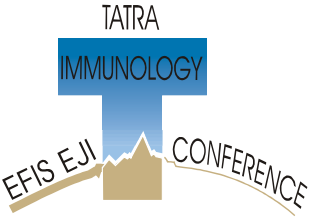
11th EFIS-EJI TATRA IMMUNOLOGY CONFERENCE

Molecular Determinants of T-Cell Immunity



September 06-10, 2014

Štrbské Pleso, Slovakia

PROGRAMME
ABSTRACT BOOK

European Federation of Immunological Societies

11th EFIS-EJI TATRA IMMUNOLOGY CONFERENCE

Molecular Determinants of T-Cell Immunity

CONFERENCE VENUE

Hotel Patria, Štrbské Pleso, High Tatra Mountains, Slovakia

September 6-10, 2014

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)
A. Hayday (London)
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PROGRAMME

Saturday, 6th September - Arrival of participants

Sunday, 7th September

8:30 - 8:40	Opening the Conference
SESSION 1	**Chairperson: H.Stockinger (Vienna)**
8:45 - 9:30	Michael Dustin (Oxford): *Role of TCR enriched microvesicles in T cell communication with antigen presenting cells.*
9:30 - 10:15	Nancy Hogg (London): *How LFA-1 guides the immune response*
10:15 - 10:30	**Tea/Coffee break**
10:30 - 11:15	Bernard Malissen (Marseille): *Toward an integrative biology of T cell-dendritic cell interactions*
11:15 - 12:00	Nikolaus Romani (Innsbruck): *From the Low Tatra in 1994 to the High Tatra in 2014: The lows and highs of Langerhans cells during this period.*
12:10 - 13:00	**Lunch**
13:00 - 16:10	**Afternoon trip**
SESSION 2	**Chairperson: J.Ivanyi (London)**
16:30 - 17:15	Robert Wilkinson (Cape Town): *Transcriptional profiling in human tuberculosis*
17:15 - 18:00	Carl Figdor(Nijmegen): *Dendritic cells in cancer-immunotherapy*
18:00 - 18:45	Thomas Hünig (Würzburg): *From JJ316 to TGN1412 to TAB08: CD28 superagonist therapy before, during and after the storm*
19:00 - 23:00	**Welcome Party**

Monday, 8th September

SESSION 3	**Chairperson: G.Wick (Innsbruck)**
8:45 - 9:30	René van Lier (Amsterdam): *Transcriptions factors involved in the regulation of human CD8$^+$ T cell effector functions*
9:30 - 10:15	RadekŠpíšek (Prague): *Immunotherapy of the prostate cancer using dendritic cells loaded with immunogenic tumor cells*
10:15 - 10:30	**Tea/Coffee break**
10:30 - 11:15	Ethan Shevach (Bethesda): *Two decades since the rebirth of T regulatory (suppressor) cells--Where are we going?*
11:15 - 12:00	Manuela Battaglia (Milan): *Autoimmune diseases: very different disorders yet immunologically very similar*
12:10 - 13:00	**Lunch**
13:00 - 16:10	**Afternoon trip**
SESSION 4	**Chairperson: A.Hayday (London)**
16:30 - 18:00	Selected poster presentations (6 speakers, each 15 min.): NathaliKaushansky (Rehovot) Anna Ohradanova-Repic (Vienna) Wolfgang Paster (Oxford) Jan Wiegers (Innsbruck) Deborah Yablonski (Haifa) PolinaZjablovskaja (Prague)
18:30 - 19:30	**Dinner**
20:00 - 22:00	**Poster session (with refreshments and wine)**

Tuesday, 9th September

SESSION 5	**Chairperson: V.Hořejší (Prague)**
8:45 - 9:30	Johannes Huppa (Vienna): *How T-cells recognize antigens - an imaging approach*
9:30 - 10:15	Janie Borst (Amsterdam): *The mechanistic insights in T-cell costimulation that guide CD27 targeting in cancer immunotherapy*
10:15 - 10:30	**Tea/Coffee break**
10:30 - 11:15	Hannes Stockinger (Vienna): *Ultrasensitive imaging and live cell biochemistry of early T cell reactions*
11:15 - 12:00	Salvatore Valitutti (Toulouse): *Immunological synapses in human health and disease*
12:10 - 13:00	**Lunch**
13:00 - 16:10	**Afternoon trip**
SESSION 6	**Chairperson: R.Špíšek (Prague)**
16:30 – 16:50	Dominik Filipp (Prague): *Gastrointestinal autoimmunity is associated with the loss ofAIRE-mediated central tolerance to enteric alpha-defensins*
16:50 – 17:15	OndřejŠtěpánek (Basel): *Co-receptor scanning by T-cell receptor provides a mechanism for T cell tolerance*
17:15 - 18:00	Paul Garside (Glasgow):*In vivo imaging of immune responses*
18:00 - 18:45	Adrian Hayday (London): *Maintaining the front line: the regulation of T cells at epithelial surfaces*
18:45 – 19:15	**Closing of the conference**
19:30 - 23:30	**Farewell Party**

Wednesday, 10th September - Departure

ABSTRACT BOOK

SPEAKERS

AUTOIMMUNE DISEASES: VERY DIFFERENT DISORDERS YET IMMUNOLOGICALLY VERY SIMILAR

Manuela Battaglia

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The etiology and pathogenesis of type 1 diabetes (T1D) – one of the most frequent chronic, life-debilitating, autoimmune diseases in humans – have long fascinated endocrinologists, pathologists and biologists alike. Currently conventional wisdom – dominated by the highly resilient T-cell–centered approach – portrays T1D as T-cell mediated autoimmune disease that leads to the specific destruction of pancreatic insulin-producing β-cells.
In recent years, the immunological perspective of several autoimmune diseases (e.g., SLE, psoriasis, vasculitis) has changed significantly. In contrast, the immunological view of autoimmune type 1 diabetes (T1D) that was delineated in the 1970s continues to prevail, more or less unchanged, today: the paradigm is obdurately tenacious. I am convinced that our focus has been overly narrow and that our anatomization of all possible T-cell mediated mechanisms involved in T1D pathogenesis has reached a dead end. Said mechanisms represent only one aspect of the much more complex immune response that affects patients with T1D. This is indeed what distinguishes the immune system from other "organs": it hosts a complex interaction between several "players", each with its own specific role, that act collectively to win "the game" (which is, in T1D, pancreas destruction).
I will present our recent studies that provide new insight into the hypothesis that neutrophils might be key in T1D initiation and perpetuation with the attempt to suggest that different autoimmune diseases might share common pathogenic mechanisms.

THE MECHANISTIC INSIGHTS IN T-CELL COSTIMULATION THAT GUIDE CD27 TARGETING IN CANCER IMMUNOTHERAPY

Jannie Borst, *Tomasz Ahrends, Nikolina Babala, Yanling Xiao*.

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In immunotherapy of cancer, the goal is generate a cytotoxic T cell(CTL) response against the tumor cells. To achieve this goal, T cells that can recognize the tumor need to be activated, kill the tumor cells and ideally remain as memory cells. In this lecture, we will outline the important roles thatcostimulatory TNF receptor/ligand systems - including CD27/CD70- play in shaping the different phases of the CTL response. We will highlight the molecular mechanisms these receptors employ to mediate T-cell survival and to generate optimal memory T cells. By analyses at the cellular and molecular level, we have proven that CD27/CD70 costimulation promotes survival and metabolism of proliferating T cells, survival of effector T cells in non-lymphoidtissue and formation of memory T cells. In addition, the CD27/CD70 system plays a key role in mediating CD4 T-cell help for the CTL response and memory programming. Our combined data provide the arguments why CD27engagement is critical to optimize strategies of cancer immunotherapy.This can be achieved by agonistic antibodies or by optimizing CD70 expression on antigen presenting cells.Data from mouse modelsdemonstrate the efficacy of these approaches.

POLARIZED BUDDING AND RELEASE OF RECEPTOR ENRICHED MICROVESICLES IN THE IMMUNOLOGICAL SYNAPSE

Kaushik Choudhuri, Jaime Llodra, Kesley Attridge, Salvatore Valvo, Jones Tsai, Lance C. Kam, David Stokes and ***Michael L. Dustin***

Skirball Institute, New York University School of Medicine, New York, NY USA; Dept. of Biomedical Engineering, Columbia University, New York, NY USA; Kennedy Institute, NDORMS, University of Oxford, Oxford, UK
(michael.dustin@med.nyu.edu)

We performed correlated light and electron tomography studies on the immunological synapse formed with supported lipid bilayers (SLB). Surprisingly, the compartment that is enriched in the T cell antigen receptor (TCR) and major histocompatibility complex (MHC), was not a simple close contact region, but was instead an extracellular compartment packed with TCR enriched microvesicles. Microvesicle formation contributes to TCR down-regulation and can participate in activation of the antigen-presenting cell. Microvesicle formation requires TSG101 and could also be blocked by TSG101 interacting protein HIV Gag, which replaces TCR in the central microvesicles when expressed in T cells. We further analyzed the components of the microvesicles under selected physiological conditions including the introduction of ICOS-ligand (ICOS-L) and analysis of the GFP-anchored type II transmembrane protein tetherin. We find that ICOS is incorporated into the central microvesicles in a ICOS-L dependent manner, particularly in follicular helper T cell isolated from human tonsils. We further have found that tetherin is highly concentrated in the central microvesicles and may account for apparent tethering of the vesicles to the T cell in immunological synapses formed by human T cells. Tetherin is a host-factor that restricts the release of HIV particles into the extracellular space. Our results suggest that tetherin also restricts the release of microvesicles that bud at the plasma membrane and improve specificity for synaptic transfer and perhaps generate a distinct set of antigen specific signals.

DENDRITIC CELLS IN CANCER-IMMUNOTHERAPY

Carl G. Figdor, *Gerty Schreibelt, Harm Westdorp, Kalijn Bol, Steve Boudewijns, Winald Gerritsen and Jolandade Vries*

Department of Tumor Immunology, Radboud University Medical Center, The Netherlands
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Vaccination is a most effective way of protection against microorganisms. No wonder that immunologists spend great effort to exploit vaccination to fight devastating disease like cancer. The main problem is that cancerous cells are much alike normal cells, making development of effective vaccines extremely difficult. In addition to cancer cell derived or synthetic vaccines, Dendritic Cell (DC)-based immunotherapy is explored worldwide in clinical vaccination trials with cancer patients.In this presentation I will provide insight in the state of the art and novel directions to design even more effective vaccines. So far, predominantly ex vivo-cultured monocyte- or Cd34+ derived DCs have been used. Although during the past 15 years the concept of DC vaccination has been clearly proven and found safe, the number of patients that have long-term benefit is limited.
Instead of monocyte derived DC, we recently performed studies with two major types of naturally occurring DCs: myeloid DCs (mDCs) and plasmacytoid (pDCs). Despite their low abundancy in the peripheral blood, initial results indicate that these cells are extremely potent in initiating immune responses in cancer patients.
I will discuss the the future perspective of DC based cancer immunotherapy, also in view of the recent introduction of immune checkpoint inhibitors in the clinic and the need for biomarkers that may predict which patients might benefit most from immunotherapy.

GASTROINTESTINAL AUTOIMMUNITY IS ASSOCIATED WITH THE LOSS OF AIRE-MEDIATED CENTRAL TOLERANCE TO ENTERIC ALPHA-DEFENSINS

Dominik Filipp, *Jan Dobeš, Martina Benešová, Matouš Vobořil, Annamari Ranki, Kai Krohn, Nicolas Kluger, Antonela Meloni,*

Institute of Molecular Genetics AV CR, Prague, Czech Republic
(dominik.filipp@img.cas.cz)

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is caused by the loss of central tolerance due to mutations in the autoimmune regulator (AIRE) and the escape of self-reactive T-cells to immune periphery. Enteric α-defensins belong to a superfamily of antimicrobial peptides secreted by intestinal Paneth cells and are essential for the gut homeostasis. While gastrointestinal symptoms and sero-reactivity with secretory granules of Paneth cells are common in APECED patients, the role of enteric α-defensins in the intestine-related autoimmunity is unknown. Our data demonstrated that extra-intestinal expression of enteric α-defensins localized to medullary thymic epithelial cells and was dependent on AIRE. Consistent with a crucial role for AIRE in tolerance towards defensins, some APECED patients lacked Paneth cells, and sero-positivity for defensin-specific autoantibodies correlated with the frequent occurrence of diarrhea. Importantly, we demonstrated the presence of defensin-specific T-cells in AIRE-deficient mice. Adoptive transfer of these T-cells into athymic mice resulted in the infiltration of T-lymphocytes into the gut, loss of Paneth cells, microbial dysbiosis exemplified by a dramatic enrichment of segmented filamentous bacteria and the induction of Th17 autoimmune responses that together resembled those observed in APECED patients. Our findings provide a mechanistic link between the disruption of central tolerance to enteric α-defensins and intestinal autoimmunity in APECED patients.

ASSESSING IMMUNE CELL INTERACTIONS IN VIVO

Paul Garside

University of Glasgow
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Cell interactions and their nature underpin many of the fundamental processes of the immune system. They are crucial in processes ranging from central tolerance to effector cell function and occur between many different cell types in a variety of tissues. I will discuss how these interactions can be assessed in vivo, how they are influenced by the location in which they take place and ways in which they may influence the outcome of immune responses.

T CELLS IN TISSUES AND IN INNATE IMMUNITY; NEW MOLECULAR INSIGHTS INTO IMMUNOTHERAPY

Adrian Hayday

Kay Glendinning Professor of Immunobiology, King's College London, and Senior Group Leader, London Research Institute, Cancer Research UK
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We conventionally view lymphocytes as circulating cells that become activated in secondary lymphoid organs by highly specific, antigen receptor-mediated interactions; that take time to clonally expand; and that then migrate to effect function at sites of infection, while others enter the memory pool. This is the essence of adaptive immunity. However, many T lymphocytes constitutively reside within epithelia where they make rapid, afferent responses to infections or other forms of dysregulation because of their capacity to recognize "stress-antigens" on stromal cells. These data place these lymphocytes within the early, innate immune phase of a response, implying that cell-cell interactions between epithelial cells and T cells are major orchestrators of immunogenicity. In one such interaction, epithelial cells display stress-induced, MHC-like ligands for the non-clonotypic activating receptor NKG2D, promoting the rapid activation of local T cells. Conversely, in the second type of interaction, normal epithelial cells express molecules in the steady state that engage the T cell antigen receptor (TCR), thereby maintaining tissue-resident T cells in a state of controlled readiness to respond to stress ligands. Consistent with this, molecules mediating the second type of interaction are down-regulated by stress. These molecular data-sets depict how epithelial cells act as primary regulators of immune cells at both steady state and during dysregulation. They cast a revised perspective on T cell receptor biology and have implications in the context of inflammation, cancer, and allergic disease. In particular, tumour-infiltrating lymphocytes (TILs) should be re-evaluated as tissue-associated T cells rather than simply as systemic T cells within tumours.

HOW LFA-1 GUIDES THE IMMUNE RESPONSE

Nancy Hogg

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To fulfil their role in an immune response, circulating immune cells must first adhere to the vasculature and then migrate into sites of infection or injury. They make use of the integrin adhesion receptor LFA-1 to orchestrate the change of environment from circulation to tissue. The behaviour of circulating immune cells is mimicked *in vitro* by the shear flow assay which reveals the cells to roll using selectins, then attach using LFA-1 to ligand ICAM-1, first transiently and then firmly. Three conformations of LFA-1 bind ICAM-1 with different strengths and are predicted to be involved in different lymphocyte activities. Whether LFA-1 is used for migration within the low shear environment of tissues is more problematic, but we have found that it has a role, not only in entering a lymph node, but also in decision making at the point of leaving a lymph node. Thus T cells use LFA-1, not to exit, but for shuttling back into the node parenchyma. This behaviour is predicted to enhance immune surveillance by providing opportunities for further encounters with foreign antigen. The importance of active integrins to an immune response and thus to general well-being is highlighted by Leukocyte Adhesion Deficiency-III (LAD-III) patients that are characterised by life-threatening bleeding and infections. Such patients express normal levels of the integrins on their haematopoietic cells, but the integrins are inactive due to defective signalling. Mutation in the *FERMT3* gene that specifies the kindlin-3 protein is the cause of LAD-III disorder. Kindlin-3 and its homologues kindlin-1 and kindlin-2 are scaffold-like FERM domains, intersected with a classical pleckstrin (PH) homology domain with phosphoinositide-binding motif. As this single protein has such a controlling effect on the immune response, it is essential to understand where it is involved in the sequence of events regulating active LFA-1.

TAB08: CD28 SUPERAGONIST THERAPY BEFORE, DURING AND AFTER THE STORM

Thomas Hünig

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Over a decade ago, we reported that treatment of rats with strongly agonistic CD28-specific mAb (CD28 superagonists, CD28 SA) results in a transient wave of polyclonal Treg activation. Based on these findings, the therapeutic potential of CD28 SA has been demonstrated in multiple rodent models of autoimmunity, inflammation, transplantation and tissue repair. In contrast to these encouraging results, a phase I trial of the human CD28 SA TGN1412 resulted in a life-threatening cytokine release syndrome, interrupting further clinical development. I will review why this reverse reaction occurred and why it had not been anticipated, and report recent preclinical and clinical results indicating that if appropriately dosed, TGN1412, now called TAB08, efficiently expands regulatory T-cells without toxic cytokine release. Finally, the mechanistic basis for the preferential activation of regulatory T-cells at low CD28 SA doses will be discussed.

HOW T-CELLS RECOGNIZE ANTIGENS - AN IMAGING APPROACH

Johannes B. Huppa

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T-cells are remarkably sensitive towards antigen; they can detect the presence of even a single antigenic peptide/MHC complex among thousands of non-stimulatory peptide/MHC complexes on the surface of antigen-presenting cells (APCs). Of note, TCR-peptide/MHC interactions are only of moderate strength when measured in solution and 2-3 orders of magnitudes weaker than typical antibody-antigen interactions. How can we then explain the phenomenal degree of T-cell antigen sensitivity, a hallmark of the adaptive branch of immunity?

We think that the specific microenvironment within the immunological synapse, where TCR-peptide/MHC binding takes place, provides at least in part the answer. Binding parameters are severely influenced because receptors and ligands are pre-oriented, to some extent clustered and moreover subjected to cellular forces. To account for these nonlinear properties of the contacting cells, we have devised a non-invasive ultrasensitive live-cell imaging approach, in which synaptic TCR-pMHC binding events are directly detected and quantified *in situ.* We find TCR-binding indeed significantly increased within the synapse with both an accelerated association and, due to cellular forces, an accelerated dissociation. Moreover, TCR affinities vary substantially within different synaptic regions and also between different cells. These observations imply that TCR-peptide/MHC binding and the entire process of antigen recognition are controlled through not well-understood cell-biological parameters, which might also be subject to regulation. Identifying these parameters and quantifying their effects on the efficacy of T-cell antigen recognition stands in the forefront of our research.

TOWARD AN INTEGRATIVE BIOLOGY OF T CELLS AND DENDRITIC CELLS

Bernard Malissen

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T cells probe the surface of dendritic cells (DCs) in search of cues reflecting the antigenic and inflammatory status of the body tissues. It remains a daunting task to understand how T cell activation is regulated through the summation of a multitude of positive and negative inputs and how their integration contributes to the unfolding of appropriate T cell responses. One of our major objectives is to understand how mutations that reduce TCR signaling output paradoxically lead to severe immune pathologies in both the mouse and human species. More specifically, we would like to elucidate the mechanisms through which during physiological, antigen-driven T cell responses some signaling "hub" used by the TCR leads first to activation of intracellular signaling pathways and then exerts with a temporal delay a feedback inhibition that leads to rapid attenuation of the whole TCR signaling pathway. In the absence of such negative feedback, T cell responses evolves into chronic pro-inflammatory T cell responses that perpetuate themselves in a TCR-independent manner and induce the production of massive amounts of autoantibodies. After giving an overview of the major actors of T cell activation, we will present some recent genetic and proteomic approaches that we have developed to tackle the complexity of T cell activation under physiological conditions and at the systemic levels.

FROM THE LOW TATRA IN 1994 TO THE HIGH TATRA IN 2014: THE LOWS AND HIGHS OF LANGERHANS CELLS DURING THIS PERIOD.

Nikolaus Romani

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Since their discovery in 1868 by Paul Langerhans, our views on ontogeny and function of this conspicuous antigen-presenting cell of the epidermis have changed considerably. Especially the past few years have seen tremendous progress in our knowledge on Langerhans cells. This was mainly due to the development and availability of sophisticated and valuable methods (mainly in mice) to study ontogeny and function. These developments will be reviewed. Moreover, the perspectives to harness the immunological potential of Langerhans cells for immunotherapy (e.g., of cancer) will be discussed. Targeting antigens to antigen-uptake receptors (C-type-lectins) on the surface of Langerhans cells or other skin dendritic cells is a promising strategy to improve and regulate immunization via the skin for vaccination purposes, including immunization against cancer.

CONTROL OF T REGULATORY (TREG) CELL HOMEOSTASIS

Ethan M Shevach, *Michael Holt, AminaMetidji, Mathew Sebastien, Angela Thornton*

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T cells expressing the transcription factor Foxp3 play a critical role in controlling all aspects of immune responses both in health and in disease. Although studies over the past two decades have dramatically increased our understanding of the biologic properties of this critically important T cell subpopulation, many aspects of Treg cell biology remain unknown. One uniquecharacteristic of Tregsis that a major subpopulation is proliferating at a rapid rate in vivo. The goal of our studies is to determine the cell extrinsic and cell intrinsic factors that control Treg homeostasis. We have identified two distinct populations of Treg-one quiescent and one rapidly dividing. While treatment of mice with anti-MHC class II or absence of microbial antigens have no effect, blockade of IL-2 moderately reduces, but blockade of CD80/CD86 derived signals profoundly reduces Treg proliferation. One additional extrinsic factor capable of modulating Treg function, particularly under conditions of "stress," are type I interferons (IFN). Treg lacking the IFNAR exhibit defects in cell survival and function. Lastly, a major cell intrinsic factor regulating Treg homeostasis is the transcription factor, Helios. Selective deletion of Helios in Tregsresults in the gradual development of a systemic autoimmune disease characterized by T effector cell activation, differentiation to a Th1 phenotype, and enhanced germinal center formation. A further understanding of Treg homeostasis is critical to their use in cellular therapy and manipulation of their function in vivo with drugs or biologics.

IMMUNOTHERAPY OF THE PROSTATE CANCER USING DENDRITIC CELLS LOADED WITH IMMUNOGENIC TUMOR CELLS

Radek Spisek

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Immunotherapy has emerged as another treatment modality in cancer. The goal of immunotherapy in advanced cancer patients does not have to be the complete eradication of tumor cells but rather the restoration of a dynamic balance between tumor cells and the immune response. Appropriate combination of tumor mass reduction (by surgery and/or chemotherapy) and neutralization of tumor-induced immunosuppression might set the right conditions for the induction of anti-tumor immune response by active immunotherapy. We initiated two Phase I/II clinical trials using mature dendritic cells (DCs) pulsed with killed LNCap prostate cancer cell line in patients at two distinct stages of the prostate cancer. In the first trial, patients with biochemical relapse, defined as three consecutively rising levels of 3rd generation PSA, are treated with continuous subcutaneous administration of DCs. Second trial is designed for patients with hormone refractory prostate cancer and patients receive alternate treatment with DC-based vaccine and palliative chemotherapy with docetaxel to reduce the tumor cell burden. After one year of follow up, we observe a significant prolongation of the PSA doubling time in the cohort of biochemical relapse patients when compared to the historical controls. In patients with hormone refractory prostate cancer, the alternate administration of DC-based cancer immunotherapy and docetaxel results in the stabilization of the disease progression and longer than expected survival rather than to the reduction of the tumor cells burden. We conclude that the continuous DC-based cancer immunotherapy can represent an efficient adjuvant treatment modality for prostate cancer patients.

CO-RECEPTOR SCANNING BY T-CELL RECEPTOR PROVIDES A MECHANISM FOR T CELL TOLERANCE

Ondrej Stepanek, *Arvind S. Prabhakar, Celine Osswald, Carolyn G. King, Anna Bulek, Dieter Naeher, Marina Beaufils-Hugot, Michael L. Abanto, Virginie Galati, Barbara Hausmann, Rosemarie Lang, David K. Cole, Eric S. Huseby, Andrew K. Sewell, Arup K. Chakraborty, and Ed Palmer*

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Thymocytes recognizing self-antigens with high affinity are eliminated from the pool of developing T cells, but how thymocytes measure self-antigen affinity is poorly understood. Here we show that very few coreceptor molecules are coupled with the signal-initiating kinase, Lck. To initiate signaling, an antigen engaged TCR scans multiple coreceptor molecules to find one that is coupled to Lck. MHCII-restricted T-cell receptors (TCRs) require a shorter antigen dwell time (~0.2s) to initiate negative selection compared to MHCI restricted TCRs (~0.9s) because more CD4 coreceptors are Lck-loaded compared to CD8. Coreceptor scanning is the first and rate-limiting step in a kinetic proofreading chain of events that eventually leads to TCR triggering and negative selection. Based on experimental data and mathematical analysis, we generated a model (Lck come&stay/signal duration) that accurately predicts the experimentally observed antigen dwell-time thresholds used by MHCI- and MHCII-restricted thymocytes to initiate negative selection.

ULTRASENSITIVE IMAGING AND LIVE CELL BIOCHEMISTRY OF EARLY T CELL REACTIONS

Hannes Stockinger

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The knowledge of the molecular mechanisms processed in the synapse between T cells and antigen presenting cells is crucial to understand decisions of T cells to tolerize antigen or mount an adaptive immune response. Uncovering the underlying pathways is prerequisite to identify targets for diagnosis and therapy of immunological diseases such as allergy, autoimmunity, chronic inflammation. We have made recently technological advances with superresolution imaging allowing mobility, dynamic, stoichiometry and lifetime measurements of proteins and lipids and their complex formation in the living cell at length scales of a few nanometers and a sub-millisecond time scale. Using these techniques we followed the motion of co-regulatory- and submembranesignaling molecules of the T cell antigen receptor (TCR), in particular glycosylphosphatidylinositol (GPI)-anchored proteins and the Src-protein tyrosine kinase Lck. We visualized a considerable fraction of these moleculesas dimers in living cells. Designing constructs that allow controlled dimer induction, we arenot only able to show that dimerization of Lckis a level of functional regulation of this key signaling molecule of TCR but also that lipid rafts are the platforms for priming of dimerization. In summary, by using non-invasive ultrasensitive imaging techniques in living cells we now can gain new insights into molecular modification and cellular positioning of molecules for regulation of cell signaling and function.

IMMUNOLOGICAL SYNAPSES IN HUMAN HEALTH AND DISEASE

Salvatore Valitutti

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We have a long-standing interest in the study of the morphological and functional aspects of human immune cell immunological synapses.
In the first part of my talk I will expose our recent findings on the molecular mechanism of human cytotoxic T lymphocyte interaction with target cells. I will discuss: i) how CTL secrete lytic granules in the absence of microtubule organizing center re-polarization at the immunological synapse; ii) how within a clonal population a few individual CTL are able to exert an extremely efficient killing activity after prolonged interaction with target cells iii) a novel "synaptic" mechanism of tumor cell resistance to CTL attack; iv) the impact of CTL infiltration in non-Hodgkin lymphoma progression.
In the second part of my talk I will describe a novel effector mechanism of mast cells: the antibody-dependent degranulatory synapse (ADDS). ADDS is initiated by the clustering of ITAM-containing Fc receptors and leads to polarized degranulation towards adjacent cells targeted by either IgE or IgG antibodies. Polarized exocytosis of secretory granules allows mast cells to accumulate bioactive molecules at the cellular interface with targeted cells. Remarkably, ADDS against IgG-targeted *Toxoplasma gondii* tachyzoite triggers polarized mast cell degranulation resulting in tryptase-dependent parasite death.

BLOOD AND BEYOND: (HUMAN) CD8$^+$ TISSUE-RESIDENT MEMORY T-CELLS

René A. van Lier

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The epithelial cells that line the respiratory tract are constantly exposed to the external environment. Respiratory viruses target epithelial cells as initial site of entry and replication. After infection, a specialized population of memory CD8$^+$ T-cells resides in the epithelium to maintain constant immune surveillance and protection against recurring respiratory infections. We determined the transcriptional profile of T_{RM} retrieved from human lung resection samples. A comprehensive set of transcription factors was identified that characterizes lung resident T_{RM}. To this set belongs Hobit (ZNF683), a transcription factor highly homologous to Blimp-1. Interestingly, in mice the concerted action of both BLIMP-1 and Hobit was found to be necessary for Trm maintenance in skin and liver. Furthermore, the expression of both the NOTCH1 receptor and a large number of NOTCH1 target genes is high in lung derived T-cells and even more pronounced in those that express CD103. The relevance of NOTCH1 for the formation of T_{RM} was shown in an influenza model in NOTCH1/2 deficient mice. Our data illustrate the adaptation of lung resident T-cells to the requirements of the respiratory epithelial environment. Defining the molecular imprinting of these cells is important for rational vaccine design and may help to improve the properties of T-cells for adoptive cellular therapy.

TRANSCRIPTIONAL PROFILING IN HUMAN TUBERCULOSIS

Robert J Wilkinson

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This talk will review the contribution of whole genome based transcriptomic profiling to increase knowledge of the pathogenesis of tuberculosis and to highlight translational consequences that may arise from this work. Tuberculosis is an important disease with 8.6 million cases per year and 1.3 million deaths. Suboptimal understanding of pathogenesis exacerbates existing control strategies that rely, in the absence of a vaccine that reduces transmission, on chemotherapy of the most infectious cases. The latter strategy is in turn compromised by suboptimal diagnostic methods. In various collaborations we have described an ex vivo whole-blood transcriptomic signature for active TB that is dominated by a neutrophil-driven interferon (IFN)- inducible gene profile, consisting of both IFN-gamma and type I IFN-alpha/beta. the signature correlates with radiological extent of disease and reverts to that of healthy controls after treatment. The latter feature allows exploration the concept of 'molecular distance to health'(i.e. the evolution of over- and under-represented transcripts over time). Within patient normalisation to baseline allows the calculation of a decreasing 'temporal molecular response' during successful antitubercular therapy, an attribute that might allow better monitoring of novel treatment regimes. In an extension of this work we have recently investigated the converse: divergence in transcriptomic signature between HIV-tuberculosis patients who commence ART who do and do not develop the immune complication of immune reconstitution inflammatory syndrome. TB-IRIS is characterised by signature dominated by innate receptor signalling which gradually evolves during the first two weeks of ART.

POSTER PRESENTATIONS AND SHORT ORAL PRESENTATIONS

VARIATION IN SELECTED TOLL-LIKE RECEPTORS IN ANCIENT CHICKEN BREEDS

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Toll-like receptors (TLRs) belong to innate immunity sensors of evolutionarily known potential danger. Although they trigger immune response by recognizing conservative microbial molecular patterns, they have been shown to be polymorphic and this variability has been associated with susceptibility or resistance to various diseases in several species. Domestic chicken (*Gallus gallus domesticus*) with its enormous economic importance serves as a basic model in avian immunological research. Much of the present investigation in chicken TLRs has been conducted in inbred lines. However, these lines exhibit only limited genetic variability that might be immunologically relevant. In contrast, ancient chicken breeds kept by fancy breeders represent a suitable highly diversified source of genetic polymorphism available for the research of functionally significant variation in *TLRs*. In our study we sequenced two intracellular (*TLR3* and *TLR7*) and two extracellular TLRs (*TLR4* and *TLR5*) in 110 chickens belonging to 25 breeds kept in Europe. The highest level of polymorphism was found in *TLR3* (41 SNPs; 19 non-synonymous /ns/ SNPs), followed by the other intracellular receptor, *TLR7*, with 30 SNPs (14 nsSNPs). In *TLR4* we identified 28 SNPs (12 nsSNPs) and the lowest number of SNPs was detected in *TLR5* (20 SNPs; 11 nsSNPs). Although majority of amino acid substitutions in the protein sequence are located apart from ligand binding regions, in TLR4 and TLR5 we revealed several substitutions in proximity to predicted functionally important sites of the receptors. Their possible impact on receptor function awaits to be tested.
This research has been supported by the Czech Science Foundation (project GACR P502/12/P179).

THE POOL OF PREACTIVATED LCK IN THE INITIATION OF T CELL SIGNALING:
A CRITICAL RE-EVALUATION OF THE LCK STANDBY MODEL

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The initiation of T-cell receptor (TCR) signaling, based on the co-binding of TCR and CD4-Lck heterodimer to a peptide-MHCII complex (pMHCII) on antigen presenting cells, represents a classical signaling model of T-cell biology. Less known, however, is the mechanism which translates TCR engagement to the phosphorylation of ITAM motifs on CD3 chains and how this event couples to the delivery of Lck function. Recently, the "standby model of Lck" proposed that resting T-cells contain an abundant pool of constitutively active Lck which is required for TCR triggering and this amount, upon TCR engagement, remains constant. Here, using different biochemical and genetic approaches, we show that while the maintenance of the limited pool of pY394Lck is necessary for rapid generation of TCR proximal signals in time-restricted fashion, the amount of cellular pool of pY394Lck is much smaller than previously reported. We provide evidence that high levels of pY394Lck found in the original study are likely the consequence of spontaneous phosphorylation of Lck occurring after cell solubilization. Additional discrepancies can be accounted by the sensitivity of different pY394Lck-specific antibodies and the type of detergent used. These data suggests that reagents and conditions used for the quantification of signaling parameters must be carefully validated and interpreted. Thus, a significantly diminished size of pY394Lck pool in primary T cells invites for quantitative parameters of the standby model of Lck to be adjusted and the mechanism by which a limited pool of pY394 contributes to the generation of proximal TCR signaling should be re-evaluated.

hAAT AND TEMPORAL T CELL DEPLETION SYNERGIZE TO EXPAND TREGS AND TO EXTEND ALLOGENEIC SKIN GRAFT SURVIVAL

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Introduction: The xenoimmune response involves macrophages and CD4 T-cells. Alpha-1 antitrypsin (AAT), an anti-inflammatory circulating protein that lacks a direct effect on T-cells, has been shown to synergize with temporary T-cell depletion in favor of rat-to-mouse islet xenograft survival, in a yet unknown mechanism. Aim: To elucidate the protective mechanism behind combined AAT and temporary T-cell depletion during xenoimmune response. Methods: C57BL/6 and hAAT+/+ mice (both H-2b) were used as graft recipients. Temporary T-cell depletion was performs using anti-CD4 and anti-CD8-depleting antibodies. Circulating T-cell subtypes were monitored. Bone-marrow-derived IL-7 expression was determined by real-time PCR. Rat islets were grafted into either non-depleted untreated mice, or depleted mice that were either untreated or treated with hAAT (Glassia, Kamada, Ltd.). To examine whether protection relates to antigenic load, skin allotransplantation was performed. Results: After depletion, hAAT+/+ mice displayed significantly higher levels of CD4+ cells (6.47±1.56-fold compared to WT mice) and 6.18±0.86-fold more foxp3+ cells. Post-depletion IL-7 mRNA levels were elevated in all groups, and to a greater extent in the hAAT group (1.49±0.1-fold compared to WT, $p<0.05$). Non-depleted, untreated mice rejected islet xenografts on day 15.3 (mean, n=6); CD4-depleted mice rejected on day 26.4 (mean, n=5), and CD8-depleted mice on day 15.5 (mean, n=6). None displayed graft survival after day 35. hAAT+/+ mice exhibited graft function after day 35 in 67% CD8-depleted and 40% CD4-depleted mice. Skin allografts were protected only in the combined approach. **Conclusion**: The combined treatment appears to favor regulatory T-cell expansion, which may be mediated by elevated IL-7 levels. hAAT also addresses antigenic load that is otherwise resistant to temporary T-cell blockade. This novel combination between two safe therapeutic approaches should be further evaluated in other, similarly challenging immunological responses.

Interaction of tumor cells withantibodies against human carbonic anhydrase I

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There is a group of patients with different types of malignancies who developed an aplastic anemia like syndrome after a high-dose-therapy and autologous stem cell transplantation, but even though they appeared to have spontaneous tumor regression without relapse. It was found that these patients have high titers of serum autoantibodies against carbonic anhydrase,isoform I (anti-CAI) and the presence of the anti-CAIis therefore associated with malignancy regression [1]. This study is focused on monitoring of the interactions between anti-CAI antibodies and tumor cells. Especially we study the influence of these antibodies on viability of tumor cells byxCELLigence and then we try to reveal the mechanism of action of this process by identification of proteins, which react with anti-CAI and could be involved in the process. For this we usedthe interactions between anti-CAI antibodies and tumor cells lysates analyzed by two-dimensional gel electrophoresis followed by western blot analysis. The experiments demonstrated that the antibodies interact not only with their specific antigen (CAI) and that they affect cell grow of tested tumor cell line. It could explain the effect of repression on tumor cells and clarify the phenomenon of an aplastic anemia and spontaneous tumor regression in the patients.

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OPAL1: FROM B CELL LEUKEMIA MARKER TO E3 UBIQUITIN LIGASE ADAPTOR

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Ubiquitination is a modification regulating protein turnover and trafficking, as well as cellular signal transduction. Critical role in the process is played by E3 ubiquitin ligases, which recognize the target protein and facilitate ubiquitin transfer. Their activity and interactions with various substrates are regulated in many cases by adaptor proteins. We describe an identification of a novel adaptor protein for NEDD4 family E3 ubiquitin ligases, Outcome Predictor of Acute Leukemia 1 (OPAL1). OPAL1 was initially identified as a marker predicting a favorable outcome of childhood Acute Lymphoblastic Leukimia (ALL) treatment, but its function was unknown. We show that OPAL1 interacts with several members of NEDD4 family and this interaction enhances their activity. The interaction is mediated by WW domains and PPxY motifs present in the NEDD4 ubiquitin ligases and OPAL1, respectively. By recruiting several NEDD4 family members OPAL1 is involved in the regulation of chemokine receptor CXCR4 in leukocytes. ShRNA-mediated downregulation of OPAL1 expression resulted in significant attenuation CXCR4 proximal signaling after stimulation with CXCR4 ligand SDF-1α in murine macrophage progenitors, as well as in REH cell line (human pre B-ALL). This phenotype was also associated with increased chemotaxis towards SDF-1α. Altogether, our data suggest the role of OPAL1 in the regulation of CXCR4 expression and/or activity via the recruitment of NEDD4-family ubiquitin ligases and allow us to propose the hypothesis that increased OPAL1 expression in certain childhood leukemias may reduce CXCR4-dependent homing of leukemic cells to the bone marrow resulting in an improved response to treatment.

EFFECT OF VITAMIN D ON INFLAMMATORY MARKER EXPRESSION IN SARCOIDOSIS

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A link between vitamin D levels and sarcoidosis was proposed over 50 years ago. In our previous study (2013) we identified an increased expression of TREM-1 and TREM-2 on the surface of myeloid cells in pulmonary sarcoidosis. In present study we tried to explore the link between D vitamin and expression of TREM-1 and TREM-2 receptors. 43 patients with pulmonary sarcoidosis were prospectively enrolled in the study. The TREM-1 and TREM-2 expressions on the cell surfaces in bronchoalveolar lavage fluid (BALF) were investigated using anti - TREM-1 and anti - TREM-2 monoclonal antibodies and flow cytometry. Subsequently, the level of vitamin D3 was analysed in the sera of patients and compared with TREM expressions in patients with normal and decreased levels of vitamin D3. Plasma levels of vitamin D3 statistically significantly correlated with total TREM-2 expressions ($p=0.0457$). We found significantly higher expressions of TREM-2 in patients with normal plasma D3 vitamin levels compared with patients with decreased vitamin D levels ($p=0.0039$). However, we did not find significant difference in TREM-1 expression in patients with normal vitamin D3 compared with patients with low vitamin D3 levels ($p=0.7358$). Our results suggest that vitamin D participates in immunopatogenesis of pulmonary sarcoidosis and influences TREM-2 receptor expression.

THE ROLE OF THE LINKER FOR ACTIVATION OF T CELLS (LAT) IN THE MECHANICAL CHARACTERISTICS OF JURKAT T CELLS

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The transmembrane adaptor protein LAT is required for the development of T cells and T cell receptor (TCR) signaling. Upon TCR activation LAT becomes phosphorylated by ZAP-70, and associates with molecules, like PLCγ, Grb2 and GADS to promote further downstream signaling. These functions of LAT depend on its palmitoylation close to the transmembrane domain, which is required for the transport of LAT to the plasma membrane. In addition, LAT is also interacting with the actin cytoskeleton, and is involved in the polarization of the microtubule-organizing center (MTOC). Here, we used atomic force microscopy (AFM) to measure the elasticity of Jurkat T cells E6-1, derived from a patient with acute T cell leukemia, and of J.CaM2 cells, a LAT-deficient mutant of Jurkat cells. Analysis of the force curves with the Hertz model showed that Jurkat E6-1 cells were ~1.5 times softer than J.CaM2 cells (Young's modulus ~110 Pa for E6-1 *vs.* ~170 Pa for J.CaM2). In order to verify the role of LAT in cell elasticity, we reconstituted the LAT-deficient J.CaM2 cells stably with a LATwt-GFP fusion protein. These cells had similar cell elasticity to Jurkat E6-1 cells with endogenous LAT. Reconstitution of J.CaM2 cells with a non-palmitoylated variant of LAT (LAT(C26/9A)-GFP) failed to rescue the increased cell stiffness. These findings suggest that LAT is involved in the organization of the actin cytoskeleton (i), that LAT has to present in the plasma membrane to influence cell elasticity (ii), and surprisingly that this function of LAT seems to be independent of TCR activation as it was observed in resting cells (iii).

ORAL ADMINISTRATION OF ANTI-CD3 ANTIBODIES LEADS TO A REDUCTION IN OF ATHEROSCLEROSIS AND AN INCREASE IN REGULATORY T CELLS IN APOE-/- MICE

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Oral application of antigens leads to tolerance towards the given antigen. Surprisingly, orally applied CD3 specific antibodies (Abs) can also induce tolerance and this approach is now under investigation for the treatment of various autoimmune diseases.
To elucidate the mechanism of the Ab action, animals were fed with a monoclonal anti-CD3 Ab or with control IgG (5µg/feeding) on 5 consecutive days and sacrificed on day 8. Mesenteric, inguinal and axillary lymph nodes as well as spleen were analyzed by means of flow cytometery for the presence of regulatory T cells. Small intestine was taken for immunofluorescence investigation at different time points after feeding in order to scrutinize the fate of the ingested anti-CD3 Abs.
Oral feeding with anti-CD3 Abs prevents atherosclerosis in ApoE-/- mice. The CD3-specific Abs can be found in the small intestine already 30 minutes after administration, indicating Ab uptake. Lastly, an increase in regulatory T cells was detected in anti-CD3 Abfed mice.
Our findings indicate that orally administered anti-CD3 Abs areatheroprotective. The Abs are present in the digestive tract and induce LAP^{+}Tregs, which are considered to be instrumental in the reduction of atherosclerosis. The exact functional mode of action is now under investigation.
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NEW ROLE FOR AN OLD DRUG: SURPRISING ANTICANCER EFFECT OF THE ANTIPARASITIC DRUG MEBENDAZOLE.

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A promising approach in the field of cancer research lies in repurposing compounds already used in human medicine for cancer treatment. Such research can draw on readily available data and advance through clinical trials for a new indication much easier, faster and cheaper than researching completely novel drugs.

One example of a repurposed drug is mebendazole, an antihelminthic from the family of benzimidazoles, widely used in human and veterinary medicine since early 1970s and marketed worldwide as Vermox or Mebendazole. The anticancer properties of mebendazole have first been documented in 2002 (1) and have so far been reported in numerous cancer types, including colorectal carcinoma, melanoma and NSCLC (2), (3). Although the exact mechanism of function in mammalian tumor cells has not been fully described, the effect seems dependent on microtubules and Bcl-2 inactivation (2), (3). Due to the fact that the drug is extremely hydrophobic, parenteral application has so far not been reported and the drug is routinely used perorally.

In our study, we further tested *in vitro* and *in vivo* efficacy of mebendazole in several murine cancer cell lines. Mebendazole has shown *in vitro* activity comparable with that of doxorubicin in LL2 lung carcinoma, EL4 T-cell lymphoma and Bcl-1 B-cell leukemia. Our main goal is to prepare a polymeric conjugate based on N-(2-hydroxypropyl) methacrylamide) where mebendazole is covalently bound through a defined linker allowing controlled release *in vivo*. Such conjugate would allow administering mebendazole via i.v. injection and, moreover, anticancer activity of the drug should be significantly augmented due to extended half-life in the circulation (4) and passive accumulation in solid tumors via EPR effect (5).

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THERAPEUTICAL EFFECT OF XENOGENIC VACCINE IN MURINE MELANOMA AND LUNG CARCINOMA MODELS. PRECLINICAL TRIAL DATA.

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An antitumor vaccine comprising testicular and fetal-derived components is described. Such a cell-based vaccine may be used in the treatments and prophylaxis of cancer, particularly a lung cancer and melanoma. The preparations may be defined as vaccines comprising a mixture of cells derived from different xenogenic normal tissues. Preparations are prepared from tissues harvested directly from animals. A vaccine consisting of glutaraldehyde-treated cells prepared from sheep testis and fetal lung epithelium has been found to be effective in inducing antitumor cell-mediated responses, as well as in prolonging the survival of mice with lung cancer and melanoma. A vaccine comprising a soluble extract from xenogenic antigens tissue is also provided. In the absence of immunoadjvants this vaccine was found to be ineffective in inducing antitumor immune responses.

EXPRESSION OF IMMUNOGENIC MARKERS ON DENDRITIC CELLS GENERATED BY TWO DIFFERENT MATURATION COCKTAILS

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Recently, specific active immunotherapy of cancer have received more and more attention. One of its most promising directions is therapeutic dendritic cells (DCs) vaccination. Therapeutic DC vaccination is applied in various clinics of the world and is prescribed in case of melanoma, prostate, breast, stomach cancer, renal carcinoma, sarcoma, lymphoma, non-small cell lung cancer. Clinical response to treatment with DCs is reached in 54% of patients. However, immunotherapy still should be optimized in order to achieve the maximum clinical effect. DCs vaccines (DCV) are produced individually for each patient by differentiating monocytes to DCs, which are later matured with the help of different cytokines and loaded with autologous tumor antigens. Currently, the suitability of DCV for clinical application is evaluated only by the expression of biomarkers reflecting maturation and activation of DCs (CD83, CD80, CD86, HLA-DR etc.). The effectiveness of DCV depends on the cytokine cocktail used for maturation of the DC cells *in vitro*. Therefore, to accurately assess the effectiveness and clinical effects of DCV, it is necessary to thoroughly characterize the functional properties of DCs.

In our study we evaluated the immunogenic potential of DCs, generated by two different maturation cocktails (cocktail 1 - IFN-α; cocktail 2 - IFN-α and IFN-γ). The analysis of immunogenic biomarkers of DCs (CD80, CD83, CD11c, HLA-DR) was performed using flow cytometry . The DCs cytokine secretory profile (IL-12p70, IL-1β, TNF- α, TGF- β, IL-10) was assayed by cytometric bead array. The participants of our study were patients with glioblastoma and breast carcinoma (age 24-73 years). Our results show that using the second maturation cocktail (IFN-α and IFN-γ), 92% of the cells were positive for all four immunogenic biomarkers in comparison to 60% of cells, generated by first maturation cocktail (IFN-α).

THE ROLE OF ADAPTOR PROTEIN PSTPIP2 IN PREVENTING AUTOINFLAMMATORY DISEASE

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Leukocyte signaling is one of the most important proximal events in mounting immune response against pathogens or danger signals in the body. Proper regulation and balancing of the signaling is also crucial for immune response not to turn pathological. Phosphorylation of lipids and proteins represents significant part in leukocyte signaling. While phosphorylation is carried out by kinases, dephosphorylation is performed by phosphatases. These two groups of enzymes are regulated at multiple levels, including direct inhibition or activation, as well as spatial distribution in the cell. Their correct localization is arranged by adaptor proteins which frequently serve as a scaffold binding multiple effector proteins and enzymes. PSTPIP2is an adaptor protein present mainly in the myeloid lineage. Absence of PSTPIP2 in mice results in focal sterile inflammation in external parts of the body such is tail, paws and ears, generally described as chronic multifocal osteomyelitis. This state closely resembles CRMO (chronic recurrent multifocal osteomyelitis) in humans. Part of PSTPIP2 inhibitory function is probably mediated by phosphatases from PEST family known to interact with this protein. Now we show that PSTPIP2 also bindsinhibitory enzymesCsk and Ship1. Interaction with Ship1 is probably of high importance as it binds to the critical region at the C-terminus of PSTPIP2 known to be important for its function various cellular systems. Our data support the hypothesis that absence of PSTPIP2 leads to dysregulation of signaling where cooperation of Ship1 and PSTPIP2 is required. This leads to increased signaling through PI3-kinaseand MAP kinase pathways and over responsiveness of myeloid cells, eventually culminating in the development of autoinflammatory disease.

N-TERMINAL DOMAIN OF 26 KDA TNF AND CKIP-1: INTERACTING PARTNERS WITH OPPOSING ROLES IN REVERSE SIGNALING

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When transmembrane forms of members of the TNF superfamily interact with their cognate receptors signaling pathways are activated in both participating cells leading to activation, differentiation or apoptosis. These bidirectional events, called reverse signaling seem to influence almost every decision during the immune responses. Though more and more data suggest the importance of reverse signaling the participating molecules and their functions remain largely unknown. Here we examined the role of CKIP-1, a protein that was identified as an interacting partner of the N terminal intracellular domain of mTNF in yeast two hybrid system, in inflammation and TNF reverse signaling.

TNF reverse signaling or the over-expression of the N-terminal peptide of the mTNF molecule induced a massive translocation of CKIP-1 from the plasma membrane to intracellular compartments and lead to apoptosis in HEK cells and THP-1 monocytes. Overexpression of CKIP-1 prevented this phenomenon. We demonstrated that CKIP-1 expression was induced by LPS in human monocytes, which was not relaxed when TNF reverse signaling was elicited. CKIP-1 transactivated the human TNF promoter in a HEK293 model system and proved to be a cooperating partner of c-Jun in this activation. Expression of the N-terminal peptide of the mTNF molecule in this system interfered with TNF promoter activation and induced the intracellular translocation of overexpressed CKIP-1. Our findings suggest the involvement of CKIP-1 in both pro-inflammatory activation pathways and TNF reverse signaling providing a protective function upon apoptotic challenge.

AGE-RELATED CUT-OFF VALUES OF SERUM S100B AND MELANOMA INHIBITORY ACTIVITY (MIA) PROTEINS – PRELIMINARY STUDY

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S100B and melanoma inhibitory activity (MIA) proteins are two valuable serum biomarkers for prognosis of the very aggressive skin melanoma, for early diagnosis of melanoma metastasis and for disease monitoring during antineoplastic treatment. We previously showed that in melanoma patients, a strong correlation between S100B and MIA is associated with an unfavorable clinical evolution.
This study aimed to investigate serum S100B and MIA in relation to age and immune status. Serum levels of S100B and MIA were measured by ELISA from a group of healthy adults (HA), a group of healthy children (HC) and a group of children with respiratory infections (IR). A significantly higher cut-off value for serum MIA was found in HC group vs HA. Regarding S100B protein we didn't obtain a statistically significant difference between HC and HA, and also our preliminary results showed no significant differences between HC and IR for the two analyzed proteins, but this could be due to the limited number of children in this study. However, both HC and IR groups showed higher S100B and MIA cut-off values in children ranging 1 to 4 years old compared with children ranging 5 to 13 years old. There were no differences between girls and boys. Our results bring valuable evidence that age is extremely important in the interpretation of S100B and melanoma inhibitory activity (MIA) protein levels.

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THE INVESTIGATION AND MODIFICATION OF TNF REVERSE SIGNALING ON MONOCYTES AND MACROPHAGES

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Intracellular signaling elicited by interaction of TNF with receptor molecules or blocking antibodies (reverse signaling) has essential role in the regulation of the innate and adaptive immune response.
In this study we examined how reverse signaling acts on LPS stimulated monocytes and macrophages. LPS challenge alone resulted in enhanced cytokine and chemokine production in human and mouse monocytes. Simultaneous induction of reverse signaling by TNF neutralizing antibodies (namely infliximab, IFX and certolizumab pegol, CZP, respectively) resulted in marked relative gene expression decline in case of inflammation-related cytokines as compared with LPS treated samples. Moreover, this effect seemed to be independent from the presence of the Fc fragment in case of IFX, as in IgG treated samples gene expression decline was not detected. Additionally, in LPS-induced human monocytes, CZP treatment also resulted in significant relative gene expression alterations in a time-dependent manner.
In case of PMA induced primary mouse macrophages, LPS stimulation robustly enhanced the relative gene expression of the inflammation-related effector molecules. In accordance with the results obtained from human monocytes, IFX treatment resulted in marked relative gene expression decline as compared with LPS stimulated samples.
Although TNF blocking antibodies are widely used in the clinical practice in many autoimmune diseases, little is known about their mechanism of action. Our results suggest that reverse signaling has pivotal role in the beneficial effects of TNF neutralizing monoclonals by down regulating inflammatory processes. Furthermore, the application of reverse signaling may have therapeutic potential in clinical oncology by interfering with M2 polarized macrophages.

HMGB1 AND INFLAMMATION IN PATIENTS WITH MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is an autoimmune disease of the central nervous system.Althought he etiology of MS is unknown, both genetic and environmental factors contribute to the pathogenesis. From the immunological point of view an important prognostic factor is to monitor an inflammation, particularly through the latest inflammatory markers such as HMGB1 (High Mobility Group Box 1 protein). HMGB1 has been known for its intracellular function, contributes to stabilization of nucleosomes, mediates DNA bending and is regarded tohave an essential role in DNA repair. After it's release from necrotic cells or actively production by various cell types,HMGB1 acts as an alarmin and is responsible for production of pro-inflammatory cytokines. The knowledge that multiple sclerosis is an inflammatory disorder has prompted us to investigate the plasma levels ofthis new inflammatory marker. The levels of HMGB1 were analysed by sandwich ELISA test in the cohort of 165 patients with multiple sclerosis and 31 healthy controls. Our results revealed more than 4,5 times higher plasma levels of HMGB1 in patients with MS (13,529 ng/ml ± 19,076) compared with healthy subjects (2,999 ng/ml ± 2,147; $p<0,0001$). Higher levels of HMGB1 were found also in women (15,098ng/ml± 18,673) than in men (12,571ng/ml ± 20,058) and patients with at least one active MRI lesion in the brain. Moreover, levels of HMGB1 correlated with number of active lesion (19,667ng/ml ± 24,777; $p=0,0209$) in the brain. Higher levels of HMGB1 were found in patients with EDSS $\geq$ 3 (17,549ng/ml±23,310) than in patients with EDSS $\leq$ 2,5 (11,648 ng/ml± 19,752; $p<0,0001$). Elevated plasma levels of HMGB1 reflect severity of MS and the presence of chronic inflammation in MS patients. HMGB1 can represent a potential marker in MS monitoring.

ANTI-CANCER PROPERTIES OF GASTROPODAN HEMOCYANINS IN MURINE MODEL OF COLON CARCINOMA

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Background: Various immunotherapeutic approaches have been used for the treatment of cancer. A number of natural compounds are designed to repair, stimulate, or enhance the immune system response. Among them are the hemocyanins (Hcs) - extracellular copper proteins isolated from different arthropod and mollusc species. Hcs are oxygen transporter molecules and normally are freely dissolved in the hemolymph of these animals. Hemocyanins are very promising class of anti-cancer therapeutics due to their immunogenic properties and the absence of toxicity or side effects. KLH (*Megathura crenulata* hemocyanin) is the most studied molecule of this group setting a standard for natural carrier protein for small molecules and has been used in anti-tumor clinical trials.
Results: The Hcs isolated from marine snail *Rapana thomasiana* (RtH) and the terrestrial snail *Helix pomatia* (HpH) express strong *in vivo* anti-cancer and anti-proliferative effects in the developed by us murine model of colon carcinoma. The immunization with RtH and HpH prolonged the survival of treated animals, improve humoral anti-cancer response and moderate the manifestation of C-26 carcinoma symptoms as tumor growth, splenomegaly and lung metastasis appearance.
Conclusion: Hemocyanins are used so far for therapy of superficial bladder cancer and murine melanoma models. Our findings demonstrate a potential anti-cancer effect of hemocyanins on a murine model of colon carcinoma suggesting their use for immunotherapy of different types of cancer.

INCORPORATING TOLL LIKE RECEPTORS (TLRs) AGONISTS INTO CONJUGATED PLGA-PEI AS A VACCINE DELIVERY SYSTEM FOR IMPROVING THE IMMUNE RESPONSES

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To accomplish successful vaccination, it is essential to design new strategies to develop vaccines that can effectively penetrate the cells particularly when the designed vaccine can be co-localized in an antigen-presenting cell (APC) and produce more specific immune responses for antigen. Therfore it is important to use vectors which can co-deliver antigen and adjuvant to APC and help increasing their uptake and presentation. Toll like receptors (TLRs) agonists are promising vaccine adjuvants with the ability to enhance and modulate innate as well as adaptive immune responses in great potentials.The use of non-viral vectors as delivery carriers for oligonucleotides and antigens, enhance vaccine immungenecity which can lead to protective immunity. An effective way for co-delivery of antigens and CpG oligodeoxynucleotides (CpG ODN) as TLR9 agonist is to encapsulate both in one carrier to ensure that they both enter one cell at the same time. In this study, OVA protein as antigen was encapsulated in PLGA (poly-lactic-co-glycolic acid) which was covalently conjugated using amide chemistry to the surface of 10% covered polyethylenimine (PEI) followed by addition of CpG ODN which is a 22 mer oligonucleotide in order to produce a nanoparticulate vector for vaccine delivery. The resulting complex was characterized in terms of size, zeta potential and the structure was confirmed by FT-IR spectroscopy. The vaccine delivery potentials of the resulting complex are under investigation and the preliminary resuls have been promising. It ir concluded that OVA protein encapsulated in PLGA covalently conjugated with PEI and followed by addition of CpG ODN non-covalently, could ideally be utilized as a nano-adjuvant to sucessfully deliver vaccine antigens into the desired cells.

INTERFERON REGULATORY FACTOR 4 EXPRESSION IN THYMIC EPITHELIUM REGULATES PERIPHERAL TOLERANCE BY CONTROLLING THYMIC REGULATORY T-CELL OUTPUT

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Interferon regulatory factor 4 (Irf4) has been shown to play a critical role in the differentiation and function of a diverse group of peripheral immune cell populations including B-cells, macrophages, dendritic cells and several T-cell subpopulations. Despite its high expression levels in thymic epithelial cells (TECs) Irf4 has not been studied in the context of thymic microenvironment and its consequent effect on the generation of an immunocompetent set of peripheral T-cells. To address this issue we crossed *Irf4* $^{flox/flox}$ mice with *FoxN1-Cre* mice eventually resulting in TEC-specific Irf4-deficient progeny (Irf4$^{-/-}$). Here we show that the morphology of Irf4-deficient thymi is in general unaffected and the numbers and phenotype of a majority of T-cell populations originating from the thymus appear to be comparable to the control mice. We witnessed however a 50% decrease in the numbers and percentages in regulatory T (T_{reg}) cells in the thymi of 2 months old Irf4$^{-/-}$ mice. This decrease was also present in the periphery where Irf4$^{-/-}$ mice had a 20% decrease in splenic T_{reg} cell population. Aged Irf4$^{-/-}$ mice also showed an increased incidence of autoimmune infiltrations in the salivary glands compared to the controls. Altogether these results indicate a role for TEC-specific Irf4 expression in avoiding peripheral autoimmunity by regulating the maturation process of thymic regulatory T-cells.

A NOVEL IMMUNOFLUORESCENCE METHOD REVEALS STRONG CORRELATION BETWEEN ULCERATIVECOLITIS DISEASE ACTIVITY AND COLONIC $CD4^+CD25^+FoxP3^+$ T CELL COUNTS

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Regulatory T cells (Tregs) are key mediators of immunity. Their quantitativeassessment is of vast medical interest since many inflammatory and autoimmune diseases have been linked to changes in Tregnumbers and/or function. Still, a tool for a precise quantitative and depictive investigation of Tregs in theaffected tissue is missing.In this study we established a novel immunofluorescence (IF) method for regulatory T cells *in situ*, allowing staining of three markers(CD4, CD25, FoxP3) in one section. Biopsies inhealthy subjects (n=15) and in patients diagnosed with ulcerative colitis (n=13 for IF, n=11 for FACS)were assessed for Treg numbers with IF and FACS. $CD4^+CD25^+FoxP3^+$ T cell numbers assessed by IF revealed asubstantially lower frequency of these cells in healthy controls compared to UC patients, correlatingwith results obtained by FACS analysis. In ulcerative colitis patients we observed a strong correlationbetween disease activity scores and percentage of $CD4^+CD25^+FoxP3^+$ T cell (R^2=0.6832,p<0.0001). Correlating results were obtained from FACS analysis (R^2=0.7169, p<0.0001).This new immunofluorescence method allows depiction and quantification of Tregs in paraffinembeddedhuman biopsies. Since validation against the standard method of Treg quantification wassuccessful, this new method will provide further insight into the role of Tregs in autoimmunediseases in humans.

NK CELL RECEPTORS VIEWED BY X-RAY CRYSTALLOGRAPHY

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There are many ways to identify the molecular binding partners active in immunological-processes. However, only protein crystallography offers direct experimental visualization whether the expected interactions are realistic. It namely offers a chance for rational design of modulation of immunological functions. Other advantage of the method is that the inspected proteins are under similar stress as in the tissue (protein concentrations in "crystals" are similar to those in tissues). Drawbacks: (1) preparation of complex crystals may be difficult in some cases, (2) if the receptor-ligand interactions are not stable in time, they should be stabilized, (3) in the restricted two-dimensional space of the proteins anchored inside cell membranes, there are fewer interactions possible than one can observe in solution or in the crystal, and thus more experiments or modelling must be used to verify the correct complexation mode.
The following examples of application of protein crystallography concentrate on: (1) IgG interactions with receptors, self-aggregation of immunocomplexes via IgG-Fc, and the role of glycosylation on their stability, (2) the structures of selected NK-cell receptors and ligands (NKRP1A, NKRP1F, Clr-g, CD69, CD94, Ly49A, and NKG2d) and their supposed interactions.

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PHARMACOLOGICAL TARGETING OF MEK-ERK SIGNALING TRIGGERED BY ITAM-COUPLED REGULATORY RECEPTORS AS A STRATEGY TO RESTORE PLASMACYTOID DENDRITIC CELL FUNCTIONALITY

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Recent studies reported that production of type I IFN and other cytokines by plasmacytoid dendritic cells (pDC) triggered by Toll-like receptors 9 (TLR9) agonists is abrogated by crosslinking of ITAM-coupled regulatory receptors (RR) of pDC, such as CD303 (BDCA2) or CD85g (ILT7). Crosslinking of ITAM-coupled RR initiates in pDC a B cell receptor (BCR)-like signaling, characterized by phosphorylation of SYK, BLNK and MEK-ERK. Here we addressed the question whether pharmacologic targeting of BCR-like signaling can restore functionality of pDC abrogated by ligation of ITAM-coupled RR. To this end we specifically inhibited MEK in pDC exposed to TLR9 agonist CpG-A simultaneously with BDCA2 ligand, hepatitis C virus particles, or with ILT7 natural ligand, CD317 (BST2/tetherin). Our results demonstrate that the immune response of pDCs depends on spatio-temporal characteristics of ligand-pDC interaction, i.e. on the sequential order in which TLR9 or ITAM-coupled RR are engaged with their agonists. Inhibitors of ERK phosphorylation restored TLR9-mediated production of type I IFN blocked by ligation of ITAM-coupled RR. In contrast, production of TNF-alpha, which was also abrogated by ligation of ITAM-coupled RR, was not restored by inhibitors of ERK phosphorylation. Pharmacologic targeting of TLR and BCR-like signaling may constitute an attractive new approach for modulation of pDC activation in pathophysiological conditions.

HELIX POMATIA HEMOCYANIN- A NOVEL BIOADJUVANT FOR VIRAL AND BACTERIAL ANTIGENS

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The killed or subunit viral and bacterial vaccines are both able to induce specific immune response. Unfortunately, killed virus vaccines and bacterial toxoids are weakly immunogenic and need adjuvants.
The hemocyanins are widely used as immune modulators. In the present study we promote the hemocyanin, isolated from the terrestrial gastropod *Helix pomatia* (HpH) as protein-carrier as well as bio-adjuvant. We have analysed its ability to generate protective immune response in Balb/C mice.
The purified HpH was combined with standard antigens and a construct of HpH with influenza virus hemagglutinin intersubunit peptide (IP) or HpH – tetanus toxoid were used for immunization. The combination of HpH and TT led to increased levels of key cytokines for development of T1 or T2-related immune response.
The results observed revealed the potency of HpH to obtain vigorous humoral response stimulating generation of specific antibody-producing B lymphocytes as well as long lasting cellular immunity with antigene specific CTLs.
The obtained data suggests that HpH could be assumed as an alternative adjuvant to conventionally used Alum with similar effectiveness and less side effects. HpH is acceptable as a potential bio-adjuvant for subunit vaccines and it could be used as natural adjuvant or protein-carrier.

LYMPHATIC ENDOTHELIAL VESSELS IN THE TUNICA MEDIA – A PART OF THE VASCULAR ASSOCIATED LYMPHOID TISSUE?

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INTRODUCTION: Atherosclerosis is an autoimmune disease mediated by heat shock protein 60 (HSP60)-reactive T cells. However, even before the onset of disease, mononuclear cell (MNC) accumulations can be found in the intima and they are assigned to be a part of the vascular associated lymphoid tissue (VALT). Up to now we assumed that they reached the intima coming from the blood stream. Based on our discovery of so far unknown lymphatic vasa vasorum (*lvv*), we hypothesize that the first MNCs reach the intima through these vessels which are coming from the lymphatic foci found in the surrounding adventitia.
METHODS: Paraffin sections of normal and atherosclerotic lesions were stained immunohistochemically with antibodies specific for vascular endothelial cells (CD31 and von Willebrand Factor (vWF)) and lymphatic endothelial cells (Podoplanin and LYVE-1), for the demonstration of classical *vv* and *lvv*, respectively.
RESULTS: *Lvv* are present in the aortal adventitia and the media even when classical *vv* are absent. Furthermore, for the first time, *lvv* were discovered in the media of normal arteries and in atherosclerotic lesions, without the presence of classical *vv*. MNC were identified in the lumen of *lvv*, but their exact phenotype remains to be determined.
CONCLUSION: This newly discovered network of arterial *lvv* may represent a so far unknown connection between the outer adventitial and inner intimal VALT, and this play a decisive role in atherogenesis.

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MONITORING OF PROTECTIVE CD8+ T-CELL RESPONSE TO CMV ENABLES SELECTION OF PATIENTS FOR ADOPTIVE TRANSFER OF CMV-SPECIFIC T-CELLS IN A CLINICAL TRIAL.

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Cytomegalovirus (CMV) reactivation often occurs in recipients of hematopoietic stem cell transplants (HSCT) and causes significant morbidity and mortality. We have shown that CMV specific CD8+ T-cells producing both, interferon-gamma (IFNg) and interleukin-2 (IL-2) control CMV reactivation (Król et al. 2011). In order to gain insight into the course of virus – T-cell interaction we have evaluated 16 episodes of CMV reactivations from a cohort of 199 patients after HSCT. In our cohort, we assessed T-cell response in 16 patients with more than one CMV reactivation episode. In 6 patients, we detect presence of dual positive interferon-γ and interleukin-2 CD8+ T-cells one week after a DNA viremia peak and consequently control of CMV reactivation was established (no further DNA viremia). On the other hand, 10 patients didn't respond and suffered from prolonged CMV reactivations and might benefit from adoptive transfer of CMV specific T-cells. We optimized a method of CMV-specific T-cell separation from apheresis product of healthy CMV-immune donor and we are currently recruiting patients to phase I clinical trial EudraCT number: 2012-001335-31 using "Streptacells" - CMV specific donor lymphocytes enriched by imunomagnetic seperation. This work was supported by grant 13-22777S by GACR.

"ANTIGEN-SPECIFIC THERAPY WITH SYNTHETIC MULTI-EPITOPE TARGETING AGENT PROMOTE TOLERANCE OF MS LIKE DISEASE BY INDUCTION OF CD11C$^+$CD11B$^+$GR1$^+$ MYELOID-DERIVED DENDRITIC CELLS"

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Specific neutralization of the pathogenic autoimmune cells is the ultimate goal in therapy of autoimmune diseases. However, the pathogenic autoimmunity in MS, can be directed against several major target antigens, and therefore targeting pathogenic T-cells directed against a single target antigen is unlikely to be effective. We have recently showed that concomitantly targeting multiple pathogenic T cells reactive against all known major target antigens in MS, via a synthetic "multi-epitope-targeting" protein (designated Y-MSPc), encompassing multiple disease-related myelin epitopes, is by far more effective in reversing chronic EAE than via a single peptide or peptide cocktail representing disease-related myelin epitope(s). The Y-MSPc was found superior to peptide(s) in concomitantly downregulating pathogenic T cells, through its efficacy in the induction of effective peripheral tolerance mechanisms, which include cytokine shift, anergy, and induction of CD4$^+$Foxp3$^+$CTLA4$^+$ regulatory T-cells.To elucidate the efficacy of Y-MSPc in suppression of MS-like-disease via induction of tolerogenic DCs, we characterized DCs from spleen and CNS of Y-MSPc treated mice. We now show that both suppression and treatment of ongoing EAE by tolerogenic administration of Y-MSPc is associated with a remarkable increase in a unique subset of dendritic-cells (DCs), CD11c$^+$CD11b$^+$Gr1$^+$-myeloid derived DCs in both spleen and CNS of treated mice. These DCs, which are with strong immunoregulatory characteristics and are functional in down-modulation of MS-like-disease displayed increased production of IL-4, IL-10 and TGF-b and low IL-12. Functionally, these myeloid CD11c$^+$CD11b$^+$Gr1$^+$ DCs suppress the in-vitro proliferation of myelin-specific T-cells and more importantly, the cells were functional *in-vivo*, as their adoptive transfer into EAE induced mice resulted in strong suppression of the disease, associated with a remarkable induction of CD4$^+$FoxP3$^+$ regulatory cells. These results, which highlight the efficacy of "multi-epitope-targeting" agent in induction of functional regulatory CD11c$^+$CD11b$^+$Gr1$^+$myeloid DCs, further indicate the potential role of CD11c$^+$CD11b$^+$Gr1$^+$myeloid DCs in maintaining peripheral tolerance and their involvement in downregulation of MS-like-disease.

TRANSMEMBRANE ADAPTOR PROTEIN SCIMP AND ITS ROLE IN IMMUNE CELL SIGNALLING

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SCIMP (SLP65/SLP76, Csk-interacting membrane protein) is a palmitoylated member of transmembrane adaptor protein family (TRAPs). TRAPs are important components of cell membrane specialized for recruiting of cytoplasmic proteins to the proximity of the cell membrane and thus enabling signal initiation. SCIMP, in contrast to majority of known TRAPs, is not present in lipid rafts. In contrast, it is found in tetraspanin-enriched microdomains. SCIMP is strongly expressed in immune cells, including dendritic cells, macrophages and B cells. In B cells, SCIMP is involved in signal propagation after MHCII crosslinking via the recruitment of BLNK/SLP65 adaptors. In macrophages and dendritic cells SCIMP expression is highly upregulated after proinflammatory stimuli and, above all, after GM-CSF treatment. In these cells SCIMP phospohylation is strongly enhanced after exposure to zymosan (nonspecific Dectin-1 agonist). Protein tyrosine kinases of Src and Syk families are responsible for SCIMP phophorylation after Dectin-1 receptor engagement. Considering the fact that Dectin-1 is presented in tetraspanin-enriched microdomains, we imply SCIMP role in Dectin-1 signalling. For further understanding of SCIMP function under the physiological conditions, detailed analysis of SCIMP-deficient mice will be presented.

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NOVEL CYTOMETRIC DRUG EFFLUX ASSESSMENT MODEL FOR MORE ACCURATE IDENTIFICATION OF CANCER STEM CELLS

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Cancer stem cells (CSC) are drug resistant and survive chemotherapy treatment, contributing to tumor relapse in some patients. Therefore, identifying and targeting, those cells is thought to improve therapy outcome, as well as enhance the general understanding of cancer. Among the methods of CSCs identification is cytometric side population (SP) approach. It takes advantage of molecular pumps, the ATP-binding cassette (ABC) transporters, which cause CSCs to appear "on the side" of the main population of cells when stained with certain dyes. SP method directly highlights the drug-resistant fraction of the cell population, since, in theory, drugs undergo efflux from cells the same way that the dyes used in the procedure. Novelty of the research presented by the authors of this study lies within introducing to the procedure an actual drug, doxorubicin, instead of currently used substitute dyes. The substitute approach is flawed, because there is no clear image of what to simulate: actual drugs use more than one ABC transporter and current knowledge of their efflux process is incomplete. Due to this fact using actual drug to visualise CSCs and drug resistant cells is much more likely to produce reliable results.

A new protocol for SP was created with the use of doxorubicin – a commonly used and naturally fluorescent drug. Procedure was tested on ovarian, breast and colon cancer cell lines. Results show that doxorubicin efflux pattern is different from those created with rhodamine123 or Vybrant DC Violet and most likely is facilitated by different transporters. This undermines the reasoning behind using drug substitutes in SP studies and proves that suggested novel protocol may be the way to reliable identification of CSCs and drug resistant cells.

B LYMPHOCYTES PARTICIPATE ON INNATE IMMUNE RESPONSE AGAINST INTRACELLULAR BACTERIAL PATHOGEN *FRANCISELLA TULARENSIS*

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Francisella tularensis, as an intracellular bacterial pathogen, adhere, interact, and enter the range of phagocytic and non-phagocytic cells. Recently, we have demonstrated, using *in vitro* model, that both, vaccine strain *F. tularensis* LVS is able to adhere and even to enter the human (Ramos) and mouse (A20) B cells where survive in non-replicative state. The entrance of *F. tularensis* into B cells required active participation of bacterium and engagement of B cell receptor. The *in vivo* analysis of the cellular response during early stages of *F. tularensis* LVS i.p. infection on murine model demonstrated that the cells responding early are the B1a cells in peritoneum. These cells are almost infected and overexpress activating markers as MHC II, CD25, CD54, CD69, CD71, CD80 and CD86 very early, 12 and 24 hours, after infection. At the same intervals sorted CD19+ cells from peritoneum of infected mice produce proinflammatory cytokines IL-1β, TNF-α, IFN-γ, IL-12, IL-17, IL-23, anti-inflammatory IL-4 and IL-10 and pleotropic cytokine IL-6. Twenty-four hours after infection murine splenic B cells produce the specific anti-*F. tularensis* antibodies. Moreover, the co-cultivation of A20 B cells with the *F. tularensis* virulent strain FSC200 rendered them to be potent antigen presenting cells presenting *F. tularensis* antigens. Thus, considering the protective efficacy of circulating antibodies, production of cytokines, and potent antigen-presenting function of B cells, B cell-mediated, as well as T cell-mediated immunity plays an equivalent role in control of *F. tularensis* infection in mice.

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THE CD2 - CD58 AXIS IS THE PRIMARY COSTIMULATORY PATHWAY IN HUMAN CD28 NEGATIVE CD8 T CELLS

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Aging and repeated antigen encounter eventually leads to a state known as immune senescence. Loss of CD28, the most potent costimulatory receptor on human T cells, is generally regarded to be a main predictor of biological aging of the human immune system. Since CD28 negative T cells cannot receive signals via this potent costimulatory pathway, insufficient activating signals might contribute to the senescence state of these subsets. There are a number of other alternative costimulatory pathways (e.g. CD58/CD2, 41BBL/41BB, ICOSL/ICOS,..) which can efficiently stimulate human T cells. Currently, little is known on the role of these costimulatory ligands in the function of CD28-negative T cells.
In this study, we addressed whether alternative costimulatory signals are able to overcome this senescent phenotype and restore immune function of CD28-negative T cells.
Using an experimental system termed T cell stimulator cells we activated CD28-negative CD8 T cells from elderly individuals in the presence of the most important costimulatory ligands CD58, 41BBL, ICOSL, CD166, CD70, OX40L, GITRL and CD54. We found CD58 to generate the most potent costimulatory signals in these T cells. Furthermore, we demonstrated that CD2 signals potently stimulated the *in vitro* expansion of CD28-negative T cells and their differentiation to effector cells.
The CD2-CD58 axis has an important role for the activation of CD28 deficient T cells.

PLASMINOGEN SYSTEM IN APOPTOSIS AND EFFEROCYTOSIS

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Apoptosis, a genetically-programmed process of cell death, is an essential physiological process engrossed, for example, in tissue remodelling or haematopoiesis; when dysregulated, it contributes crucially to development of pathologies. Apoptosis involves many cellular signalling pathways triggered by a range of various stimuli, intracellular proteolytic cascades, DNA cleavage, followed by cytoplasmic condensation, membrane blebbing and coordinated formation of apoptotic bodies. The apoptotic program culminates in the removal of apoptotic bodies – they are rapidly phagocytosed by macrophages or other surrounding cells, in the process also called efferocytosis. The extracellular proteolysis is also involved in apoptosis, although it is less well understood than the intracellular machinery. Here, we analyze a role of the plasminogen activation system in regulation of apoptosis and efferocytosis, and show that the plasminogen system represents a crucial component of the anti-inflammatory apoptotic milieu.

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ESTABLISHING A MODEL OF AMBROSIA ARTEMISIIFOLIAPOLLEN-INDUCED ALLERGIC ASTHMA IN BALB/C MICE

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Ambrosia artemisiifolia, commonly known as ragweed, is a highly invasive plant with pollen that causes severe allergy. We sought to establish an experimental mouse model of ragweed pollen-induced allergic disease to study factors that alter pollen allergenicity. Using Ambrosia artemisiifoliapollen samplesfrom commercial (Allergon, Sweden) and collected sources, we compared *in vivo* allergic responses in mice.We administered either 4 or 6 doses of pollen suspended in PBS intranasally (i.n.)to 6-week old female BALB/c mice over a 3-week period and 72 h after the last pollen challenge,we evaluated lung and airway inflammation, mucus secretion and serum Ambrosia-specific IgG1. We found that both protocols induced allergic airway and lung inflammation and mucus hypersecretion, and antibody production, but lowerserum pollen-specific IgG1 antibodies after 4 doses compared to 6 doses. When we administered6dosesof 100, 10, 1, and 0.1 µg/50µl of pollen suspended in PBS,we determined that the 10 µg/50µl was the most reliable dose leading to approximately 30% eosinophils in bronchoalveolar lavage fluid.We additionally observed that there were differences in the allergic responsiveness between Ambrosia pollens tested from different sources, which suggested that allergenicitymight be correlated with specific environmental factors. Taken together, we have established a reliable Ambrosia-pollen induced experimental mouse model that provides new knowledge and opportunities to further understandthe interaction between pollenand the respiratory tract.

PIX RhoGEFs REGULATE T CELL MOTILITY REQUIRED FOR DEVELOPMENT AND FUNCTION

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Lymphocyte development proceeds in stages that are dependent on the correct positioning of precursors in microcompartments of lymphoid organs where they can make the necessary cell-cell contacts for receiving maturation signals. Our interest in RhoGEFs, activators of Rho GTPases and cytoskeletal rearrangements, led us to investigate how thymocytes develop and function in the absence of PIX RhoGEFs. αPIX (arhgef 6) is an activator of Rac and Cdc42 that is specifically expressed in immune cells, although it has a widely-expressed homolog called βPIX (arhgef7). Both PIX proteins bind to PAK1 and PAK2 and form a stable complex with the ArfGAPs GIT1 and GIT2. These bind to paxillin and can localize the PIX-GIT complex to focal adhesions. We found that thymocyte development in αPIX knockout mice is compromised due to greatly increased migration speeds of αPIX knockout thymocytes that preclude effective cell-cell contacts in the cortex. At a cellular level, αPIX knockout T cells show abnormal localization of βPIX and activated Rac, suggesting that a balance between αPIX and βPIX regulates localization and activation of GTPases. Knock down of αPIX in HSB2 and Jurkat T cells confirmed these results. On a molecular level, size exclusion chromatography and Blue Native PAGE of αPIX knockout thymocytes showed defects in the PIX-GIT complex which is larger and dominated by an increase in βPIX. Our aim is to determine how the mechanisms controlled by αPIX restrain lamellopodia formation and migration speed of lymphocytes.

MECHANICAL PROCESSING OF TISSUE SUPPRESSES NK CYTOTOXCICITY OF RESIDENT LEUKOCYTES THROUGH THE RELEASE OF HUMORAL FACTORS

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We previously reported that marginating-pulmonary (MP) cells, leukocytes which reside within and adhere to the lung capillaries, are unique in persistently containing activated NK cells and in exhibiting high lyses capacity against various autologous "NK-resistant" target cells. Here we attempted to delineate the relative significance of MP-NK cells within the entire lung NK cytotoxic ability. Thus, we compared the cytotoxicity of MP-NK cells, collected by forced lungs perfusion, to cytotoxicity of the entire lung cell population following mechanical mincing of the lungs tissue. Whereas MP-leukocytes exhibited profound cytotoxicity (50%-80%), the entire lungs cell population displayed minimal levels (up to 20%), although containing equivalent number of NK cells ($CD161^{++}$), suggesting that some processes induced by lung mincing suppress NK cytotoxicity. Indeed, washing of this preparation, or Ficoll-Paque-based enrichment and washing, increased lysis capacity, but only slightly. Moreover, co-incubating MP-NK cells with supernatant harvested from minced lungs tissue or with IL-10 and TGF-β markedly reduced MP-NK cytotoxicity in a dose and time dependent manner. The supernatant of minced lungs contained high levels of IL-10, IL-6, and IL-1-beta. These compounds, as well as TGF-beta and PGE2, suppressed MP-NK cytotoxicity in vitro. Similar findings were observed following mincing of liver tissue.
Overall, these findings indicate that the procedure of tissue mincing entails a release of certain NK-suppressing factors, which are currently targeted in our on-going studies, factors that may distort ex-vivo findings from processed tissue.

IMMUNOSUPPRESSIVE, STEMNESS AND DRUG RESISTANCE PROPERTIES OF OVARIAN TUMORS

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The aim of this study is to evaluate the relationship between drug resistance, stemness and immunosuppressive properties in ovarian cancer patients. For this study, 20 pathologically verified tumor specimens and peripheral blood samples were obtained from women with serous epithelial ovarian cancer. Flow cytometry was used to phenotype immunosuppressive T lymphocyte subpopulations in peripheral blood, to determine tumor leukocyte infiltration and to evaluate the expression of a panel of stemness-associated markers and multidrug resistance proteins in tumor cells. Immunosuppressive enzyme IDO in serum and tumor lysate was quantified by an ELISA method. qPCR was used to quantify the relative expression of drug resistance-associated genes. The expression of all markers differs significantly among patients. After applying correlation analysis, it was shown that the quantity of immunosuppressive enzyme IDO in tumor lysate correlates significantly with the expression of multidrug resistance proteins ABCC1 ($R=0,91$, $p<0,05$, $n=9$) and ABCG2 ($R=0,74$, $p<0,05$, $n=11$) in tumor cells. Also, statistically significant correlation was found between tumor leukocyte infiltration and stemness-associated markers CD44 ($R=0,76$, $p<0,05$, $n=15$) and CD133 ($R=0,54$, $p<0,05$, $n=16$) expression in tumors. It follows that the more tumor cells possess drug resistance markers ABCC1 or ABCB1, the greater quantity of immunosuppressive enzyme IDO is detected in these tumors. Moreover, the higher the tumor leukocyte infiltration level, the more tumor cells express stemness-associated CD44 or CD133 surface markers.

AUGMENTED RESPONSE TO VACCINIA VIRUS (VV) IN CD69 DEFICIENT MICE

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CD69, induced upon activation, regulates leukocyte migration and cytokine and chemokine secretion in models of pathologies mediated by inflammatory and immunity reaction. CD69 is also an inhibitor of Sphingosine-1-phosphate receptor 1 ($S1P_1$). FTY720, or Fingolimod, is currently being used in multiple sclerosis clinical trials, and is an agonist of $S1P_1$. FTY720 causes the retention of lymphocytes in the lymph nodes, and has been found to reduce and improve infection outcome (Walsh et al., 2011 and Teijaro et al., 2011). The aim of this study was to relate the extent of leukocyte activation and recruitment to viral titers and to examine whether CD69 plays a role attributed to activation and migration of leukocytes in viral infection outcome. To this end, we evaluate the immune response to VV infection in CD69-/- mice and at different times, flow cytometry was used to measure changes in immune cell populations in the spleen, peritoneum and the blood. The CD69 deficient mice are significantly more efficient in removing the virus when comparing, CD69+/+ and CD69-/- mice ($2x10^7 \pm 0.5$ vs. $5x10^7$ ± 1 pfu; $P<0.05$) , as well as CD69+/+RAG-/- and CD69-/-RAG-/- mice($2,5\ x10^7 \pm 1$ vs. $4,5\ x10^7 \pm 2$ pfu; $P<0.05$) at seven days after infection. We found that the reduced viral titer in CD69 deficient mice correspond with a higher total cell number, that included B, T and NK lymphocyte and an increase of INFg and TNFa production. When INFg was blocked or NK cells were depleted in CD69+/+RAG-/- and CD69-/-RAG-/- mice, the CD69 deficient mice lost their greater capacity to eliminate the VV. Though, NK cells and INFg seemed not to be as important in the immune response to VV in CD69+/+ and CD69-/- mice, because after NK cell depletion or INF g blocking, CD69 deficient mice continued to being better eliminating VV. In infected mice, CD69 deficient NK cells showed an augmented proliferation measured by BrdU incorporation, but no differences were found in CXCR4 or T-bet expression. In conclusion, CD69 deficient mice reached decreased viral titers and undergo changes in immune cell populations in the lymphoid organs following infection. Thus, CD69 may play a role in controlling anti-viral response. Further studies will investigate the role of CD69 in the inflammatory mRNA profile, the activation level of antigen presenting cells, and $S1P_1$ protein expression at different time points in these mice following infection.

CD69 TARGETING CONTROL THE RESPONSE TO VACCINIA VIRUS INFECTION

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CD69 is a membrane receptor that was first described as a leukocyte activation marker. However, studies with CD69 knockout mice suggested that CD69 has an important function in the control of the immune response and the inflammatory phenomenon. The role of CD69 as immune regulator opened the possibility for therapy of immune diseases by targeting CD69. In our lab, we obtained several mAbs anti-CD69 recognizing murine CD69. To check the possible importance of targeting the CD69 molecule in immune response against vaccinia virus infection, we used the mAb anti-CD69-2.2 that previously proved to be effective in eliminating murine induced tumors (Blood 2005). We found that the infected mice treated with anti-CD69 2.2 had higher cell number in the spleen and thymus but less in bone marrow compared to untreated mice. In addition, anti-CD69-2.2 treatment induced an increase of more than two fold in TNF and IFN g production in NK cells and had increased cytotoxic granules measured by CD107a marker. These mice treated with anti-C69 2.2exhibited lower viral title and therefore greater ability to virus clearance. Thus, these results indicate that CD69 could be consider as a target molecule for the therapy of infection diseases.

INVESTIGATING THE NUCLEAR FUNCTION OF INTERLEUKIN-1ALPHA

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Interleukin-1alpha (IL-α) is a well-known key proinflammatory mediator acting as a secreted molecule. Interestingly, a significant proportion of pre-IL-1α molecules is translocated to the cell nucleus and increasing evidence suggests their non-canonical nuclear function, which classifies IL-1alpha as a "dual function cytokine". Despite the importance of IL-1α, little is known regarding its binding targets and functions in the nucleus. We have previously found that IL-1α interacts with histone acetyltransferase complexes (Buryskova et al., JBC 2004). Recently, we took advantage of the evolutionary conservation of the histone acetyltransferase (HAT) complexes throughout eukaryotes and employing the power of yeast genetics, we mapped the IL-1α-binding site to the HAT/Core module of the *Saccharomyces cerevisiae* SAGA complex (Zamostna et al., PLOS ONE 2012). We also predicted the 3-D structure of the IL-1α N-terminal domain and, by employing structure similarity searches, we found a similar tertiary structure within the C-terminal regulatory region of the AMP-activated/Snf1 protein kinase, which interacts with HAT complexes both in mammals and yeast. This finding is further supported by the ability of IL-1α to partially rescue growth defects of *snf1Δ* yeast strains on agar plates containing 3-Amino-1,2,4-triazole, a competitive inhibitor of His3. Our data allow us to hypothesize that IL-1α can compete with AMP-activated/Snf1 protein kinase for the same binding site on the eukaryotic histone acetyltransferase complexes, which would broaden IL-1& function to regulation of metabolism and response to metabolic stresses.

PHENOTYPICAL AND FUNCTIONAL DIVERSITY OF FOLATE RECEPTOR B-POSITIVE MACROPHAGES

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Macrophages are important cellular components of innate immunity, which potently influence T cell responses by direct antigen presentation or cytokine release. Macrophages also contribute to tissue homeostasis by clearing up cell debris or tissue remodeling and can efficiently transit between various activation states according to local needs. But when this balance is perturbed, exacerbated macrophage responses underlie the pathology of various diseases. One disease-associated macrophage subset found in both rheumatoid arthritis- and cancer-affected tissues was identified in folate receptor β (FRβ) positive cells. Nevertheless, it has not been not clear how this subset contributes to disease pathology. To clarify the function of $FR\beta^+$ macrophages, we differentiated human peripheral blood monocytes to $FR\beta^+$ and $FR\beta^-$ macrophages. We determined their phenotypic characteristics and phagocytic capacity by flow cytometry, measured released cytokines and tested their stimulatory capability by coculture with T cells. We observed that FRβ is present on several functionally distinct subtypes. By gaining the possibility to generate *in vitro* macrophages that resemble those in diseased tissues we can comprehensively study their biology and find ways for their elimination or reprogramming.

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CHARACTERIZATION OF MEMORY T CELLS AND PLASMA CELLS NICHES IN THE BONE MARROW

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Recent studies indicate that the bone marrow plays a key role in the survival of immunological memory mediated by cytokine/chemokine producing stromal cells. Our group has demonstrated that, during aging, there is a decline of naïve and an accumulationof effector memory $CD8^+$ T cells, which are maintained by IL-15 producing cells.Moreover, the numbers of plasma cells, which home to $CXCL\text{-}12^+$ stromal cells, decrease in the bone marrow with aging. Studies in mice indicated that memory $CD4^+$ T cells are in contact with $IL\text{-}7^+$ stromal cells. The aim of this study is to characterize the bone marrow niches responsible for the maintenance of $CD8^+$, $CD4^+$ memory T cells and plasma cells and to investigate how they are changing with aging and CMV infection. qPCR experiments revealed that IL-15 mRNA increased both with age and CMV infection, while CXCL-12, CD4 and IL-7 expression is reduced during aging but not affected by CMV. Immunofluorescence staining of tissue sections and FACS analysis of cell suspensions were performed to confirmresults at the protein level. We conclude that changes in the bone marrow niches and chronic CMV infection may lead to decreased antibody responses in elderly.

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A NOVEL SIGNALLING ATTENUATOR IN T CELL DEVELOPMENT AND FUNCTION

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Ligation of the T cell antigen receptor (TCR) induces formation of phosphorylation-dependent signalling networks, ultimately leading to T cell proliferation and differentiation. Composition and dynamics of the TCR signalosome are still incompletely understood. Others and we have recently identified the T-cell-specific phosphoprotein Thymocyte-expressed molecule involved in selection (THEMIS), as a key player in the double positive thymocyte selection process. The exact role of THEMIS in signalling, especially in peripheral T cells, has remained poorly characterized and controversial.

In the present study we show that THEMIS acts as signalling attenuator in the TCR-proximal signalosome. THEMIS exists in a constitutive complex with the adapter molecule Growth factor receptor-bound protein 2 (GRB-2) and the protein tyrosine phosphatases Src-homology phosphatase 1 and 2 (SHP-1 and SHP-2). Following TCR ligation, THEMIS:GRB-2:SHP complexes utilize GRB-2-SH2 domains for rapid recruitment to the immunological synapse via the transmembrane adapter Linker for Activation of T Cells (LAT). We can further show that the THEMIS:GRB-2 complex is indispensable for T cell development in vivo. Consistent with the association of SHP proteins, shRNA-mediated knockdown of THEMIS expression leads to enhanced TCR-induced phosphorylation events and an increase in expression of surface activation markers in mature human T cells and model T cell lines. Ultimately, in the absence of THEMIS, human T cells are significantly more susceptible to activation-induced cell death due to deregulated signalling emanating from the TCR. Our data suggests that THEMIS, via modulation of SHP phosphatases, contributes to a negative feedback that limits activation of peripheral T cells. These findings also have implications for the mechanism that sets the threshold between positive and negative selection during T cell development.

IN VITRO IMMUNOBIOLOGICAL ACTIVITY OF POLY(2-ISOPROPENYL-2-OXAZOLINE) AS MATERIAL FOR BIOMEDICAL APPLICATIONS

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Immunomodulative activities of polymeric biomaterials are the most relevant ones concerning their bioavailability and biocompatibility. Generally, the strategies of triggering appropriate immune responses by functional biomaterials and approaches of biomaterials that mimic the physiological extracellular matrix and modify cellular immune responses have been stressed. Poly(2-alkyl-2-oxazolines) belong to biocompatible and non-toxic polymeric materials with a high potential in biomedical application. Here, we prepared poly(2-isopropenyl-2-oxazoline) (PIPOX) by the free-radical polymerization of 2-isopropenyl-2-oxazoline.
2-Oxazoline group in the side chain allows using PIPOX as a carrier of peptides, saccharides or drugs. Bioimmunological activities of PIPOX were established based on interreactivity of polymer with naïve BALB/c mice splenocytes to reveal the impact on proliferation activity, T_H1/ T_H2/ T_H17/ T_{reg} polarization according to induced production of signature cytokines (IFNγ, IL-4, IL-17 and IL-10) either in whole splenocyte population, compared to adherent cells (dendritic cells, macrophages). PIPOX stimulation of adherent $CD11c^+$ and $CD14^+$ cells spleen cells (1^{st} adherence period), induced significantly enhanced production of IFN-γ and increase in production of IL-17 indicating immune response polarization towards Th1/Th17 over Th2 and Treg immune responses. Adherent spleen cells after the 2^{nd} adherence period, more enriched in $CD11c^+$ antigen presenting cells, produced statistically significantly higher amount of IL-10 indicating Treg polarization of immune response.

CD222 ORCHESTRATES THE INTERACTION OF T CELL SIGNALING MOLECULES

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The complex regulation of T cell activation is not fully understood, but endosomal transport pathways are increasingly recognized as crucial regulators of T cell signaling molecules in space and time. CD222, also known as the cation-independent mannose 6 phospate/insulin-like growth factor 2 receptor, is one of the central components of endosomal pathways – CD222 transports its cargo proteins from both the Golgi apparatus and the cell surface to lysosomes. Further, CD222 is thought to be involved in endosomal recycling pathways and protein transport from the trans-Golgi network to the plasma membrane. Upon T cell activation, CD222 expression is upregulated on the cell surface, yet the biological relevance of this membrane accumulation remains elusive.
In this project we aimed to investigate the impact of CD222 on T cell activation using genetic rescue experiments, mass spectrometry, coimmunoprecipitation and confocal laser scanning microscopy.
We found that knock-down of CD222 in T cells abrogated T cell effector functions, like cytokine secretion and down-regulated calcium fluxes. Via mass spectrometric analysis we identified several interaction partner candidates for CD222 known to be involved in T cell activation and whose distribution was altered upon CD222 knock-down.
Our data uncover a yet undescribed role of CD222 in T cell signaling and we will present a model for the mechanism of CD222-driven regulation of T cell activation.

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EXPRESSION OF SOLUBLE HLA-G IN BLOOD OF KIDNEY TRANSPLANTANT PATIENTS

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HLA-G antigen is a non-classical HLA class I protein characterized by restricted tissue distribution, low polymorphism and immunosuppressive properties. HLA-G binds inhibitory receptors (ILT2, ILT4 and KIR2DL4) present on distinct immuno-competent cells resulting in inhibition of their functions. For that reason the HLA-G expression helps escape of tumor or virus-infected cells from immune control mechanisms. However, HLA-G might have also a beneficial effect on autoimmunity and transplantation. Clinical data indicate that HLA-G molecules contribute to better graft acceptance after heart, liver, and combined liver/kidney transplantation. Limited data have been reported regarding the association of HLA-G and kidney allograft acceptance. Therefore in this study, we investigated the levels of soluble HLA-G antigens (sHLA-G) in serum of renal transplant patients to analyze relationship between sHLA-G expression and renal graft acceptance. We found that the pre-tranplantation levels of sHLA-G decreased in the early post-transplant period (1-2 weeks). After more than one month of post-transplantation time the values of sHLA-G dropped even more at the patients with graft rejection while a substantial increase of sHLA-G was detected at allograft recipients without any rejection episode. We found that sHLA-G values were higher at patients with graft acceptance than with rejection. This observation supports the assumption that the increase of serum sHLA-G may contribute to allograft acceptance.

GC PRODUCTION IN THE THYMUS AND ITS INFLUENCE ON T CELL DEVELOPMENT

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Glucocorticoids (GC) are a class of steroid hormones which are part of the feedback mechanism in the immune system, turning down inflammatory processes by binding to their receptor, the GR. Recent evidence indicates that GC also modulate thymic T cell selection by promoting theselection of thymocytesthat express T cell receptors (TCR) with sufficient affinity for self-peptides, that subsequently build the peripheral T cell repertoire.GC are not only synthesized by the adrenal glands, the thymus is also able to produce GC although there are discrepancies regarding the main thymic site of production: some studies point to thymic epithelial cells (TEC) while others claim thymocytes to be the main source of thymus-derived GC. In order to clarify this issue, we are investigating the potential capability of thymocytes to produce GC and to test whether this affects their own development. We address this by measuring the expression levels of the GC synthesizing enzymes CYP11A1 and CYP11B1, and the GC activating enzyme 11□HSD1 at different stages of T cell development. In contrast to previous studies, we found expression of CYP11A1 and 11□HSD1 in both, thymocytes and TEC, but levels of CYP11B2 were undetectable. In order to analyze the effects of GC on T-cell development and selection we performed fetal thymic organ cultures (FTOC) and we found that low concentrations of GC were able to increase the number of DP (CD4+CD8+) thymocytes whereas higher concentrations induced apoptosis. On the other hand, DN cells (CD4-CD8-) were also affected by GC and appeared resistant to high concentrations of GC. These findings suggest that GC affect T cell development, at least at the DN stage. We are currently investigating whether thymus-derived GC directly shape the repertoire and in this way are required for immune fitness. This would provide new tools to improve GC treatment and dampen GC-dependent side effects during the treatment of autoimmune diseases.

UBIQUITINATION OF THE PB1-F2 PROTEIN OF THE INFLUENZA A VIRUS

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Influenza A virus is important viral pathogen claiming more than half millions of lives each year. Current strategy of virus control is focused on vaccination inducing virus neutralization anti-hemagglutinin antibodies. Undesirably, influenza A virus hemagglutinin rapidly evolves and escapes neutralization by antibodies. Therefore it is important to identify and characterize novel antiviral targets. We had observed that antibodies against C-terminal part of influenza A virus PB1-F2 protein modestly protect mice against virus infection. PB1-F2 is a very small unstable protein characterized by very poor immunogenicity. We have identified central role of K73,78,85 residues for rapid proteasome degradation of PB1-F2. Substitution of these residues by R73,78,85 resulted in increased expression and enhanced humoral immune response. By DNA vaccination of Balb/c mice with plasmid expressing wt PB1-F2, PB1-F2 K73,78,85R or Stop3aa variant we have observed K73,78,85R mutant protein PB1-F2 confer enhanced protective immunity against infection. Work was supported by grants: VEGA 2/0176/12, 2/0100/13, 2/0117/11, APVV-1605-06, APVV-0250-10, No. DO7RP-0025-10.

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DEFECTIVE RECRUITMENT OF MONOCYTES Ly6C^{hi} AND PLASMACYTOID DENDRITIC CELLS (pDCs) TO THE PERITONEAL CAVITY OF CD38$^{-/-}$ MICE IN RESPONSE TO PRISTANE-INDUCED CHRONIC INFLAMMATION

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A subset of inflammatory monocytes expressing CCL2 and high levels of the surface marker Ly6C is recruited to the peritoneum in response to MCP-1 and is a major source of IFN-I in pristane-treated mice. In the present study, the role of CD38 in the early phase of the pristane-induced lupus disease was examined. Two weeks following pristane injection, the total number of peritoneal cells in pristane-treated CD38$^{-/-}$ mice was slightly lower than in wild-type mice but much higher than in untreated mice, indicating that pristane could induce an inflammatory response in CD38$^{-/-}$ mice. The effect of CD38 deficiency on the recruitment of Ly6C^{hi} monocytes (CD11b^{+}Ly6C^{hi}Ly6G^{-}) also was examined. Peritoneal exudates from pristane-treated CD38$^{-/-}$ mice contained less Ly6C^{hi} monocytes and less Gr1^{+}B220^{+}CD11b^{-}pDCs in comparison with pristane-treated B6 controls. Peritoneal neutrophils and myeloid DCs were largely unaffected. Because Ly6C^{hi} monocytes, and to lesser extent pDCs, are the major source of Type I IFN production in the inflamed peritoneum of pristane-treated mice, these results are consistent with the defective expression ofType I IFN-inducible genesfound in peritoneal cells from pristane-treated CD38$^{-/-}$ mice. These findings suggest an important role for CD38 in the response of monocytes and pDCsto pristane and their recruitment to the primary site of inflammation that is thought to trigger lupus onset in this experimental model of SLE.

MAST CELLS CONDITION DENDRITIC CELL-MEDIATED RE-STIMULATION OF T-CELLS

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Mast cells are connective tissue- and mucosa-resident immune cells that are considered to significantly participate in regulation of acquired and innate immune defense system. Increased mast cell numbers are observed in inflamed tissues and many tumors. Mast cells accumulation within tumor environment is considered to play a significant role in tumor development and regulation of tumor-targeted immune response. Previous reports have shown that activation of $CD8^+$ T-cells was enhanced in the presence of stimulated mast cells. However, this enhancement only reflected an immediate (acute) impact stimulated mast cells had on $CD8^+$ T-cell activation. Using a model of human mast cells, LAD2, and human monocyte-derived mature dendritic cells, we found that extended (chronic) exposure of mast cells to T-cells impairs the ability of $CD8^+$ T-fraction to become re-stimulated with dendritic cells. Our results showed that an extended (7 days) co-culture of mast cells with T-cells that were previously stimulated (primed) with dendritic cells compromised the ability of the $CD8^+$ T-cell fraction to become re-stimulated with the dendritic cells. This compromised ability was evidenced namely by decreased (>70%) de novo production of IFN-γ and TNF-α post the re-stimulation. These data indicate that albeit a short-term impact of mast cells on T-cells is associated with enhanced responses of $CD8^+$ T-cell fraction the long-term impact is opposite. In summary, our data suggests that the increased mast cell numbers in the vicinity of cancer cells may dampen an effective immune response to cancer cells through regulation of $CD8^+$ T-cells responsiveness to re-stimulation with dendritic cells.

A NOVEL *STAT1* MUTATION AND IMPAIRED DEVELOPMENT OF IL-17-MEDIATED IMMUNITY IN A HUNGARIAN PATIENT WITH CMCD

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Chronic mucocutaneous candidiasis disease (CMCD) is characterized by persistent or recurrent infection of the skin, nails, and oral- or genital mucosae caused mostly by *Candida albicans*. Heterozygous gain-of-function signal transducer and activators of transcription 1 (*STAT1*) mutations affecting the coiled-coil domain and the DNA-binding domain (DBD) of the protein have been shown to cause CMCD. We have examined a 26 year-old Hungarian female with severe CMCD. Genetic analysis of coding exons was performed by Sanger sequencing. We identified a novel heterozygous c.1219C>G (L407V) mutation in *STAT1* affecting the DBD of the STAT1 protein. Non-adherent leukocytes from patient and healthy control were activated with anti-CD3 and treated with different cytokines (IL-1β, IL-23, IL-6, TGF-β and IL-2). After 5 days, small percentage of circulating IL-17A- and IL-22-producing CD4+ T cells were detected by flow cytometry after intracellular staining. Negligible concentration of IL-22 by differentiated T cells and small Candida-induced secretion of IL-22 by mononuclear cells were measured by ELISA compared to healthy control. These findings suggest that the new mutant allele may result in impaired development of CD4+/IL-17+ and CD4+/IL-22+ cells causing susceptibility to *Candida* infection on body surfaces

COMPASSIONATE-USE THERAPEUTIC DENDRITIC CELL VACCINATION OF PATIENTS WITH TERMINAL-STAGE MELANOMA: CLINICAL RESULTS

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Immunotherapy remains the main treatment option for patients with advanced melanoma. Among various immunotherapeutical approaches, specific active immunotherapy or therapeutic cancer vaccination is among the most promising melanoma treatment options. It targets dendritic cells (DCs), which are the most potent antigen-presenting cells and have a unique capacity of inducing naive and central memory T cell immune response most efficiently. DCs can be therapeutically targeted either *in vivo (in situ* vaccination) or by *ex vivo* manipulations and subsequent re-injection back into the same patient. Here we provide data about therapeutic vaccination of highly pre-treated, terminal-stage melanoma patients, using autologous, monocyte-derived, mature DCs, loaded with autologous tumor lysate. **Patients:** We treated 12 patients with distant metastases to at least two sites (liver, lung, bone, CNS), progressive after treatment with surgery, interferon-α, chemotherapy, irradiation of brain metastases, cryoablation, radiofrequency ablation of liver or pulmonary metastases. Control group included clinically-matched patients (n=12), who received standard of care for end-stage, treatment-refractory melanoma (no specific antitumor treatment, only palliative care). **Results:** no complete responses were observed. Partial responses were observed in 2/12 vaccinated patients (17%), lasting for at least 8 and 10 months. Stable disease was observed in 7/12 vaccinated patients (58%), lasting for a median of 8 months (range 3-14 months), among them 2 patients had mixed responses. Progressive disease was observed in 3/12 vaccinated patients (25%). Hence, overall clinical benefit (objective response + stable disease) was achieved in 9/12 (75%) vaccinated patients. Median overall survival (mOS) was at least 11.95 months for vaccinated pts vs 4.7 months for controls (p=0.00016). In the vaccine group, mOS was at least 13 months for clinical responders vs 8.2 months for non-responders. Six-month survival rate was 100% in vaccinated patients vs 41.6% in controls. One-year survival rate was 58.3% in vaccinated patients vs 0% in controls.

RESPONSE OF CD4+ T CELLS IN CVID PATIENTS AFTER STIMULATION

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Common variable deficiency (CVID) is a heterogeneous group of disorders characterized by the impairment in antibody production. Underlying genetic defects are not yet well defined. Apart from defects in B-cell function one of the possible mechanisms could be a failure in T cell - B cell activation and interaction. Crucial for the production of immunoglobulins is the interaction betweencostimulatory molecules CD154-CD40 onCD4+ T cells and B cells, respectively. For this reason we studied CD4+T-cell activation after 4 hour stimulation with ionomycine (IM) and phorbolmyristate acetate (PMA) and after 24 hour stimulation with anti-CD3 and anti-CD28 monoclonal antibodies.In addition, we performed 3 day proliferation tests using incorporation of ^{3}H thymidine after stimulation with mitogens Phytohemagglutinin, Concanavalin A and combination of anti-CD3 and anti-CD28.

We found statistically significant decrease in the percentage of T helper cells which expressed CD69 or CD154 between CVID patients and healthy controls.Reducedproliferation activity was present for all mitogens used in this study. Furthermore,there was no correlation between the intensity of proliferation for all the stimuli and PMA- and IM-triggeredexpression of CD154 in a group of CVID patients while such a correlation was observedin thehealthy control group ($p<0.02$).

The fact that,unlike in healthy controls,in CVID patients we see decreased activation (CD69) and/or reduced expression of CD154 together with reduction in proliferation activity without correlation could indicate that more pathwaysleading to actual proliferation after stimulation are affected.

B7 COSTIMULATION AND INTRACELLULAR INDOLEAMINE 2,3-DIOXYGENASE (IDO) EXPRESSION IN UMBILICAL CORD BLOOD AND ADULT PERIPHERAL BLOOD

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Alterations in the expression of B7 costimulatory molecules and their receptors as well as differences in the tryptophan catabolic pathway may influence immunological reactivity of umbilical cord blood (UCB) compared to adult peripheral blood (APB) T lymphocytes. We determined the frequency of activated (CD11b+) monocytes expressing B7-1, B7-2, B7-H1, and B7-H2, and that of T cells and CD4+ T helper cells expressing CD28, CTLA-4, PD-1, and ICOS in UCB and APB samples using flow cytometry (BD FACS Aria). We also examined the intracellular expression of indoleamine 2,3-dioxygenase (IDO) applying flow cytometry and plasma levels of tryptophan (TRP), kynurenine (KYN) and kynurenic acid (KYNA) using high-performance liquid chromatography. The level of CTLA-4 expression on CD4 cells was higher in UCB compared to APB, indicating that the possibility of CD28-mediated costimulation may be decreased. The level of the corresponding costimulator molecule, B7-2 was also elevated. Therefore, this inhibitory relation may function to a higher extent in UCB than in APB. The plasma KYN to TRP (K/T) ratio was two-fold higher in UCB compared to APB. However, the capacity of UCB monocytes compared to APB monocytes was lower to produce IDO, and reverse signalling via B7-2 in UCB monocytes was found to be immature, which suggests that the observed increase in K/T ratio may be due to placental rather than fetal overexpression of IDO in competent cells. These factors may all contribute to the previously observed reduced reactivity of UCB T lymphocytes compared to APB T cells.

PRODUCTION AND TESTING OF ANTI-ERBB2 F(AB')2-S

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Trastuzumab, a humanized anti-ErbB2 antibody is a specific targeted therapy against ErbB2 positive tumors, with a history of both success and a high rate of therapy resistance. Another humanized antibody, pertuzumab inhibits ErbB2 heterodimerization. Combined application of the two antibodies may improve treatment outcomes by either enhancement at the molecular level (e.g. by hypercrosslinking-based enhanced internalization of ErbB2), or offering sterically complementary docking sites for NK cells implementing ADCC. Distinction between these routes can be ascertained by also testing the $F(ab')_2$ fragments of these antibodies.
$F(ab')_2$ from trastuzumab and pertuzumab was produced with pepsin-agarose and separated with chromatography optimized for exclusion size and ionic strength. Affinity and lack of Fc fragment on $F(ab')_2$ was tested with immunofluorescence in flow cytometry. In vitro Ki was assessed with an MTT based assay. ADCC in the presence of the whole antibodies and absence thereof with $F(ab')_2$ was tested in a real time adherence assay. The effect on proliferation of in vitro sensitive BT-474 and resistant JIMT-1 cell lines and was not affected by removing FC region. The only measurable difference was a slightly enhanced dimerization inhibition by the smaller pertuzumab $F(ab')_2$ as opposed to its intact parent antibody. Also, intact antibodies mediated ADCC-based killing of both tested cell lines, while $F(ab')_2$ fragments did not.
The set of trastuzumab and pertuzumab whole antibodies and their $F(ab')_2$s are ready for in vitro and in vivo testing of their possible synergistic effects.

SYNERGISM BETWEEN GM-CSF AND IL-17 CAUSES ENHANCED JOINT PATHOLOGY VIA THE PRODUCTION OF IL-6 AND IL-23

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Recent studies highlight a surprising role for T-cell derived granulocyte-macrophage colony stimulating factor (GM-CSF) in the pathogenicity of Th17 cells. We examined the mechanism by which IL-17 and GM-CSF contribute to cartilage- and bone damage of synovial joints during experimental arthritis, and provide a rationale for combination therapy in auto-inflammatory conditions. Collagen-induced arthritis (CIA) was elicited in DBA/1J mice. Neutralizing antibodies to IL-17 and/or GM-CSF were administered after onset of disease for 14 days. Combined therapeutic treatment of mice early after the onset of CIA ameliorated disease progression and joint inflammation. Simultaneous blocking of GM-CSF and IL-17 was also the most effective treatment in the prevention of radiological bone damage and histological cartilage destruction. To provide further insight in local additive or synergistic effects of IL-17 and GM-CSF, overexpression of IL-17, GM-CSF or the combination was achieved with adenoviral vectors. Inflammatory infiltrate and cartilage- and bone damage developed in all groups from day 1 after adenoviral transfer, with the most severe effect observed in the combination group. Overexpression of GM-CSF alone induced IL-1β, which was enhanced by IL-17. Interestingly, overexpression of IL-17 alone caused a clear increase in synovial IL-6 production, which was dramatically enhanced in the co-presence of GM-CSF. In addition, a strong synergistic effect of combined overexpression was seen on the Th17 differentiation factor IL-23. We show that IL-17 and GM-CSF cause joint damage through synergistic effects on inflammatory mediators in synovial joints. Combined inhibition of IL-17 and GM-CSF might be an interesting option for RA patients that do not fully respond to inhibition of the separate cytokines.

B-REGULATORY CELLS IN CVID PATIENTS

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B-regulatory cells (Breg cells) were described in human as B-cells with the phenotype CD19+CD24++CD38++IL10+. This phenotype that has been previously associated with immature transitional B cells, it comprises the highest fraction of IL-10-producing B-cells upon CD40 stimulation in human peripheral blood in healthy individuals. Bregcells suppress production of TNFα+ and IFNγ+ by CD4+ T-cell.CD40 signalling and the engagement of CD80 and CD86 are pivotal for the generation and function of Breg cells. Defect of CD40 ligand expression on T-cells and low levels of CD80 on B-cells was described in CVID patients. For these reasons we have examined the number and function of Breg cells in CVID patients. We have investigated the production of IL10+in B-cells and intracellular TNFα+ and IFNγ+ CD4+ T-cells in 28 CVID patients and 24 healthy controls. We did not find differences between IL10 positive B-cells in CVID patients and control group.When comparing CVID patients with healthy controls the frequency of CD19+CD38++CD24++IL10+ Breg cells were decreased in patients with CVID ($p<0,0001$).We did not observe correlation for subpopulations CD19+CD38++CD24++IL10+ Breg cells and CD4+TNFα+ and CD4+IFNγ+T-cells, but we found negative correlations between percentage of 19+IL10+ B-cells and frequencies of CD4+TNFα+ and CD4+IFNγ+ T cells ($p=0,0166$ and $p=0,0405$).We did not find these correlations in the control group. Our experiment showed that the population of IL10+ B-cells but not CD19+CD38++CD24++IL10+ B-cells inhibit proinflammatory cytokine production by CD4+T-cells in CVID patients. This inhibition is proportional to the increaseof number of IL10+ B-cells in CVIDpatients.

DEFECTIVE CELL DEATH AFFECTS TREG CELL DEVELOPMENT ANDFUNCTION

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Defects in cell death caused by overexpression of anti-apoptotic Bcl-2 or loss of pro-apoptotic Bim facilitates the appearance of autoimmunity in mice and is also associated with autoimmune diseases in humans. As the elimination of autoreactive lymphocytes in the thymus (central tolerance) is not complete, peripheral mechanisms involving regulatory T (Treg) cells keep these autoreactive cells in check (peripheral tolerance). Despite the well-established role of Bcl-2 family proteins in shaping the immune system and their frequent deregulation in autoimmune pathologies, it is poorly understood how these proteins affect Treg cell development and function.Here we define a key role for the Bim/Bcl-2 axis in Treg cell development, homeostasis and function but exclude a role for apoptosis induction in responder T cells as relevant suppression mechanism. Notably, only lack of the pro-apoptotic BH3-only protein Bim or Bcl-2 overexpression led to accumulation of Treg cells while loss of pro-apoptotic Bad, Bmf, Puma or Noxa had no effect. Both Bim-deficient and Bcl-2 overexpressing Treg cells displayed reduced levels of key Treg cell markers compared to WT Treg cells. Remarkably, apoptosis resistant Treg cells showed reduced suppressive capacity both *in vitro* and in an *in vivo* model of T cell-driven colitis, posing a *caveat* for the use of such long-lived cells in possible therapeutic settings.

BRIDGING THE GAP: UNEXPECTEDLY COMPLEX REGULATION OF GADS FINE-TUNES SIGNALING THROUGH THE TCR SIGNALOSOME

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T cell antigen receptor (TCR) signals are interpreted by a complex of three adaptors: LAT, Gads and SLP-76, with Gads bridging the interaction between the other two. A Grb2-family adaptor, Gads binds constitutively to SLP-76, and recruits this adaptor to the membrane, via its TCR-inducible, SH2-mediated interaction with membrane-bound LAT. Despite its established role in lymphocyte signaling; the posttranslational regulation of Gads has hardly been considered. We conducted a phospho-mass spectrometry analysis, which revealed a large number of Gads phosphorylation sites, some of which were TCR-inducible. One of the sites, T262, negatively regulated TCR responsiveness. In addition to regulation by phosphorylation, we present evidence that Gads undergoes oligomerization, which profoundly influences its binding to phospho-LAT. Functional analysis of the regulatory mechanisms impinging on Gads requires a clean and tractable genetic system; to this end, we developed a Gads-deficient T cell line using TALEN-mediated genome engineering. Our initial analysis of this cell line revealed Gads to be a rate limiting factor for TCR responsiveness; that determines the sensitivity of cells to weak TCR stimuli. Functional analysis of mutant forms of Gads in this genetic background revealed the contribution Gads domains, oligomerization state, and phosphorylation sites to the fine tuning of T cell responsiveness.

DIFFERENCES IN PROMOTER DNA METHYLATION AND mRNA EXPRESSION OF INDIVIDUAL ALLELES OF HLA CLASS II DQA1 GENE AND THEIR RELATION TO TYPE 1 DIABETES MELLITUS

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BACKGROUND: Large polymorphism of HLA class II genes is not restricted to coding region only, but it also applies to the linked promoter region of the gene, where it forms the basis for different level of individual alleles expression. Different expression of HLA class II alleles has been postulated to influence risk of developing autoimmune disease, as is type 1 diabetes mellitus (T1DM). In addition to genetic variability, variation in epigenetic modifications can be another cause of uneven expression of individual alleles.
OBJECTIVE: We aimed to determine whether HLA-DQA1 gene DNA methylation and mRNA expression differ between 1) individual DQA1 alleles 2) diabetic patients and healthy individuals.
METHODS: 97 healthy donors and 30 T1DM patients were included into study. HLA-DRB1, -DQB1 and -DQA1 genes were genotyped using PCR-SSP. Genomic DNA was converted by bisulfite and target segment in DQA1 gene promoter was PCR amplified. PCR product was cloned into *Escherichia coli* and individual clones were sequenced. mRNA expression of individual DQA1 alleles in peripheral blood leukocytes was quantified by Real-Time PCR.
RESULTS: In both groups tested, the most methylated promoter alleles were QAP 2.1 (DQA1*0201-DR7) and QAP 4.2 (DQA1*0401-DR8), the least methylated allele was QAP 4.1B (DQA1*0501-DR3). The most expressed allele was DQA1*03 (DR4 haplotype). We observed one difference in site-specific DNA methylation between healthy individuals and patients.
CONCLUSION: We found correlation between genotype and epigenotype of DQA1 gene alleles. No clear relationship between alleles DNA methylation, mRNA expression and donors disease status was observed.

EVI2B, A NOVEL C/EBPA TARGET GENE, IS REQUIRED FOR MURINE NEUTROPHILIC DIFFERENTIATION AND CONTROLS HEMATOPOIETIC STEM CELL MAINTENANCE

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EVI2B encodes a transmembrane protein, which is ubiquitously expressed in hematopoietic cells. We recently identified *EVI2B* as one of the genes belonging to the C/EBPa signature, suggesting it might be required for granulocytic differentiation. Nevertheless, the function of Evi2b in hematopoiesis remains unknown. Here, we aim to determine the function of Evi2b in neutrophilic differentiation and hematopoietic stem cell (HSC) maintenance. We demonstrated that C/EBPa binds to and transactivates *EVI2B* promoter in a dose-dependent manner, and that C/EBPa upregulates *EVI2B* expression. In line with these results, we showed that Evi2b expression positively correlates with C/EBPa upregulation during granulocytic differentiation, and that downregulation of Evi2b leads to a block of neutrophilic differentiation in 32D/G-CSF-R cells. Next, we observed that downregulation of Evi2b in murine HSC impairs the ability of these cells to form colonies in semi-solid cultures. Bone marrow transplantation experiments demonstrated reduced repopulating ability of Evi2b-silenced cells in comparison to controls. In addition, we observed reduced HSC proliferation after Evi2b silencing, and accordingly, we showed that Evi2b knockdown increases the percentage of HSC cells in the G_0 quiescent cell cycle phase. Altogether, our data demonstrates that Evi2b is an essential regulator of granulocytic differentiation and HSC maintenance.

ALTERATION OF INFLAMMATION-RELATED MICRORNAS IN PLASMA AND PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PSORIASIS PATIENTS

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MicroRNAs (miRNAs) regulate gene expression and can be detected in circulation.Gene expression levels of inflammation-related miRNA-155, let-7i, miRNA-21, miRNA-146a, and miRNA-223, in peripheral blood mononuclear cells (PBMC); and miRNA-21, miRNA-146a, and miRNA-233 in plasma, were evaluated by quantitative real-time polymerase chain reaction, from eleven plaque-type psoriasis patients; which were treatment-naive or had undergone a 4-6 weeks washout-period for topical, systemic or biological treatments. We examined whether there was any changes after 11-(9-12)-months [(median (25-75th percentile range)] methotrexate or topical (betamethasone + calcipotriene) treatments. Eleven healthy controls age- and gender-matched were studied. At the start of the study, miRNA-155, let-7i, miRNA-21, miRNA-146a and miRNA-223 PBMC gene expressions were up-regulated in psoriasis patients. MiRNA-21, miRNA-146a, and miRNA-223 patients' plasma expressions were also increased. Patients' miRNA-155 PBMC expression was correlated to Psoriasis Area Severity Index (PASI); plasma miRNA-146a and let-7i PBMC expression. All patients responded to treatments (PASI reduction ≥50%). After treatments, patients' plasma miRNA-21, miRNA-146a, and miRNA-223 were down-regulated; miRNA-155 and let-7i PBMC up-regulated expressions were decreased; and a further increase of PBMC'miRNA-146a expression, together with no changes in miRNA-21 and miRNA-223 PBMC expressions, wereobserved. In psoriasis patients there was a similar trend of alteration both in plasma and PBMCs of inflammation related-miRNAs. Patients' plasma miRNAs levels were largely reversible on disease remission. However, differential changes in the expression patterns of miRNAs in PBMCs were observed.

THE ROLE OF THE UROKINASE RECEPTOR IN T CELL ACTIVATION

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T cell activation and migration of T cells are two central processes in adaptive immunity. We are interested in one particular molecule namely CD87 (urokinase receptor, uPAR),which seems to equip T cells with features important in both of them. CD87 is akey regulator of the plasminogen activation system and usually it ishardly found on resting lymphocytes. However, it has already been shown that T cells start to express CD87 upon activation (Nykjaer et al 1994). Furthermore, there is evidence that CD87 is important in lymphocyte migration to the lung (Gyetko et al 2001) and also to tumors (Edwards et al 2006).Yet, detailed mechanisms as well as CD87s role in other aspects apart from migration have so far not been investigated. We therefore examine CD87s function in T cell activation and furtherscrutinize the role of CD87 in migration in more detail.We observed that T cells overexpressing CD87 show a higher response to T cell receptor stimulation as measured by calcium mobilization. In addition, the expression of several characteristic surface molecules is altered upon CD87 overexpression. In our current experiments we want to clarify functional consequences of CD87 overexpression.Further, we intend to dissect CD87s signaling pathways in T cells and elucidate the molecular mechanisms responsible for our observations.