for attachment of the virus to the host cellular receptor. Interaction of WNV through DIII with unknown receptor on hBMEC is likely the main route of WNV entering to the CNS. In this work, we prepared a recombinant DIII (rE-DIII) to confirm its interaction with hBMEC and evaluate its ability to evoke a specific transcriptomic response, which may help WNV to enter into CNS. 6x His tagged rE-DIII was overexpressed in *E. coli* M15. Interaction of rE-DIII (2 μg/well) with hBMEC lysate was confirmed by ELISA using anti-His-HRP, in which the strong interaction was found (RFU rE-DIII 3816; RFU negative control 933). This interaction was then confirmed by immunocytochemistry (70% hBMEC monolayer) with anti-6X His tag® antibody (FITC). rE-DIII was used further for stimulation of hBMEC *in vitro* for 1 hour at 37 °C and 5% $CO_2$. Expression of selected genes (CEACAMs, actin-related protein, adhesins, ILs etc.) were evaluated using qRT-PCR, where β-microglobulin served as housekeeping gene. Among studied genes, CEACAM3, CEACAM6, IL1A, actin-related protein 1 were found upregulated, while CEACAM4 and 20, ICAM-1, IFNA1, NFKB1, PECAM1 and thrombomodulin were downregulated. In our study we acknowledged the interaction of rE-DIII with hBMEC. We also confirmed ability of DIII to induce the cellular response, probably responsible for WNV invasion into CNS. The molecular mechanism of neuroinvasion of WNV is not completely clear yet. Detailed analysis of the cell signaling events is important in complete understanding of mechanisms of WNV entry into CNS.

Acknowledgements: This work was supported by APVV-14-0218 and VEGA 1/0439/18

# MECHANISM OF IMMUNE SYSTEM DYSREGULATION – THE ROLE OF T HELPER CELLS

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**Background:** Common Variable Immunodeficiency (CVID) is a heterogenous group of disorders characterized by impaired immunoglobulin production and dysregulation of immune system manifesting as lymphoproliferative, granulomatous or autoimmune diseases. The changes in B cell development are well described. On the contrary, the role of Tcells have not been entirely revealed yet. Therefore, we initiated this study to characterize T helper cell compartment and its role in the immune system dysregulation. **Methods:** Upon izolation of peripheral blood mononucler cells the first portion was stimulated with ionomycine and Phorbol myristate acetate for 6 hours including protein inhibition by Brefeldin A. We measured expression and production of activation markers (CD69, CD154, HLA-DR), chemokine receptors (CXCR3, CRTH2, CCR6), transcription factors (T-Bet, GATA-3, ROR-gamma) and intracellular cyto**k**ine production (IFN-gamma, IL-5, IL-17) using standard flowcytometric protocols. In the second portion of PBMC T helper (using expression of CD3, CD4, CD8, CD45RO, CCR7 ) and B cells subpopulations were determined (using expresson of CD19, CD21, CD24, CD27, CD38, IgM). All flowcytometric data were statistically analyzed **Results:** We revealed significant differences in the expression of chemokine receptors and transcription factors as well as in the production of intracellular cytokines favouring the T helper 1 immune response. There were also differences in T helper cell compartment with reduction of naive and expansion of mature forms. Moreover, CVID patients had higher expression of HLADR and limited response to stimulation with reduced increase of CD69 expression. **Conclusions:** We revealed disturbed development of T helper cells with increased number of mature forms. Based on expression of activation markers we also found signs of chronic stimulation of immune system and its exhaustion with limited response to stimulation. There is also skewing towards T helper 1 immune response. We assume, all these changes contribute to development of pathological conditions such as autoimmune diseases, where similar changes have been described.

**ASSESSMENT OF ANTI-INFLAMMATORY EFFECT OF β-CARYOPHYLLENE IN HUMAN *IN VITRO* INFLAMMATORY KERATINOCYTE MODEL SYSTEMS**

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Inflammatory cutaneous disorders impair the quality of life of millions worldwide. Dermatitis is a general term for skin inflammation in which the epidermis is affected where the epidermal keratinocytes are the key players of cutaneous homeostasis. β-caryophyllene (BCP) is a natural bicyclic sesquiterpene which is a constituent of several spices and also Cannabis sativa. It is an effective medicine in the treatment of anxiety, depression, arthritis, it can reduce pain, inflammation, it has antimicrobial, antioxidant, neuroprotective and anti-cancer effects. Despite the extensive usage of BCP, its potential anti-inflammatory effects in human keratinocytes are still poorly investigated.

The goal of our study was to assess the potential cutaneous anti-inflammatory effect of BCP in previously optimized *in vitro* epidermal keratinocyte models. We found that the viability of HPV-KER cells was not reduced in any of the applied BCP concentrations. Importantly, however, expressions of certain pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, IL-8) were significantly down-regulated upon the administration of BCP in all models.

Our study provides the first evidence that BCP exerted anti-inflammatory actions on human epidermal HPV-keratinocytes. These intriguing data invite further pre-clinical and clinical studies to exploit the therapeutic potential of BCP in a various cutaneous inflammatory conditions, like dermatitis.

## *IN VITRO* EVALUATION OF IMMUNOMODULATORY PROPERTIES OF PIDOTIMOD

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Pidotimod is a synthetic dipeptide molecule known for its immunomodulatory properties. According to literature, biological activity of pidotimod is focused both on innate and adaptive immune responses. Several experimental evidences postulate immuno-stimulatory role of pidotimod but still the mechanism is not well clarified. Clinical studies show beneficial effect of pidotimod when associated to antibiotics on children suffering with acute and recurrent respiratory tract infection, and also in preventive measure with reduction of the number of recurrences, reducing intensity of sign and symptoms of disease. Since pidotimod appears to modulate the immune system, we hypothesize possible beneficial effects as an enhancer of anticancer response. Aim of this study is to evaluate role of pidotimod using *in vitro* and *in vivo* models. In *in vitro* model BALB/c mice splenocytes activated with lipopolysaccharides followed by 100µg/ml pidotimod for twelve hours, to evaluate the mechanism behind the stimulation and/or inhibition of immune cells by the molecule. *In vivo* model will be evaluating if there are changes in the tumour (B16 melanoma and CT26 colorectal carcinoma) behavior e.g. rate of growth, dimension of tumour in BALB/c mice, and differences in immunity between control and animals treated with pidotimod will be assessed by monitoring cytokine production, variation in subpopulation of immune cells, cytotoxicity, resistance of immune cells to apoptosis. Preliminary results of *in vitro* treatment of splenocytes with pidotimod shows upregulation of $CD3^{+}CD4^{+}$ and $CD3^{-}NKP46^{+}$ cells expressing CD69 marker.

**PLATINUM SENSITIVITY OF OVARIAN CANCER CELLS DOES NOT INFLUENCE THEIR ABILITY TO INDUCE M2-TYPE MACROPHAGES**

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Development of platinum resistance in ovarian cancer is mediated by both cancer cells and tumor microenvironment. Activation of epithelial-mesenchymal transition program in cancer cells may lead to enrichment for resistant clones. These processes can be affected by tumor-associated macrophages, a highly plastic population of cells that participate in tumor progression and response to treatment by shaping the microenvironment. We aimed to study how platinum resistance influences the crosstalk between macrophages and ovarian cancer cells. Using cisplatin-sensitive ovarian cancer cell line A2780, we developed and characterized cisplatin-resistant A2780Cis and cisplatin and doxorubicin co-resistant A2780Dox cell lines. Next, we set up an indirect co-culture system with THP-1 cell line-derived M0-type-, M1-type- and M2-type-like polarized macrophages. We monitored the expression of genes associated with cellular stemness, multidrug resistance, and epithelial-mesenchymal transition in cancer cells, and expression profile of M1/M2 markers in macrophages. Development of drug resistance in ovarian cancer cell lines was accompanied by increased migration, clonogenicity and upregulated expression of transcription factors, associated with cellular stemness and epithelial-mesenchymal transition. Upon co-culture, we noted that the most relevant changes in gene expression profile occurred in A2780 cells. Moreover, M0- and M1-type macrophages, but not M2-type macrophages, showed significant transcriptional alterations. Our results provide the evidence for bidirectional interplay between cancer cells and macrophages. Independent of platinum resistance status, ovarian cancer cells polarize macrophages towards M2-like type, whereas macrophages induce epithelial-mesenchymal transition and stemness-related gene expression profile in cisplatin-sensitive, but not cisplatin-resistant cancer cells.

# MICROGLIA CELLS LOCALIZATION DETERMINE THE CONTENT OF RELEASED EXOSOMES AND THEIR BIOLOGICAL FUNCTION

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Microglia cells represent 5-10% of total central nervous system (CNS) cells. They are derived from hematopoietic stem cells present in the yolk sac at E7.5-E8, then migrate into the early brain at stage E9.5 and become resident cells through life. The study aim is to understand whether the microglia cells located in different areas of CNS undergo the same maturation processes.

Using proteomics analyses we demonstrated that microglia cells issued from two sources (cortex and spinal cord) did not express the same phenotype i.e. anti-inflammatory and neurogenesis/tumorigenesis profile for cortex microglia and inflammatory profile, involved in injury mechanisms, for spinal cord microglia. We confirmed these results by performing proteomic analyses and biological tests for microglia derived exosomes. Using neurites sprouting and outgrowth assays, as well as 3D spheroids cultures for glioma proliferation analysis, we established that the microglia exosomes effect is different, in correlation with microglia cells source. Furthermore, exosomes of spinal cord microglia cells, treated with lipopolysaccharide, inhibit tumor proliferation and increased neurites sprouting, whereas the ones from cortex microglia promote glioma sprouting and stimulate neurites outgrowth. Cortex microglia exosomes possess markers of their neonatal nature by presenting neuroectodermal markers (Spp1, Lgals1, Gpx1) associated with other proteins involved in brain development such like metalloproteases (Mmp2 and Mmp9). By contrast some of these factors are absent in spinal cord microglia exosomes like NOV, Lgals1, Vim.

Taken together, we established that microglia present in spinal cord reveal a phenotype different to the one in cortex. The results can lead to new therapeutic strategy based on microglia cells exosomes content.

Supported by APVV-15-0613 (DC), Stefanik (MS), SK-FR-2015-0018 (DC), ERANET- AxonRepair.

**NANOCAPSULES OF TARIN, A LECTIN PURIFIED FROM TARO (COLOCASIA ESCULENTA) ENCAPSULATED IN LIPOSOMES: DESIGN OF A NUTRACEUTICAL WITH ANTITUMORAL AND IMMUNOMODULATORY ACTIVITIES**

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Tarin (TR), a mitogenic lectin purified from taro corms exhibits immunomodulatory and antitumoral activities, which makes it a novel therapeutic candidate. TR was loaded into the aqueous core of small unilamellar vesicles from hydrated-phospholipid films resulting in encapsulation of TR with efficiency superior to 80%. Scanning electron microscope showed production of round-shaped vesicles. The average diameter of nanocapsules estimated by dynamic light scattering was around 150nm and the estimated polydispersity index close to 0.1 evidenced vesicles with homogeneous size. Liposomes were stable for 6 months under storage at 4°C. At pH ranging from 4.6 to 7.4 and 36°C, TR release obeyed an exponential kinetic achieving 50 to 84% of release after 6 h. Encapsulated TR exhibited no *in vitro* cytotoxic effects against mice bone marrow cells. Optical microscopy images from cells, in the presence of free or encapsulated TR (20µg/mL), displayed large, elongated cells with distinct morphology from untreated cells. Flow cytometry revealed a transitory enhancement in erythroid progenitors (TER119) after 7 days of tarin addition. 50µg/mL of encapsulated TR inhibited 65% and 41% of human glioblastoma cells (U87MG) and human breast adenocarcinoma (MDAMD231) growth, respectively. Administration of encapsulated or free TR (5mg/kg) to C57BL/6 mice increased the number of peritoneal cells after 3 and 5 days of treatment and bone marrow cells after 5 days. No change in peripheral leukocyte counts was observed. Encapsulated TR exhibited superior pharmacological activities when compared to free TR and may be applicable as a nutraceutical or an adjuvant medicine associated to chemotherapeutic drugs

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**_ACE_ I/D POLYMORPHISM AND ALZHEIMER´S DISEASE IN SLOVAK POPULATION**

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An insertion (I) / deletion (D) polymorphism in the gene for angiotensin 1-converting enzyme (ACE) that can interact with amyloid β, may influence the onset and course of Alzheimer's dementia (AD). Plasma ACE levels may vary depending on the genotype. There are ethnic differences in the distribution of *ACE* alleles and also differences between AD patients and controls. In the majority of studies, the allele I was increased in AD. In the publication from Slovakia (patients from Eastern Slovakia) this association was not confirmed. Contrary, in *APOE**Ɛ4 individuals, the genotype *ACE* DD was a supporting factor for AD in patients compared to controls. The aim of our study was to compare frequencies of *ACE* I/D polymorphisms in AD patients and controls in the Slovak population. The group consisted of 61 AD patients (mean age: 80 years, male/female ratio 43:18) and 99 controls without dementia (mean age: 73 years, female/male ratio 62:37), all from Slovakia. For identification *ACE* I/D alleles PCR was used. The results were analyzed by the $\chi 2$ test. The frequencies in the AD group were: allele I 44.26%, D 55.74%, genotype II 18%, ID 52.46%, DD 29.51% and in the controls: allele I 52.02%, D 47.98%, genotype II 26.26%, ID 51.51%, DD 22.2%. The differences were not statistically significant, but there was higher frequency of D allele in patients. The plan is to extend the data and to study the interactions of *ACE* with the other genes and clinical parameters in AD. Grant support: VEGA 1/0240/16

**FEEDING NOVEL PROTEINS DURING THE ACUTE PHASE OF AN INFLAMMATORY BOWEL DISEASE**

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INTRODUCTION: Food consumption is one of the main activities of human life and the choices or eating habits directly affect our health. Studies from our group showed that the introduction of a new food in an intestinal inflammatory context results in sensitization to this food. Thus, our aim was to evaluate the kinetics of induction of oral tolerance or allergy to new food proteins in the acute phase in the specific antigen intestinal inflammation. METHODS AND RESULTS: Male mice C57BL / 6 will be divided into 10 groups (n = 10 / group). For the induction of allergy all animals (excluding the negative control group) will receive two doses of 100 µg of percutaneous protein peanut extract, with 21 days apart, with the addition of adjuvant only in the primary. All groups will receive peanuts *ad libitum* for 40 days (challenge diet-CD) for the intestinal inflammation induction, except the negative control group, which will continue to receive the commercial chow. As the new protein, we use fresh egg white diluted 1: 5 v / v in distilled water, which will be offered 48h, 24h, 12h and 06h before and after the start of the diet challenge We will also offer co-OVA or saline to the beginning of the P-CD. Later, all animals (except the negative control) will receive two doses of 100µg percutaneous ovalbumin 5X (OVA), 21 days apart, with the addition of adjuvant only in primary followed by O-CD (egg white chow) until the end of the experiment. We evaluate: body weight, food consumption, IgG titers (total) OVA and anti peanuts, duodenum histomorphometry and lymphocyte profile T and B (LT and LB) spleen and mesenteric lymph nodes (LM) and also duodenum immunohistochemistry and RT-PCR. This study was submitted to the CEUA. No significant differences were observed in food consumption between the groups. During the P-CD period the animals within each group lost significant weight when compared to the beginning of the P-CD ($p<0.05$). Anti OVA IgG titers were analyzed by ELISA and the co-OVA diet group showed significant

higher IgG titers when compared to the others groups. Preliminary histomorphometric analysis showed normal characteristics of the animals of the 6h Antes group and inflammatory characteristics in the Positive Control group. CONCLUSION: New proteins introduced concomitant to an allergenic protein are able to develop of multiple allergies; Administration of a new food protein in a physiological context of the gut mucosa generates tolerance for this new protein.

# MHCII ARE MONOMERIC, HIGHLY MOBILE AND RANDOMLY DISTRIBUTED FOR SENSITIZED T-CELL DETECTION

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It is still unclear how T-cells manage to efficiently detect even a single agonist peptide/MHC (pMHC) among millions of structurally similar endogenous pMHCs displayed on the surface of professional antigen presenting cells (APCs), especially when considering the moderate affinity of T-cell antigen receptors (TCRs) for nominal pMHCs. Despite lack of direct evidence, pMHC enrichment and oligomerization in nanoscopic membrane domains as well as co-agonism mediated by endogenous pMHCs interacting with bystander TCRs have been regarded critical for sensitized T-cell antigen recognition. We have applied molecular live cell microscopy to investigate this in detail and found pMHCs on the surface of professional APCs (i) at high densities (200 – 2000 molecules per $\mu m^2$), (ii) monomeric, (iii) randomly distributed and (iv) rapidly diffusing ($D = 0.2 – 0.6\ \mu m^2\ s^{-1}$) even when newly released from internal transport vesicles. Quantitative FRET-based measurements of synaptic binding between TCR and endogenous pMHCs failed to detect meaningful interactions, consistent with our observation that such ligands neither affected synaptic TCR-engagement of rare agonist pMHCs nor the ensuing T-cell response both at early and later stages of synapse formation. Combined, our findings render pMHC clustering and co-agonism through endogenous pMHCs unnecessary for sensitized antigen detection by scanning T-cells. Instead, they highlight the capacity of individual agonist pMHCs to elicit the full response of scanning T-cells in an autonomous fashion, which is facilitated by fast redistribution of newly arriving pMHCs on the APC surface.

# NEUTROPHILS PROMOTE T-CELL-MEDIATED INFLAMMATION IN ALLERGY

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Lately we identified HLA-DR-positive neutrophils in allergen-induced cutaneous late phase reactions (LPR) in vivo. Moreover, we demonstrated in vitro that allergen-pulsed neutrophils from allergic individuals that had been activated with cytokines present in the allergic LPR were capable of inducing proliferative and cytokine responses of HLA-DR-restricted allergen-specific CD4+ T cells. Notably, HLA-DR-positive neutrophils did not express CD80 and CD86 which represent primary ligands for CD28. CD28 is relevant for optimal activation and clonal expansion of CD4+ T lymphocytes. T cell receptor (TCR) signaling in the absence of CD28-costimulation results in clonal anergy. Anergic T-cells poorly proliferate to subsequent TCR stimulation even in the presence of costimulation. Here we studied whether neutrophils induced clonal anergy in allergen-specific T cells.

Allergen-specific T cells were at first stimulated with either allergen-pulsed autologous HLA-DR-positive neutrophils or PBMC or synthetic peptides containing their epitopes. When re-stimulated with PBMC plus allergen, T cell cultures that had been primarily stimulated with peptide poorly proliferated. In contrast, T cells stimulated with neutrophils plus allergen demonstrated similar responses to allergen-specific re-challenge as those initially exposed to PBMC plus allergen. To elucidate the cause for the non-induction of anergy, the expression of various costimulatory molecules on the surface of neutrophils was assessed. They constitutively expressed CD58. The addition of anti-CD58 antibodies resulted in a markedly reduced proliferative response of T cells to specific stimulation by neutrophils. Together, these results indicate that neutrophils engage CD58 for productive T-cell activation which might amplify T-cell-mediated allergic inflammation. The latter was confirmed in a chimeric human/mouse model of birch pollen allergy. We conclude that neutrophils are relevant amplifiers of T cell-mediated inflammation in allergic diseases.

**COMPARISON OF HLA-G AND MMP TRANSCRIPTION IN HUMAN TUMOR CELL LINES**

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HLA-G antigens and matrix metalloproteinases (MMPs) are expressed in various tumors and both can be involved in tumor growth and metastasis. It was observed, that in some ovarian carcinoma cell lines HLA-G expression may induce transcription of MMP15 and increase cell migration. The main goal of our work was to establish whether in other tumor cell lines of different origin exist correlation between HLA-G and MMP transcription.
We examined relationship between transcription of HLA-G and MMPs in choriocarcinoma and leukemia cell lines. It was found that choriocarcinoma cell lines JEG-3 and JAR express similar panel of MMPs except MMP12. Transcript MMP12 was exclusively detected in HLA-G expressing JEG-3 cells but lacking in HLA-G deficient JAR cells. Induction of HLA-G expression in JAR cells (by 5-aza-2´-deoxycytidine - AZA) had no effect on MMP12 transcription. Relationship between HLA-G and MMP transcription was investigated in human leukemia cell lines. Leukemia cells (lacking HLA-G expression) were converted to their HLA-G positive counterparts by AZA-treatment or by HLA-G transfection. In all examined leukemia cell lines we did not find any correlation between HLA-G and MMP transcription.

**SELECTION OF NOVEL MIMOTOPE AS POTENTIAL LYME DISEASE DIAGNOSTIC MARKER**

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Lyme disease (LD) is caused by *Borrelia burgdorferi* sensu lato complex that is widely distributed in Europe. Early diagnosis and treatment of infected individuals is important to limit serious damage to the nervous and musculoskeletal systems. Currently available test kits for detection of LD using borrelial cell extracts or recombinant proteins to capture immunoglobulin M and G antibodies in patients' serum commonly lead to false positive results. Thus, selection of short peptide fragments detectable by *Borrelia*-specific antibodies is highly needed. The objective of this study was to identify mimotopes of neuroinvasive *Borrelia bavariensis* strain SKT-7.1 using combinatorial phage display library (Ph.D.-C7C). Various overexpressed borrelial immunodominant antigens in *Escherichia coli* were tested for their binding affinity to antibodies from human sera. Selected LD negative sera were immobilized on Protein G/A-magnetic beads and pre-incubated with phage library. Unbound phages were panned against beads-serum from patient with neuroborreliosis mixture. Additional negative selection using serum from patient with LD arthritis and excessive washing steps were carried out. Random 300 phage clones were screened using phage ELISA and phage Dot blot against sera from healthy subjects. Based on the specific RFU signals phage clones were selected for next round of the phage ELISA against sera from Lyme arthritis, neuroborreliosis and from patients showing various neurological symptoms (but not Lyme disease). Selected specific mimotopes were also sequenced and derived peptides were analyzed to exclude non-target related peptides using SAROTUP 2.0 server and visualized in three-dimensional structures with the help of PEP-FOLD3 program. Finally, a unique 7-mer cyclic peptide (C**RGGGETA**C, note that cysteine flanking enables intramolecular disulphide bridge and thus cyclic nature of peptide) was selected by phage Dot blot that minimum nonspecific signals with sera from healthy individuals

(RFU 0, relative fluorescence unit), Lyme arthritis (RFU -504) and from patients showing various neurological symptoms (RFU -1,800) when compared with serum from neuroborreliosis (RFU 12,100) using Odyssey CLx (LI-COR). This mimotope can be suitable candidate to differentiate neuroborreliosis from other forms of Lyme disease and antibodies from patients with other neurological disorders.

Acknowledgements: This work was supported by APVV-14-0218 and VEGA 1/0258/15.

**ANALYSIS OF THE IMMUNE RESPONSE ASSOCIATED WITH LOW DOSE ORAL ALLERGEN EXPOSURE IN AN EXPERIMENTAL MODEL**

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Background: It is widely accepted that oral exposure to food antigens is primarily resulting in prevention of food allergy by tolerance development. However, even to date mechanistic knowledge remains limited and has been mainly evaluated after single or repeated high dose antigen exposure in experimental models. Thus, we aimed at evaluating the immunological changes during oral tolerance induction in mice after oral exposure to low amounts of food proteins, which were previously described to be associated with food allergy development.

Method and results: For oral tolerance induction 24 mice were fed daily with 200µg of the model allergen ovalbumin (OVA) for 2 weeks. Immediately afterwards, one group (n=8) was sacrificed for evaluating the OVA-specific local and systemic immune response. Two groups were subsequently either subjected to food allergy induction by OVA feeding under gastric acid suppression or received repeated cycles of oral OVA gavages. All measured values were compared to the background levels determined in naïve controls. After 14 days of oral OVA feeding we observed a significantly elevated local OVA-specific IgA production measured in intestinal lavages and higher percentage of Foxp3 positive CD4 T cells. Even though the mice were protected from development of OVA-specific IgE and IgG levels after OVA feeding under gastric acid suppression, we measured significantly elevated OVA-specific IgG1 and IgG2a levels after exclusive oral OVA gavages.

Conclusion: Oral low dose exposure to food allergens is associated with profound induction of oral tolerance and changes in local and systemic immune response in an experimental model.

The work was supported by the BMWFW-AWS PRIZE Project P1621673.

**LYMPHOCYTE-SPECIFIC TYROSINE-PROTEIN KINASE LCK HOMO-DIMERS IN THE COMPLEX CONTROL OF T-CELL RECEPTOR SIGNALING**

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Engagement of the T-cell antigen receptor (TCR) initiates a signaling cascade resulting in T-cell activation, proliferation and differentiation. Intracellular lymphocyte-specific kinase (Lck) plays a pivotal role in this process, transducing initial TCR/CD3 stimulation into tyrosine phosphorylation, calcium fluxing, synapse formation, and altered gene expression. Lck activity is regulated on multiple intercalated levels, including its subcellular localization (by transporters), 2D nano-domain distribution within the plasma membrane, and its phosphorylation status that is directly linked to its enzymatic activity. A potential mechanism of Lck regulation not investigated so far is its homo-dimerization. Noteworthy, ligand-induced homo-association followed by trans-activatory auto-phosphorylation is an established principle for transmembrane receptor tyrosine kinases. Employing a super-resolution imaging technique - Thinning Out Clusters while Conserving the Stoichiometry of Labeling - we identified a significant amount of Lck dimers in living T-cells. Furthermore, homo-association of membrane-anchored Lck was confirmed by co-immunoprecipitation. To investigate the role of Lck homo-dimers in T-cell signaling, we established an inducible Lck-dimerization system in human Jurkat T-cells after CRISPR/Cas9 knock-out of endogenous Lck. Controlled and specific dimerization of Lck by a membrane-permeable X-linking agent significantly altered its phospho-status and enzymatic activity in a titratable fashion, modulating early as well as late TCR signaling events. In conclusion, homo-dimerization of Lck represents a novel regulatory mechanism controlling Lck kinase activity and thus stimulatory thresholds for T-cell activation.

# SECRETED ASPARTIC PROTEASES FROM *CANDIDA PARAPSILOSIS* REGULATE INNATE IMMUNE EVASION AND INFLAMMASION

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*Candida parapsilosis* is an opportunistic fungal pathogen responsible for approximately 30% of candidaemia episodes in new-borns, and 10%–15% of *Candida* infections in adults worldwide. Fungal extracellular hydrolytic enzymes, especially secreted aspartyl proteinases (SAP) are important virulence factors of *Candida* species, that contribute to the development of disseminated candidiasis. SAPs cause **significant damage to epithelial cells, promote the escape of fungal cells during host attacks by cleaving host complement proteins, modulating inflammasome activation and also the secretion of inflammatory cytokines from imune cells.** Although, several studies have investigated the role of *C. albicans* secreted aspartyl proteinases in its virulence, less is known about the precise role of *C. parapsilosis* secreted aspartyl proteinases (*SAPP*s) in host invasion, especially during complement evasion. Thus, we aimed to study the function of *C. parapsilosis* secreted aspartyl proteinases during host attack.

To investigate the role of *SAPP* genes, a deletion mutant strain (*sapp*$^{-/-}$) was generated, lacking all functional *CpSAPP* ORFs. To examine the effect of complement proteins on the growth of the *C. parapsilosis* GA1 (wild-type) and *sapp*$^{-/-}$ strains, their growth was measured in YPD and YCB liquid medium supplemented with 20% of either normal human plasma (NHP) or heat-inactivated human plasma (HiP) at 30 and 37$^0$C. In order to examine the effect of gene deletion on viability and appearance, we also tested their growth on different complex, minimal and stressor containing media.Virulence properties of the mentioned strains were also tested using an epithelial cell line, the human monocytic cell line THP-1 and human macrophages (PBMC-DMs). *In silico* analyses were also performed to evaluate efficiencies of HIV protease inhibitors against Sapp's.

Our results clearly indicated similar growth rates of the *sapp*$^{-/-}$ strain and the wild-type strain in both YPD and YCB medium regardless of the temperature used, whereas addition of NHP significantly inhibited the growth of the mutant strain only. Deletion of *CpSAPPs* neither affect biofilm formation, nor the morphological attributes of *C. parapsilosis.*

Although, the *sapp*$^{-/-}$ strain showed significant differences in its virulence when compared to the wild-type. THP-1 and PBMCDM's killed and phagocytosed *sapp*$^{-/-}$ cells more efficiently. Mutant cells also induced less damage to human epithelial cells, THP-1 cells and to PBMC-DMs compared to the wild-type strain. Furthermore, when examining host cytokine responses, **the wild-type strain induced higher levels of IL-1β, TNF-α and IL-8 than the** *sapp*$^{-/-}$ mutant strain indicates SAPP mediated immune modulation during C. parapsilosis infection.

To summarize, our preliminary data suggest that *CpSAPPs* do not effect fungal viability, morphology or biofilm formation, however significantly contribute to the virulence of *C. parapsilosis in vitro*, thus play an important role in *C. parapsilosis* virulence.

**EFFECTS OF PSEUROTIN ALKALOIDS ON POLYMORPHONUCLEAR NEUTROPHILS**

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Pseurotin A is a secondary metabolite produced by many species of fungi, mainly by *Aspergillus sp.* and *Penicillium sp*. During the pseurotin A biosynthesis, a large number of closely related bioactive compounds, such a pseurotin D or synerazol are also formed. Natural pseurotins have antimicrobial and antiparasitic activity. Interestingly a few studies suggested effects of pseurotins in eukaryotes e.g. antiangiogenic activity in chick chorioallantoic membrane assay. In this study, we focused on effects of natural pseurotins A and D on physiological functions of polymorphonuclear neutrophils (PMNL), the most abundant leukocytes in human blood. The effects of pseurotins on control and phorbol 12-myristate 13-acetate, interleukin-6, or tumor necrosis factor-alpha activated PMNL was tested based on flowcytometric determination of surface receptors CD11b and CD66b, oxidative burst determination by luminol- enhanced chemiluminiscence (CL), and release of interleukin 6, myeloperoxidase, and elastase determined by ELISA. Interestingly, we show inhibition of both CD11b and CD66b expression induced by selected activators. Based on CL determination of oxidative burst, pseurotins induced limited, but significant activation of PMNL. On the other hand, pseurotins revealed inhibitory effects on oxidative burst induced in some combinations with selected activators. Other tested effects were also dependent on particular combination of natural pseurotin and used activator. It can be concluded that natural pseurotins are able to modulate PMNL functions and their response to activators.

**BLOOD AND SYNOVIAL CD8$^+$ T CELL IN RHEUMATOID ARTHRITIS RELY ON THE WARBURG-EFFECT TO MAINTAIN THEIR CHRONIC INFLAMMATORY PROFILE**

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In autoimmune diseases like rheumatoid arthritis (RA), the resolution phase of inflammation does not occur since autoantigens and endogenous stimuli are permanently reactivating the immune system. CD8$^+$ T cells contribute to RA by releasing pro-inflammatory and cytotoxic mediators, even in a challenging hypoxic and nutrient-poor microenvironment like the chronically inflamed synovial membrane. Here we characterize the metabolic profile underlying the chronic effector phenotype of CD8$^+$ T cells in RA. All peripheral blood and synovial membrane RA CD8$^+$ T cell subsets (naïve, memory and effector) relied on aerobic glycolysis to meet their metabolic demands with consequent massive lactate production and overexpression of Warburg-effect-linked enzymes (HK2, PKM2, HIF-1α and LDHA). The relevance of the Warburg-effect was shown by the reduction of pro-inflammatory CD8$^+$ T cell effector functions in the presence of specific glycolytic inhibitors. Moreover, metabolically restrained RA CD8$^+$ T cells lost their capacity to induce healthy B cells and non-inflammatory synovial fibroblasts to develop an activated pro-inflammatory phenotype. This glycolytic hallmark of RA CD8$^+$ T cell metabolism was used as a biomarker to distinguish RA (even with negative serological parameters) from other chronic inflammatory arthritic diseases (psoriatic arthritis and spondyloarthritis). Thus, targeting the glycolytic CD8$^+$ T cell metabolism can lead to new therapeutic and diagnostic strategies for RA.

# SIMULTANEOUS INHIBITION OF WNT/β-CATENIN SIGNALING IN LNCAP PROSTATE CANCER CELLS AND PROSTATE CANCER PATIENT′S LYMPHOCYTES BY XAV939 ALLOWS A SUSTAINED LYMPHOCYTE-MEDIATED ELIMINATION OF THE CANCER CELLS

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Wnt/β-catenin signaling plays an important role in pathogenesis of several cancers and regulation of immune cell development. Whereas in cancer cells its upregulation is associated with increased cancer cell resistance, in immune cells it is associated with either immunosupression or immunostimulation. What impact an inhibition of this signaling may have on immune cell-mediated elimination of cancer cells is therefore largely unknown. Here we show that XAV939-mediated inhibition of Wnt/β-catenin signaling in both LNCaP prostate cancer (PCa) cells and PCa patient′s lymphocytes is necessary for a sustained lymphocyte-mediated elimination of cancer cells. Using fluorescent protein(TagFP635)-transfected LNCaP cells to monitor their numbers in culture, we found that 5 μM Wnt/β-catenin signaling inhibitor XAV939 did not affect their proliferation but yet still inhibited the signaling of both LNCaP cells and the lymphocytes by 50 % as determined by the inhibion of β-catenin translocation to the nucleus. When co-culturing LNCaP cells with the lymphocytes we revealed that 2-day pre-treatment of the lymphocytes with 5 μM XAV939 accelerated elimination of LNCaP cells by 54 % after 3-day co-culturing. No significant acceleration was observed when LNCaP cells were pretreated with the inhibitor or the inhibitor was present in the co-cultures. Following a transfer of 5-day co-cultures to fresh LNCaP cells we found that, regardless of the XA939 pretreatments, the lymphocyte-mediated elimination of LNCaP cells ceased and only their expansion was abrogated. However, the co-cultures with the presence of XAV939 still showed a sustained elimination of LNCaP cells leading to elimination of 86

% of LNCaP cells in 10 days. In addition, this elimination was associated with 61 % decrease of $PD1^{+}CD8^{+}$ T cell population in the co-culture. In summary, our data indicate that whereas a short-term inhibition of Wnt/β-catenin signaling in lymphocytes licenses these cells to mediate an accelerated elimination of cancer cells, a continuous inhibition of the signaling in both the lymphocytes and the cancer cells is necessary to allow a sustained lymphocyte-mediated cancer cell elimination.

**THE ROLE OF IRF7 IN CELLS DIRECTING THYMOCYTE DEVELOPMENT**

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Interferon regulatory factor 7 (Irf7) has been known to regulate type I Ifn gene transcription in lymphoid tissues in response to viral nucleic acids. It has also been shown to regulate the differentiation and function of several immune cell populations. Despite being thoroughly studied in the peripheral immune system, not much is known about Irf7 function in the thymus. Irf7 has been shown to be expressed by thymic epithelial cells (TEC) and is required for the constitutive *Ifnβ*–expression by medullary TEC (mTEC), maintenance of thymic architecture and mTEC differentiation. However, the effect of *Irf7* and *Ifnβ* expression on TEC function and phenotype is incompletely understood. To delineate the role of Irf7 in thymic epithelium, we analyzed the thymi of WT and $Irf7^{-/-}$ mice. Our flow cytometric (FC) analysis confirmed the previously identified increase in cortical TEC in $Irf7^{-/-}$ mice thymi. However, we found no disturbances in the mature mTEC compartment either by FC or immunofluorescence. Our qPCR analysis of FACS-sorted thymic stromal cells revealed a decrease in the expression of chemokines directing early thymocyte development, but FC analysis of both early and late stage thymocytes did not show any differences between WT and $Irf7^{-/-}$ mice. We identified dendritic cells to be another major source of *Ifnβ* in the thymus, however the expression level of *Ifnβ* did not alter in $Irf7^{-/-}$ TEC or dendritic cells. Hence, by our analysis Irf7 is dispensable for TEC function and thymic *Ifnβ* expression. The role of the constitutive *Ifnβ* expression in thymic stroma remains elusive and requires the use of mice deficient in this gene.

# THE EFFECT OF *E. COLI* O83:K24:H34 ON HUMAN AND MURINE DENDRITIC CELLS.

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**A** balanced microbiome is greatly beneficial for a host. Any disturbance is associated with various problems and diseases, herefore, a number of experiments focus on correction for dysbiosis by probiotic supplementation. One of the promising probiotics appears to be *Escherichia coli* O83:K24:H31 ( *E. coli* O83) but the exact mechanism of its positive effect has not yet described. To uncover it, we focused on differences between human and murine dendritic cells (DCs) after *E. coli* O83 stimulation. Both human and murine DC-like cells were obtained by *in vitro* differentiation from progenitor cells in the presence of GM-CSF and then stimulated with probiotics and LPS for 24 hours. The appearance of DC surface markers was analysed by flow cytometry and the relative gene expression (qPCR) and secretion (ELISA) of various cytokines and enzymes were tested. The expression of DC activation markers D80, CD86 and MHCII was significantly increased after stimulation by *E. coli* O83, indicating that DC-like cells are able to engulf and process probiotic antigens and reflecting their maturation state.
Besides that, primed human DC-like cells produce higher amounts of IL-10 cytokine and express indole 2,3-dioxygenase enzyme. A slightly different mechanism occurs in the murine cells expressing also inducible nitric oxide synthase, typical for activated macrophages. Taken together, results suggest that the positive effect of *E. coli* O83 is mediated by the reinforcement of tolerogenic DCs, thus supporting T regulatory cells which play a critical role in the induction of tolerance to self antigens and also to components of the microbiome.

**RELATIONSHIP BETWEEN GYLCOSYLATION AND GLUCOSE UPTAKE IN B CELLS OF CLL PATIENTS**

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Cancer cells have to reprogram their energy metabolism to gain functions which support the maintenance of proliferation. Tumors exhibit alterations in glucose uptake and utilization which means that the metabolism of the cells shifts towards glycolysis even when oxygen supply is not limited and mitochondrial pathways are functioning. To satisfy the increased demand for nutrients, cancer cells show higher glucose uptake. Therefore, most cancers upregulate glucose transporters. Genes encoding the elements of the glycolytic pathway are overexpressed in hematopoietic malignancies as well. Metabolic pathways and signaling networks are connected at several points. Glycosylation can be considered as a path of crosstalk between these networks. Immuno- and tumor metabolism research from the last few years suggests that glycosylation acts as an intracellular nutrient sensor. Differences between the glycosylation level of normal and malignant cells have already been reported in chronic lymphocytic leukemia (CLL).
In this study we established a novel protocol for measuring glycosylation by flow cytometry. Different permeabilisation methods were tested on peripheral blood mononuclear cells (PBMC) to get the best intensity with anti O-glycosylation antibody, clone RL2. Results were compared with laser scanning microscope as well. In a second panel, we measured the glucose uptake in PBMC with a fluorescent glucose analog, 2NBDG. In our experiments the relationship between the overall level of O-linked glycosylation and glucose uptake in B cells of CLL patients and healthy individuals was investigated. Results were interpreted taking the fasting glucose, insulin and hemoglobin A1c levels.

# STUDY OF INTERACTION OF SURFACE PROTEINS OF *Borrelia bavariensis* WITH HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS

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Lyme borreliosis is one of the most common tick-borne diseases in the Northern hemisphere caused by *Borrelia* species. *Borrelia bavariensis* is one of the five borrelial species distributed in Europe able to cause neuroborreliosis, the most serious clinical manifestation of Lyme borreliosis. Borrelial outer surface proteins can interact with the receptors expressed on brain microvascular endothelial cells (BMECs) and trigger pathological processes to cross the blood brain barrier and enter into the CNS. Thus the aim of this study was to select potential ligands of *Borrelia bavariensis* and confirm their interactions to the receptors on human BMEC. In this study we selected and produced 6 outer-exposed proteins of *Borrelia bavariensis* (LysM domain protein, (BGAPBR_0326); Bacterial extracellular solute-binding protein family 5, (BGAPBR_0334); Antigen S1, (BGAPBR_A0008); Complement regulator acquiring protein 1-CRAP, (BGAPBR_A0071); Erp23 protein, (BGAPBR_Q0067); Lipoprotein, (BGAPBR_V0029) in recombinant form with GFP tag. Interaction between recombinant ligands and protein extract of human BMECs was assessed using a direct ELISA. Ligands of *B. bavariensis* bound to human BMEC were detected by incubation with anti-GFP antibody conjugated with HRP. We also confirm interaction of ligands with live cells of hBMEC, using immunocytochemistry. In immunocytochemistry, ligands were incubated with BMECs for one hour, unbound proteins were washed and cells were fixed with 4% paraformaldehyde. Bound proteins were visualized with anti-His antibody conjugated with FITC and fluorescence microscopy. These selected proteins can be candidates for development antibodies to inhibit pathological processes into host.

Acknowledgements: This work was supported by APVV-14-0218 and VEGA 1/0258/15.

**THE IMPACT OF PRENATAL STRESS AND VENLAFAXINE TREATMENT ON THE BRAIN INFLAMMASOME NLRP3 PATHWAY**

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**Introduction:** Microglia are equipped with the intracellular multimolecular NLRP3 complex and were able to produce IL-1β in response to different classical inflammasome activators (e.g. LPS, stress). Recent data have indicated the role of inflammasomes in excessive microglia activation and its implication in the pathogenesis of depression. **Aim:** The aim of this study was to assess the protein level of the inflammasome NLRP3 subunits: NLR, ASC and protease caspase-1 in the frontal cortex and hippocampus of adult male rats after prenatal stress procedure. Moreover, the impact of chronic venlafaxine administration on this system were evaluated. **Methods**: Pregnant rats were subjected to restraint stress. At 3 months of age, rats were tested for behavioral changes. After that male offspring were administered with venlafaxine (10 mg/kg). The protein level of all NLRP3 subunits was determined by ELISA kits. **Results:** Prenatal stress procedure causes long-lasting behavioral alterations and increased the concentration of all NLRP3 subunits in hippocampus. Chronic treatment of venlafaxine normalized these changes in prenatally stressed offspring. **Conclusion:** Prenatal stress procedure leads not only to persistent behavioral disturbances, but also structure dependent malfunction in brain NLRP3 inflammasone pathway. Microglial NLRP3 inflammasome pathway can be in part a new therapeutic target for prevention and treatment of depression. **Acknowledgements:** Supported by the grant no. 2015/17/N/NZ7/00924, National Science Centre, Poland.

**ABROGATION OF IFN-γ RECEPTOR DOES NOT NECESSARILY WORSEN SENSITIVITY TO PD-1/PD-L1 BLOCKADE**

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Increased expression of programmed death-ligand 1 (PD-L1) by tumor cells is one of the most frequent tumor escape mechanisms from immune surveillance. PD-1/PD-L1 blockade is used for therapy of several cancer types with efficacy up to 30%. PD-L1 expression is highly inducible by IFN-γ and in some cases, primary or secondary resistance to PD-1/PD-L1 blockade was associated with impaired IFN-γ signaling. However, IFN-γ is not the only cytokine that affects the expression of PD-L1. Therefore, the aim of our study was to asses if a loss-of-function mutation in IFN-γ receptor 1 (Ifngr1) interfere with anti-PD-L1 cancer therapy. For this purpose, we used mouse oncogenic TC-1 cell line positive for PD-L1 and derived TC-1/A9 cells with downregulated PD-L1 and MHC class I expression. Both TC-1 and TC-1/A9 cell lines enhanced PD-L1 expression after treatment with IFN-γ. Using the CRISPR/Cas9 system, we prepared TC-1/dIfngr1 and TC-1/A9/dIfngr1 clones with deactivated Ifngr1 and TC-1/dPD-L1 and TC-1/A9/dPD-L1 clones with deactivated PD-L1. While highly reduced oncogenicity of cells with non-functional PD-L1 showed the crucial involvement of PD-L1 molecules expressed on tumor cells in the suppression of anti-tumor immunity, deactivation of Ifngr1 did not cause a reduced sensitivity to PD-L1 blockade. We then found upregulation of the PD-L1 expression on TC-1/dIfngr1 and TC-1/A9/dIfngr1 cells *ex vivo* and demonstrated the induction of PD-L1 molecules on these cell lines by IFN-β. Our results suggest that the only abrogation of IFN-γ signaling is not sufficient for escape from anti-PD-L1 treatment and should not be used for the exclusion of patients from this therapy.

**EFFECT OF COLD ADAPTATION ON THE IMMUNE SYSTEM**

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Maintaining energy homeostasis at reduced temperatures is essential for the survival of the organism. In this study, we determined the influence of cold on the immune system in cold-adapted rats. The binding of noradrenaline (NE) to β-adrenergic receptors plays a key role in this process, and in the subsequent phases of cold adaptation, thyroid hormones are also thought to be involved. In our study, in the initial phase of cold adaptation, the number of T lymphocytes in peripheral blood and spleen has temporarily increased. In the later stages of adaptation, however, we observed a significant increase in the number of B lymphocytes, which correlated with the increase in B cell growth factor, interleukin 4. Changes in the expression of tyrosine hydroxylase and iodothyronine deiodinase, enzymes responsible for the activity of NE and thyroid hormones, respectively support hypothesis about participation of these factors in cold-induced modulation of the immune system. We further evaluated the effect of catecholamines and thyroid hormone on immune cells in vitro. Our results confirmed changes in the representation of individual immune populations and their activation status. Further studies are necessary for deciphering the mechanisms of thermoregulation and its influence on the immune system, understanding these processes will be beneficial for possible clinical use.

**SERUM AND URINARY LEVELS OF CD222 IN CANCER: ORIGIN AND DIAGNOSTIC VALUE**

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The mannose 6-phosphate/insulin-like growth factor 2 receptor (CD222, M6P/IGF2R) is a multifunctional transmembrane type I receptor, mostly localized intracellularly, less on the surface of all types of mammalian cells. It is known both to transport lysosomal enzymes through their mannose 6-phosphate moieties and to internalize extracellular ligands like insulin-like growth factor 2 or plasminogen. CD222 is involved in regulation of cell proliferation, migration, T cell activation, and apoptosis. Soluble CD222 has been found in higher concentrations in sera of liver disease patients. In this study, we analysed the level of CD222 present in body fluids, namely in serum and urine, of cancer patients. We found significantly elevated levels of soluble CD222 in sera of cancer patients compared to healthy controls irrespective of the type of disease. The urine CD222 levels were increased specifically in breast cancer and multiple myeloma. In contrast to serum, CD222 was present within CD222-positive exosomes in urine pointing to different origins of CD222 present in various human body fluids. Based on this work, we propose serum soluble CD222 as a general biomarker for tumorigenesis.

*The work was supported by the FWF (P22908), VEGA (2/0063/14) and APVV-16-0452 grants.*

# TOLL-LIKE RECEPTOR SIGNALING IN THYMIC EPITHELIAL CELLS INCREASES COOPERATIVE ANTIGEN TRANSFER AN ENFORCES THE INDUCTION OF IMMUNE TOLERANCE

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Immune tolerance is achieved by the presentation of self-antigens and subsequent negative selection of self-reactive T-cells or their conversion to T-regulatory cells (Tregs) by medullary thymic epithelial cells (mTECs). These cells bear the unique capacity to produce the vast majority of host`s antigens. The cooperative antigen transfer (CAT) from mTECs to thymic DCs further enforces the immune tolerance. However, the mechanisms which regulate CAT are currently poorly understood.
We observed that the intrathymic stimulation of mTECs via TLR9 specifically leads to the enrichment of migratory DCs (mDCs) in the thymus, enhancement of CAT and to the increased generation of thymic Tregs. Deep RNA sequencing identified a set of secreted chemokines from TLR9-stimulated mTECs that act through Cxcr2 receptor expressed on mDCs. This provides the explanation for mDC recruitment and their participation in CAT impacting Treg numbers and characterizes a novel signaling axis involved in the regulation of immune tolerance.

**EXPLORATION OF LACTOFERRIN BIOLOGICAL EFFECTS ON PROSTATE CANCER CELLS WITH DIFFERENT SENSITIVITY TO HORMONAL THERAPY**

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Correction of prostate cancer hormone resistance is one of the top research directions of biology exploration of this localization. One of the perspective approaches is the search of low-toxic substances that increase treatment efficacy. Our investigation proved that cultivation of prostate cancer cell lines with different sensitivity to hormone therapy (LNCaP and DU-145) with exogenous lactoferrin resulted in reduced proliferation and invasiveness of both cell lines. Also we noted reliable increase in oncogenic miR-155 and miR-205 expression which are involved in formation of hormone-refractory phenotype.

Supported by
Joint scientific project of the NAS of Ukraine and the NAS of Belarus 2015-2016 "Molecular-Biological Effects and Mechanisms of Lactoferrin Action on Tumor Cells in vitro and in vivo".

**IMMUNOSTIMULATORY PROPERTIES OF BACTERIOPHAGE DERIVED DSRNA OF DIFFERENT SIZE AND THEIR USE AS ANTICANCER VACCINE ADJUVANTS**

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Double-stranded RNA (dsRNA), regarding its origin, exhibit immunostimulatory activity and is capable of inducing innate and adaptive immune responses against viral infection and cancer. This makes dsRNA a promising candidate not only for antiviral therapy but also as an adjuvant setting for anticancer vaccines. Recent studies showed that immunostimulatory capacity of dsRNA is greatly impacted by its molecular size. The aim of our study is to evaluate the immunostimulating properties of dsRNA of different size obtained from bacteriophage-infected *E.coli* and to select the most effective adjuvant for anticancer vaccines. Immunostimulatory properties of different size dsRNA were assessed by their ability to induce *ex vivo* generated mouse dendritic cells phenotypic and functional maturation and the ability of such dendritic cells to stimulate allogeneic mouse lymphocytes proliferation. Furthermore, the use of different size dsRNA as an adjuvant for anticancer vaccine was evaluated in *in vivo* Lewis lung carcinoma model. The anticancer effectiveness of combined dsRNA and tumor lysate vaccine was measured by survival and lymphocyte subpopulation phenotypes in spleens of treated mice. We show that dsRNA capacity to activate anticancer immune response is dependent on its molecular size and the largest fraction of investigated dsRNA (500-1000 bp) possess the best immunostimulatory properties. Although dsRNA alone is not enough to evoke effective anticancer immune response, its combination with allogeneic tumor lysate shows promising results.