12th EFIS-EJI TATRA IMMUNOLOGY CONFERENCE

Molecular Determinants of T-Cell Immunity

September 03-07, 2016
Štrbské Pleso, Slovakia

PROGRAMME
ABSTRACT BOOK

European Federation of Immunological Societies

12th EFIS-EJI TATRA IMMUNOLOGY CONFERENCE

Molecular Determinants of T-Cell Immunity

CONFERENCE VENUE

Hotel Patria, Štrbské Pleso, High Tatra Mountains, Slovakia

September 3-7, 2016

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

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PROGRAMME

Saturday, 3th September - Arrival of participants

Sunday, 4th September

8:30 - 8:40	Opening the Conference
SESSION 1	**Chairperson: H.Stockinger (Vienna)**
8:45 - 9:30	**Ludger Klein (Munich):** *Functional adaptations of thymic antigen presenting cells for central tolerance induction*
9:30 - 10:15	**Jakub Abramson (Rehovot):** *AIRE*
10:15 - 10:30	**Tea/Coffee break**
10:30 - 11:15	**Anna Ohradanova-Repic (Vienna):** *Specific targeting of activated macrophages in chronic inflammatory and autoimmune diseases*
11:15 - 12:00	**Annamari Ranki (Helsinki):** *APECED as a model for autoimmune mechanisms.*
12:10 - 13:30	**Lunch**
SESSION 2	**Chairperson: J.Ivanyi (London)**
16:30 - 17:15	**Dominik Filipp (Prague):** *Extrathymic Aire-expressing cells in peripheral T-cell tolerance*
17:15 - 18:00	**Paola Romagnoli (Paris):** *Peripheral regulatory T cells recirculating back to the thymus suppress the development of their precursors.*
18:00 - 18:45	**Joanna Hester (Oxford):** *Regulatory T cells in transplantation*
19:00 – 20:00	**Dinner**

Monday, 5th September

SESSION 3	**Chairperson: G.Wick (Innsbruck)**
8:45 - 9:30	**Hermann Wagner (Munich):** *TLRs sensing nucleic acids*
9:30 - 10:15	**Greta Guarda (Epalinges):** *Emerging roles of MHC transcriptional regulators in innate and adaptive immunity*
10:15 - 10:30	**Tea/Coffee break**
10:30 - 11:15	**Christina Zielinski (Munich):** *Regulation of human T helper cell responses by tonicity signals from the microenvironment*
11:15 - 12:00	**Vladimír Leksa (Bratislava):** *Protein trafficking: a prequel to adaptive and innate immune responses*
12:10 - 13:30	**Lunch**
SESSION 4	**Chairperson: A.Hayday (London)**
16:30 - 18:00	Selected poster presentations (each 15 min. including discussion) **Maria M Klicznik (Salzburg):** *A humanized mouse model to study skin immunology* **Katarína Lopušná (Bratislava):** *Type III interferons: the surviving heroes of cell defense* **Ondřej Palata (Prague):** *Development of immuno-monitoring assays for dendritic cell-based lung cancer immunotherapy* **Karthik Subbarayan (Halle):** *Expression, interaction and antitumor activities of biglycan in Her-2/Neu mediated carcinogenesis* **Polina Zjablovskaja and Miroslava Kardošová (Prague):** *The roles of transcription factors C/EBPα and C/EBPγ in granulopoiesis* **Matouš Vobořil (Prague):** *Toll-like receptor signaling in thymic epithelial cells*
18:30 - 19:30	**Dinner**
20:00 - 22:00	**Poster session (with refreshments and wine)**

Tuesday, 6th September

SESSION 5	**Chairperson: V.Hořejší (Prague)**
8:45 - 9:30	**Michael Bachmann:** *Retargeting of T cells with BiTEs and CARs*
9:30 - 10:15	**Lorenzo Moretta (Rome):** *NK cells from the bench to the bed side and back*
10:15 - 10:30	**Tea/Coffee break**
10:30 - 11:15	**Barbara Bohle (Vienna):** *Apples can drive birch pollen-allergic patients nuts: novel insights into cross-reactivity of allergens*
11:15 - 12:00	**Martina Prelog (Würzburg):** *The immune system in old age*
12:10 - 13:30	**Lunch**
SESSION 6	**Chairperson: R.Špíšek (Prague)**
16:30 - 17:15	**Iris Gratz (Salzburg):** *Generation and maintenance of tissue regulatory T cells*
17:15 - 18:00	**Adrian Hayday (London):** *How tissues build their local T cell compartments*
19:30 - 23:30	**Farewell Party**

Wednesday, 7th September - Departure

ABSTRACT BOOK

SPEAKERS

AIRE

Anna Chuprin, Yonatan Herzig, InbalBenhar, Chamutal Bornstein, AyeletAvin, ShirNevo, Yael Goldfarb, AyeletVardi, VladislavKrupalnik, Noam Kadouri, Ben Levi, Milena Rozenberg, Jacob Hanna, Ido Amit, ***Jakub Abramson***

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Promiscuous expression of tissue-restricted self-antigens (TRAs) in medullar thymic epithelial cells (mTECs) is critical for induction of T-cell tolerance to self. This process is largely mediated by the Autoimmune regulator (AIRE). Indeed, AIRE deficiency results in a multi-organ organ autoimmune disorder - the autoimmune polyendocrine syndrome type 1 (APS-1). Although several recent studies provided very important molecular insights into how Aire operates, a more comprehensive understanding of this process still remains elusive. Similarly, our understanding of the transcriptional programs regulating the expression of Aire itself remains unclear. In this presentation I will demonstrate that Aire induces promiscuous gene expression through “hijacking” the DNA damage machinery, a step critical for opening/relaxing the closed chromatin structures at its target genes. I will also discuss in detail our most recent insights into the mechanisms that regulate the expression of Aire itself and will highlight the very complex and layered mechanisms that keep Aire expression off in the vast majority of body cells and which turn it on in mature mTECs

RETARGETING OF T CELLS TO TUMOR CELLS WITH BiTEs and CARs

Michael Bachmann

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Over the past decades various technologies were developed for retargeting of immune effector T cells to tumor cells. Two of them are in the current focus including bispecific antibodies (bsAbs) and T cells genetically modified with chimeric antigen receptors (CARs). For killing both strategies lead to a target-specific cross-linkage between effector- and tumor cells. Conventional bsAbs can simultaneously bind to the surface of the tumor cell and an activating receptor component of the T cell receptor (TCR) complex. Conventional CARs are artificial receptors consisting of an extracellular antibody domain directed to the tumor cell, a transmembrane domain, and intracellular signaling domains of the TCR complex. In parallel to conventional CARs and bsAbs in the so called BiTE (bispecific T cell engager) format, we developed a modular platform technology consisting of effector- and target modules both in the BiTE (UniMAB) and CAR (UniCAR) format. In principle, we split the bsAb or the CAR in two molecules termed effector module (EM) and target module (TM). The respective EM system recognizes a peptide epitope, while the TM is a bispecific fusion molecule recognizingthe tumor cell and containing the peptide epitope. Thus, EMs and TMs can form immune complexes which behave like conventional BiTEs or CARs. In addition to multitargeting capabilities of this platform allowing us to reduce the risk of escape variants, the obvious advantage especially in the CAR setting is that UniCAR modified cells are functionally tunable and will automatically be turned off in the absence of the TM, thus reducing dramatically the risk of side effects.

APPLES CAN DRIVE BIRCH POLLEN-ALLERGIC PATIENTS NUTS: NOVEL INSIGHTS INTO CROSS-REACTIVITY OF ALLERGENS

Barbara Bohle

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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.

EXTRATHYMICAIRE-EXPRESSING CELLS IN PERIPHERAL T-CELL TOLERANCE

Dominik Filipp, *Jan Dobeš, Tomoyoshi Yamano, Martina Dobešová, Ludger Klein*

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Central tolerance is based on negative selection which occurs during T-cell development in the thymic medulla. The key molecule of this process is the autoimmune regulator (Aire), a transcription regulator that promotes the "promiscuous" expression of otherwise strictly tissue-restricted self-antigens (TRAs), specifically in medullary thymic epithelial cells (mTECs). Despite the powerful checkpoint of central tolerance in the thymus, it is clear that negative selection fails to completely eliminate self-reactive T-cells. Recently, using Aire-EGFP-reporter mice, Aire has been suggested to fulfill an analogous function in a rare subset of lymph node cells and thereby support peripheral T cell tolerance. However, the nature, identity and functions of these extrathymicAire-expressing cells (eTACs) remain controversial. Originally, eTACs were suggested to represent a specialized, distinct and nonconventional antigen presenting population of CD45+ MHCII+ CD11clo EpCAM+ phenotype whose presence correlated with the ability to efficiently inactivate neo-antigen reactive CD8+ and CD4+ T-cells. However, the caveat of these experiments is that they failed to explore the potential phenotypic diversity of the Aire-reporter expressing eTACs and their specific function in imposing peripheral tolerance. Our data provide additional insight into the presence of several lymph node cell populations expressing the Aire-reporter construct and point to so far an uncharacterized cell-subset which among all Aire-reporter+ lymph node cells exclusively expresses detectable levels of Aire protein, which are comparable to those observed in mTECs. Phenotypic and molecular characterization of these newly identified Aire protein-expressing lymph node cells (AireP-LNCs) as well as their role in the induction of peripheral tolerance will be presented.

GENERATION AND MAINTENANCE OF TISSUE REGULATORY T CELLS

Douglas F. Pinheiro, Sophie K.Kitzmueller, Megan M. Maurano, Abul K. Abbas, ***Iris K. Gratz***

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Immune homeostasis in peripheral tissues is achieved by maintaining a delicate balance between pathogenic effector T cells (Teff) and protective regulatory T cells (Treg). However, the factors responsible for maintaining this balance – especially in the target tissues – are largely unknown.
To study immune regulatory mechanisms in T cell mediated skin inflammation we established two mouse models that feature tetracycline-inducible expression of chicken ovalbumin (Ova) in the epidermis. In these we can transfer naïve Ova-specific CD4+ DO11 T cells and follow their activation and differentiation into cytokine-producing Teff cells and Foxp3+Treg cells in vivo. Expression of antigen (Ag) in the skin elicits a T cell dependent inflammatory dermatitis that can be controlled by in vivo generated peripheral Treg(pTreg) cells. We found that IL-2 is crucial for the generation of pTreg cells. Additionally, the duration (i.e. transient versus persistent exposure) as well as the dose of tissue-Ag expression is a major determinant of the relative frequencies of Teff and Treg cells. Persistent expression of Ag, a mimic of self-Ag, lead to loss of the Teff cells with preservation of Treg in the target tissue, both of which was associated with reduced ERK phosphorylation. We further found that high doses of tissue-Ag lead to strongT cell receptor (TCR) signaling and a blockade in peripheral Treg cell differentiation, which was lifted at lower Ag doses. Reducing TCR signaling with small molecule inhibitors at high-dose Ag conditions can equally lift the blockade Treg generation in vivo, leading to the regulation of tissue inflammation. Based on these findings we developed a therapeutic immune-modulation protocol with the goal to shift the balance toward Treg cells. With this protocol we could significantly delay the rejection of skin grafts that express a foreign-antigen.
Thus our studies reveal a crucial role for the Ag and TCR signalsin maintaining appropriate ratios of Ag-specific Teff to Treg cells in peripheral tissues.

EMERGING ROLES OF MHC TRANSCRIPTIONAL REGULATORS IN INNATE AND ADAPTIVE IMMUNITY

Greta Guarda

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Nucleotide-binding oligomerization domain-like receptors (NLRs) constitute a family of innate immune receptors. Most of the characterized NLRs initiate inflammatory reactions upon sensing pathogen- or host-derived danger signals. However, through the work of several groups, ours included, it recently became clear that the NLR family member NLRC5 is a transcriptional regulator of major histocompatibility complex class I (MHC class I) genes. In fact, Nlrc5-deficient mice exhibit markedly reduced MHC class I levels, particularly on lymphocytes.

MHC class I molecules are central to cytotoxic responses by adaptive CD8+ T lymphocytes and innate natural killer (NK) cells. Whereas CD8+ T cells detect antigens presented in MHC class I molecules, NK lymphocytes eliminate targets with downregulated MHC class I expression, a phenomenon occurring upon infection or malignant transformation.

Why NLRC5 evolved to control MHC class I transcription in lymphocytes and, most prominently, in T cells remained an open question. The emerging evidence linking NK and T cell responses led us to explore whether NLRC5 regulates this crosstalk. Indeed, we uncovered a role of NLRC5 in protecting CD8+ T cells from NK cell-mediated rejection upon inflammation or viral infection.These findings relevant to immunomodulation and recent advances in our understanding of the molecular mechanisms underlying NLRC5 activity will be discussed.

NOT SIMPLY SUPERFICIAL: HOW EPITHELIAL CELLS CONTROL IMMUNITY

Adrian Hayday

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The thesis of conventional immunology is one of centralised control, whereby the response to infection within tissues is decided within lymph nodes, from which effector T lymphocytes are despatched to quell regional disturbances. But this cannot satisfactorily explain the interaction of the immune system with tissues, since many tissues at steady state are rich in T cells, a fact that raises many questions. For example, do such cells simply provide responses to infection or do they provide a more generalised means to sustain tissue integrity and ogan function? Likewise, how are such cells able to respond to acute stress but not drive constitutive tissue inflammation? And, how do immune cell–tissue interactions relate to organ physiology? To begin to answer these questions, we have adopted a molecular genetic approach to identify key receptor-ligand axes that tissues use to communicate with their local T cell compartments at rest, upon infection and upon non-infectious dysregulation. These axes show how body surface epithelia dictate the composition and activity of the local T cell compartments in an organ-specific fashion, and offer hope for highlylocalised clinical regulation of immune cells.

Strid J, Sobolev O, Zafirova B, Polic B, Hayday A. The intraepithelial T cell response to NKG2D-ligands links lymphoid stress surveillance to atopy. Science. 2011 Dec 2;334(6060):1293-7. doi: 10.1126/science.1211250. PMID:22144628.

Turchinovich G, Hayday AC. Skint-1 identifies a common molecular mechanism for the development of interferon-γ-secreting versus interleukin-17-secreting γδ T cells. Immunity. 2011 Jul 22;35(1):59-68. doi: 10.1016/j.immuni.2011.04.018. Epub 2011 Jul 7. PMID: 21737317

Vantourout P, Willcox C, Turner A, Swanson CM, Haque Y, Sobolev O, Grigoriadis A, Tutt A, Hayday A. Immunological visibility: posttranscriptional regulation of human NKG2D ligands by the EGF receptor pathway. Sci Transl Med. 2014 Apr 9;6(231):231ra49. doi: 10.1126/scitranslmed.3007579. PMID: 24718859

REGULATORY T CELLS IN TRANSPLANTATION

Joanna Hester

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Regulatory T cells play important roles in immune homeostasis and in the induction and maintenance of tolerance to self antigens. Treg can also contribute to the induction of tolerance to donor alloantigens in the context of transplantation. In transplantation, life-long immunosuppression is required to inhibit a potent and complex immune response directed towards transplanted foreign cells or organs which ultimately leads to tissue destruction and allograft rejection. However, prolonged immunosuppressive treatment results in significant side effects such as malignancy, cardiovascular diseases and nephrotoxicity. Therefore, cellular therapy with regulatory T cells, isolated from the patient, ex vivo expanded and administered back to the patient in the peritransplant period has been postulated as a potential, more beneficial way to prevent allograft rejection. Using clinically relevant in vivo model, we have demonstrated that human ex vivo expanded regulatory T cells (Treg) can protect human allografts from rejection. Human Treg accumulate and survive long-term in the allograft and can provide continuous protectionin situ. Together with other regulatory leukocyte populations, including regulatory B cells, myeloid derived suppressor cells and dendritic cells, Treg contribute to the regulation of immune responses in vivo after cell or solid organ transplantation.

FUNCTIONAL ADAPTATIONS OF THYMIC ANTIGEN PRESENTING CELLS FOR T CELL REPERTOIRE SELECTION

Ludger Klein, *ElisabettaPetroziello, Julia von Rohrscheidt*

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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.

SELF-ANTIGEN DIVERSITY IN THE THYMUS: HOW PROMISCUITY GENERATES TOLERANCE

Bruno Kyewski

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

1. Klein, L, B. Kyewski, P.M. Allen and K.A. Hogquist. 2014. Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). Nature Rev Immunology 14, 377-391.

PROTEIN TRAFFICKING: A PREQUEL TO ADAPTIVE AND INNATE IMMUNE RESPONSES

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Textbook schemes usually portray 'sequel' events of intracellular signalling cascades with individual messengers at the right time in the right place – 'ready-steady-go' for theirrace to come. However, it goes without saying that beforethe runners kneel down into starting blocks they must have been transported therein somehow. In other words, for right cellular functions it is essential to control the temporal and spatial distribution of proteins within the cell. Protein trafficking is of special importance in the context of immune cells which have to promptly react on a certain stimulusto combat pathogens and,in the same time,avoid unwanted tissue disturbances.
We have revealedso far unknownpathways controlling these 'prequel' events of intracellular signalling cascades in both adaptive and innate immune responses. First, we have found how the kinase Lck, a key signalling molecule of T cell activation, is transported to the plasma membrane. T cells represent a central branch of the adaptive immune system and controlling the distribution of Lck is prerequisitefora proper T cell responsiveness to the antigen. Second, we have identified novel mechanism of efferocytosis – the clearance of apoptotic cells by macrophages – which as well depends on intracellular transporting pathways. This is essential for homeostatic maintenance. Not only do these mechanisms contribute to our understanding of inflammation and homeostatic maintenance but also they have identified potential targets and tools for pharmacological interventions in disorders accompanied specifically with deregulated T cell activation and efferocytosis.

The research has received funding from the FWF – Austrian Science Fund (P22908), VEGA - Slovak Grant Agency (2/0063/14), and the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement NMP4-LA-2009-228827 NANOFOL.

NK CELLS FROM THE BENCH TO THE BED SIDE AND BACK

Lorenzo Moretta

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

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

THE IMMUNE SYSTEM IN OLD AGE

Martina Prelog

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The aging of the immune system (immunosenescence) is defined as a physiological progress as well as a result of differentiation and maturation from prenatal life to old age and is mainly seen as a process of specialization of the humoral and cellular reactivity to chronic antigen stimulation (i. e. cytomegalovirus) with advancing life. Immunosenescence has strong implications regarding the defense of infectious diseases, of inflammatory conditions, cardiovascular disease and of cancer.
Immunosenescence affects both the innate and the adaptive immune system but has a pronounced effect on the latter. One hallmark of T cell immunosenescence is the involution of the thymus resulting in decreased output of recent thymic emigrants, characterized by lower T-cell-receptor-excision-circles (TRECs) and CD31-expressing and naive T cells and a compensatory increase of peripheral memory and effector T cells as well as homeostatic proliferation of pre-existing peripheral naive T cells. A highly restricted T cell receptor repertoire with clonal exhaustion of effector memory T cells is found in elderly. Decreased IL-2 production and increase of pro-inflammatory cytokines are further impairments. T cell help has been shown to be less sufficient for B cell differentiation in elderly.
An age-dependent decrease of naive B cells and blockade of early hematopoietic progenitors and of B cell precursor maturation has been described in elderly. Similar to aged T cells, B cell immunosenescence is characterized by dysregulation of B cells in interaction with other immune cells, a decreased expression of co-stimulatory molecules and a loss of the diversity of the B cell receptor repertoire. B cell expansion is lower and the size of germinal centers is reduced. A shift in antibody isotypes from IgG to IgM, poor IgG responses to protein and most polysaccharides antigens and decreased persistence of IgG antibodies has also strong implications on vaccine responses in the elderly.

SPECIFIC TARGETING OF ACTIVATED MACROPHAGES IN CHRONIC INFLAMMATORY AND AUTOIMMUNE DISEASES

Anna Ohradanova-Repic, *Christian Machacek, Celine Charvet, Franck Lager, Delphine Le Roux, Eugénia Nogueira, Stephan Blüml, Miloslav Suchanek, Artur Cavaco-Paulo, Georges Bismuth, Hannes Stockinger*

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Rheumatoid arthritis (RA) is an autoimmune disease characterised by immune cell activation, chronic inflammation of synovial lining of joints and hyperplasia, which could lead to the cartilage and bone destruction and disability, if untreated. Activated macrophages are crucial players in disease pathogenicity and their numbers in inflamed synovia predict the severity of the disease. Current RA treatments have often severe side effects or are costly, therefore new therapies that specifically target activated macrophages are being developed. Folate receptor β (FRβ) was described as a marker of RA macrophages. To disect the contribution of FRβ+ macrophages to RA progression, we developed several strategies for their targeting. Firstly, we produced FRβ-specific antibodies and linked them onto liposomes using a novel non-toxic approach that involved a hydrophobic peptide fused to the antibody C terminus. We also designed bispecific antibodies, targeting FRβ and another macrophage marker present in FRβ microenvironment, since our results revealed that FRβ was expressed by several macrophage subtypes. Finally, we used folate as a targeting moiety of liposomes or carrier proteins. All formats were specific to FRβ+ cells and therefore might represent an effective way to deliver immunomodulatory drugs to the pathogenic FRβ+ macrophages and RA treatment.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 683356 and from the 7th Framework Programme under grant agreement NMP4-LA-2009-228827 NANOFOL.

APECED AS A MODEL FOR AUTOIMMUNE MECHANIMS

***Annamari Ranki*[1]**, *Nicolas Kluger*[1], *Kai Kisand*[2], *Pärt Peterson2, Kai Krohn*[1]

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APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy; OMIM 240300), also known as APS-1 (autoimmune polyendocrine syndrome type 1), is a rare and life-threatening multiorgan autoimmune disorder that is currently incurable. APECED patients need life-long therapy with hormonal substitutes and their life expectancy is reduced. Practically all patients develop chronic mucocutaneous candidiasis (CMC), hypoparathyroidism, Addison´s disease and often also gonadal failure, thyroid diseases, type 1 diabetes and gastrointestinal symptoms. APECED is found worldwide with the highest prevalence (1:25 000) and most studied patient cohort in Finland. The most common AIRE mutation, R257X, is found in almost 85% of Finnish patients, albeit close to 100 different mutations have been characterized worldwide. AIRE is a transcriptional regulator, expressed in the medullary thymic epithelial cells (mTEC). Its biological function is to drive the promiscuous transcription of tissue specific self antigens (TSAs) and thus ensue the deletion of maturing autoreactive T cells. In APECED, such potentially auto-aggressive immature T cells will mature into functional CD8+ cytotoxic T cells, leading to the destruction of multiple peripheral organs. However, AIRE is also expressed in extra-thymic cells (eTAC), in peripheral lymphoid organs, in the skin and in peripheral blood leukocytes, especially in CD14+ cells, in activated macrophages and monocyte- derived dendritic cells. The TSAs that are regulated by the peripheral versus central tolerance are different but partly overlapping. Interestingly, APS1/APECED patients also express autoantibodies with neutralizing capacity and specific for a variety of cytokines such as type I IFNs and Th-17-related cytokines. These antibodies may be beneficial, protecting the patients from certain diseases, but may also predispose to others like CMC. Also, APECED patients develop autoantibodies directed against several intracellular enzymes, involved in hormone and neurotransmitter biosynthesis, with an effect on serum serotonin and tryptophan metabolite levels and disturbed integrity of the intestinal microbiome milieu as a consequence.

RECIRCULATING PERIPHERAL Treg AFFECT THYMOCYTES DEVELOPMENT

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Our laboratory recently demonstrated that peripheral Treg can re-enter the thymus in mice and in humans. The proportion of peripheral Treg re-entering the murine thymus strongly increases with age, representing 40-50% of the thymic pool of Treg at 6 weeks of life. The recirculating cells have an activated and differentiated phenotype, suggesting that activation of Treg during immune responses in the periphery endows these cells with the capacity to migrate back to the thymus. Some of the recirculating Treg appear to have previously resided in non-lymphoid tissue. In the thymus, recirculating Treg were found in the same location as developing Treg, suggesting that they may somehow influence Treg development. Moreover, deep-sequencing analyses of their transcriptome indicated that recirculating Treg are armed with a variety of Treg suppressor-effector functions. Using several independent approaches, we could indeed establish that recirculating peripheral Treg inhibit the Il-2 dependent development of their precursors. Our data therefore demonstrated that intrathymic Treg generation is controlled by a negative feedback loop exerted by peripheral Treg recirculating back to the thymus.

TLRs SENSING NUCLEIC ACIDS

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In general, structurally conserved molecules from invading pathogens become sensed by germ-line encoded innate immune receptors such as Toll-like-receptors (TLRs). Nucleic acids (certain RNA- or DNA sequences) are principle ligands for TLR-3, TLR-7, TLR-9 and TLR-13. We will discuss the immune-biology of these TLRs. Originally, „self" versus "non-self" discrimination of nucleic acid sensing TLRs was rated as being "black and white". Yet, self-nucleic acid driven auto-immune diseases exist, and "self" RNA- or DNA sequences can activate TLRs. Perhaps it is only TLR-13 that sequence specifically recognizes 23 S r-RNA. Ligand driven dimerization of TLRs initiate signal pathways that either yield in production of pro-inflammatory cytokines, or of type -1 interferons. Very recently, the structural basis of nucleic acid recognition by the respective TLRs has been unveiled.

IN VIVO AND IN VITRO CHARACTERIZATION OF THYMOGLOBULIN

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Thymoglobulin (ATG) is a polyclonal rabbit antibody against human thymocytes used as a T cell-depleting agent in prevention and treatmentof allotransplant rejection. It has been hypothesized that due to the polyclonal character of ATG, its effect may exceed that of pure T cell depletion.
The aim of this project wasto elucidate the possible role of ATG in TREG induction both in vivoand in vitro.
For in vivo studies,humanized hCD3 BALB/c transgenic mice were i.v.injected with ATG or control rabbit immunoglobulin (Ig) and then blood and lymphoid organs were harvested.To investigate the ATG effect on the prolongation of transplant survivalwe used a murine cervical heart allotransplant model.. C57BL/6 (H2d)donors graft were transplanted into hCD3 BALB/c(H2b)transgenic mice.
For in vitro studies, human peripheral blood mononuclear cells (PBMC) were incubated with ATG for various times at 37°C. Foxp3+ TREGand monocytes were phenotypically analysed by flow cytometry and functionally by in vitro suppression assays. Cytokines were measured by Multiplex or ELISA assays.
In vivo, ATG led to T-cell depletion in peripheral blood, spleen and lymph nodes in the hCD3 BALB/c mice after 24h. After 7 days, T cells recovered in the circulation, however depletion in lymph nodes and thymus still persisted and were also observed 14 days post injection. Importantly, while naïve T cells were strongly depleted, TREG were spared. Survival and transplant function were significantly prolonged in ATG-treated mice, in comparison to mice receiving normal Ig.
In vitro, the frequencies of Foxp3+ TREG increased when human PBMC were cultured with ATG as compared with rabbit Ig or without stimulation. ATG-treated cells suppressed proliferation of autologous PBMC.Monocytes stimulated with ATG down-modulated CD16andsecreted IL-10.
Our study demonstrates that ATG has additionalimmunomodulatory propertiesfurther to T cell depletion and this includes effects on persistence of TREG and IL-10 production by monocytes.
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REGULATION OF PRO- AND ANTI-INFLAMMATORY HUMAN T HELPER CELL FUNCTIONS BY THE MICROENVIRONMENT

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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.

POSTER PRESENTATIONS AND SHORT ORAL PRESENTATIONS

Kv1.3 LYMPHOCYTE POTASSIUM CHANNEL INHIBITOR AS A POTENTIAL NOVEL THERAPEUTIC TARGET IN ACUTE ISCHEMIC STROKE

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Stroke-induced immunosuppression (SIIS) leads to severe complications in stroke patients, including an increased risk of infections. However, functional alterations of T lymphocytes during SIIS are poorly described in acute ischemic stroke (AIS). We aimed to characterize Ca(2+) influx kinetics in major lymphocyte subsets (CD4, Th1, Th2, CD8) in AIS patients without infection 6 hours and one week after the CNS insult. We also assessed the sensitivity of the above subsets to specific inhibition of the Kv1.3 and IKCa1 lymphocyte K(+) channels. We took peripheral blood samples from 12 non-stroke individuals and 12 AIS patients. We used an innovative flow cytometry approach to determine Ca(2+) influx kinetics and the surface expression of Kv1.3 channels. Our results indicate that Ca(2+) influx kinetics is altered in the Th2 and CD8 subsets in AIS which may play a role in the development of SIIS. Specific inhibition of Kv1.3 channels selectively decreased Ca(2+) influx in the CD8 and Th2 subsets of AIS patients. The surface expression of Kv1.3 channels is also altered compared to non-stroke individuals. Kv1.3 channel inhibition might have beneficial therapeutic consequences in AIS, selectively targeting two distinct T cell subsets at two different time points following the CNS insult. Within hours after the insult, it might prevent excessive tissue injury through the inhibition of CD8 cells, while at one week after the insult, it may improve the inflammatory response through the inhibition of Th2 cells, thus reducing the unwanted clinical consequences of SIIS.

RACK1 MEDIATES T CELL ACTIVATION-INDUCED REDISTRIBUTION OF LCK VIA INTERACTIONS WITH CYTOSKELETAL ELEMENTS

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The initiation of T-cell signaling pathway is critically dependent on the function of Lck kinase. We and others have previously suggested that TCR triggering requires the activation-induced redistribution of active Lck within the plasma membrane. Specifically, we showed that Lck activation outside lipid rafts (LR) results in the translocation of a fraction of Lck to LR. While such event predicates the subsequent production of IL-2, the mechanism underpinning this translocation process is unknown. In our comparative screen, we have identified RACK1 as a candidate Lck-interacting protein that is involved in the regulation of this redistribution. The formation of transient Lck-RACK1 complexes was detectable in primary CD4+ T cells with their maximum levels peaking 5-10 seconds after TCR-CD4 co-aggregation. This interaction was dependent on functional SH2 and SH3 domains of Lck. Using the mass spectrometry approach, we have identified several other components of Lck-RACK1 complex and determined the kinetics of their interaction with RACK1. In agreement with this data, various types of microscopic examination of primary CD4+ T cells and Lck-deficient Jurkat cell line expressing fluorencent protein-tagged Lck and RACK1, revealed that Lck and RACK1 briskly and transiently co-redistribute to the forming immunological synapse. The significance of interaction between activated Lck and RACK1 in the context of directing Lck to LR is further supported by previous observations that RACK1 is associated with several types of cytoskeletal elements. In this context, our data showed that microtubular cytoskeletal inhibitor nocodazole strongly affected both the outcome of activation-induced Lck translocation and the formation of RACK1-Lck complex. Importantly, Lck translocation to LR was dramatically diminished in primary CD4+ T cells by adenoviral-mediated knock-down of RACK1. Together, these results describe RACK1 as a relevant intracellular signaling component involved in the regulation of Lck redistribution within plasma membrane by linking CD4-Lck complex to the microtubular cytoskeletal network.

EARLY ACTIVATION OF TLR2 LOCUS TRACKS THE EMERGENCE OF EMBRYONIC MULTIPOTENT HEMATOPOIETIC PROGENITORS

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Toll-like receptors (TLRs) play a central role in host cell recognition and defense responses to pathogens. Recently, direct pathogen sensing of adult bone marrow hematopoietic stem and progenitor cells via TLRs has been shown to direct the cell fate towards enhanced myelopoiesis. Despite the critical role of TLRs in adult hematopoietic cells, the functional expression of TLRs in embryonic hematopoietic progenitors has not been addressed. We show that TLRs are initially detected on short-lived transplacentally transferred maternal myeloid cells, which are gradually replaced by myeloid cells of embryonic origin. Interestingly, embryonic multipotent hematopoietic progenitors, which appear at day 7.5 of mouse embryogenesis (E7.5), also express TLRs, respond to TLR2 triggering by an enhanced proliferation and myeloid differentiation rate in a MyD88 adaptor protein dependent manner. Using genetic labeling we demonstrate that the Tlr2 locus is indeed activated at early stages of embryonic development (E7.0-8.0) in emerging hematopoietic progenitors which contribute to embryonic and adult hematopoiesis. When TLR2+ progenitors were genetically labelled at E8.5 in vivo, they gave rise to erythroid, myeloid as well as lymphoid hematopoietic lineages that persisted in the peripheral blood of adult animals for >16 weeks. Moreover the progeny of these cells persisted in the peripheral blood of primary and secondary recipients for >16 weeks, suggesting their long term repopulating activity. Our results thus demonstrate that (i) activation of Tlr2 locus marks the emergence of common embryonic hematopoietic multipotent progenitors with the capacity to contribute to definitive hematopoiesis; and (ii) TLR2 triggering endows these cells with the ability to boost the production of myeloid cells, suggesting a functional link between embryonic hematopoiesis and pattern recognition receptors under inflammatory conditions.

THE EFFECTS OF CYTOKINES ON OF SUPPRESSOR ACTIVITY OF B CELLS

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The possibility to modulate immune response represents a great goal for clinical applications. The regulatory effects of T cells have been well described. However, B cells can also regulate immune response in the antibody-independent manner. In this study we analyzed the effects of cytokines on B cell development in vitro. We were aiming to describe modulatory effects of the selected cytokines on the activation of suppressor activity of B cells stimulated with lipopolysaccharide (LPS) and to determinate the mechanisms of B cell action on macrophages and their functions. Macrophages, which were co-cultivated with B cells stimulated with LPS and interferon-γ (IFN-γ), showed decreased expression of costimulatory molecule CD86 and reduced production of interleukin-6 (IL-6). These macrophages displayed also decreased ability to stimulate proliferation of activated CD8+ T cells. Furthermore, we analyzed mechanisms of B cell action on macrophages. B cells stimulated with LPS and IFN-γ produced increased concentrations of IL-10 and also expressed higher levels of the genes for Fas ligand and programmed death ligand 1. The results have shown that IFN-γ enhances activation of suppressive functions of B cells which have the ability to inhibit immune response through their effect on macrophages. The possibility to modulate suppressive functions of B cells may have a great impact for their use in a clinical setting.

ANALYSIS OF THE RESISTANCE OF B CELL ANTIGEN RECEPTOR SIGNALING TO THE INHIBITION OF SRC-FAMILY KINASES

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Signalling through antigen specific receptors BCR and TCR is crucial for the development and the function of T cells and B cells. Although, much is known about their signalling pathways a number of questions still remain to be clarified. We focused on the roles of Src-family kinases (SFKs) in the initiation of BCR- and TCR-mediated signalling. Several studies have suggested that in contrast to TCR signalling, BCR signal transduction could be initiated independently of SFKs or with only a minimal activity of these kinases. We used genetic approach to study the differences between TCR and BCR signalling apparatuses combined with inhibition of SFKs by inhibitor PP2. Using this experimental set up, we show that the differences in the roles of SFKs and inthe requirements for the levels of SFK activity needed for the initiation of BCR and TCR signalling are likely based on different composition or architecture of BCR and TCR.We further show that the SFK activity required for the initiation of TCR signalling is lower if ZAP-70 kinase is substituted with Syk kinase, which most likely reflects the higher enzymatic activity of Syk, as well as different mechanisms of Syk and ZAP-70 kinase activation.

LOCAL IMMUNITY ALTERATION RESHAPES THE STROMA OF THE COLON MUCOSAIN EARLY STEPS OF INFLAMMATION AND CARCINOGENESIS

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Chronic inflammation can induce architectural changes in the stromal scaffold of atissue leading to fibrosis. Stromal remodeling is also observed in the tumour microenvironment.We aimed to find a possible correlation between immunological network and structural changes in the site of DSS-induced inflammation or AOM-induced carcinogenesis in the colon of Wistar rats.Evaluation of the collagen scaffold was performed by using 2-photon confocal microscopy.We observed for the first time, that a very dynamic changes of connective structure rise as a consequence of the immunological changes.One month after DSS or AOM administration, it was already possible to observe modifications both in collagen density and architecture.These changes resulted to be associated to higher production of pro-inflammatory cytokines (INF-γ, IL-6, IL-1) and increase of TGF-β1 and IL-10 production.These last cytokines can modulate tissue remodeling. The stromal changes showed similar trends both in DSS and AOM rat models, underlining the importance of inflammation in allowing the tumour microenvironment development. Preliminary analysis of human mucosa samples(from surgical specimens of patients operated for colorectal adenocarcinoma) showed similar mucosal changes both in the cytokine and stromal structureas observed in the rat model. From our data, the correlation between measurable changes in various parametersof the stroma structure(density, symmetry, inter-cryptal spaces, etc.) with the local immunity changes appears as a novel promising tool for identifying pre-tumoural conditions and very initial step of carcinogenesis.

ANTIDEPRESSANT DRUGS INHIBIT PRODUCTION OF INFLAMMATORY FACTORS IN STIMULATED HUMAN HaCaT CELLS.

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Contact hypersensitivity (CHS) to haptens is an example of a cell-mediated immune response. Approximately 20% of the general population suffer from contact dermatitis. Depression is one of the most common mental disorders and is an increasing burden in western countries. In previous experimental studies we have shown the effectiveness of antidepressants in the inhibition of CHS in mice.Keratinocytes play a pivotal role in particular phases of CHS and are the source of numerous pro-inflammatory factors.The aim of present study was to elucidate if the antidepressants are involved in the inhibitory effect on pro-inflammatory cytokines production by keratinocytes.Experiments were conducted on the HaCaT cell line. Stimulation of cells was performed for 24h, with TNF-α/IFN-γ and lipopolysaccharide. Antidepressant drugs – fluoxetine, desipramine and imipramine was added to the culture. Cell lysates and supernatants were collected. The level of interleukin(IL)-1β, IL-6 and MCP-1 was measured by ELISA assay. The expression of adhesion proteins such as ICAM-1 and E-cadherin was measured by real time qPCR.The stimulants significantly increased the secretion or expression of measured cytokines, chemokines and adhesive proteins.All used antidepressants inhibit IL-1β secretion after LPS and TNF-α/IFN-γ in particular doses. After LPS stimulation, desipramine and fluoxetine in higher doses decrease IL-6 releasing, wheras after TNF-α/IFN-γ stimulation, desipramine in both doses increase IL-6 releasing by HaCaT cells and imipramine decrease secretion of this cytokine. In case of MCP-1, all doses of antidepressant drugs used inhibit secretion of this chemokine.TNF-α/IFN-γ stimulation extremely increased expression of ICAM-1 and antidepressants inhibit this upregulation. E-cadherin also was slightly elevated after stimulation, and used drugs regulated expression of this adhesive protein. It can be concluded that antidepressant drugs are effective in modulation of proinflammatorycytokines releasing by human keratinocytes, which may contribute to mechanism of suppression contact hypersensitivity.

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TRANSCRIPTION FACTOR TCF4 SUPRESSES SELF-RENEWAL OF HSCs

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The canonical Wnt pathway is mediated by β-catenin and the Tcf/Lef family of transcription factors (TCF1, TCF3, TCF4, and LEF1). In the hematopoietic system, the Wnt/β-catenin signaling pathway has been shown to be important, although its role in hematopoietic stem cell (HSC) self-renewal and maintenance has been controversial. Recently, we observed that Tcf2l7, the gene that encodes the transcription factor TCF4, is abundantly expressed in HSC. Therefore, to investigate the role of the Wnt/β-catenin signaling pathway in HSC function, we employed a mouse model expressing a dominant negative form of human TCF4 (dnTCF4) which abrogates transcriptional activation of Wnt-target genes in the hematopoietic system. We observed that introduction of dnTCF4 increases the abundance of immature cells in mouse bone marrow compared to control mice. Consistently, replating assays demonstrated that bone marrow cells isolated from dnTCF4-expresing mice possess an enhanced ability to grow in vitro. Morphological analysis revealed that dnTCF4 cultures presented an enlarged fraction of immature cells as well. Further, when transplanted into lethally irradiated mice, dnTCF4-expressing bone marrow cells engraft and repopulate recipient mice with higher efficiency compared to control. In addition, we observed that lymphoid differentiation of transplanted dnTCF4-expressing cells was significantly shifted towards B-cells. Together, our data demonstrates that Wnt/β-catenin signaling pathway, and in particular the transcription factor TCF4, negatively regulates HSC self-renewal and inhibits differentiation towards B-cells.

MODULATION OF AUTOIMMUNE T CELL REACTION IN PATIENTS WITH TYPE 1 DIABETES BY TOLEROGENIC DENDRITIC CELLS

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Tolerogenic dendritic cells (tDCs) may offer an intervention strategy to re-establish antigen-specific tolerance in autoimmune diseases, including type 1 diabetes (T1D). T1D results from destruction of β cells leading to hyperglycemia that in turn affects the patient's immune system. We prepared monocyte-derived tDCs using dexamethasone and Vitamin D2 from 26 T1D patients with optimal and 56 T1D patients with suboptimal glycemic control and assessed their tolerogenic properties in correlation with metabolic state of patients. tDCs differentiated from both groups of patients acquired regulatory phenotype, however tDCs from well-controlled patients expressed higher levels of inhibitory molecules IL-T3 and PD-L1. Moreover, GAD65-loaded tDCs from well-controlled patients decreased significantly Th1/Th17 responses, induced stable GAD65-specific T cell hyporesponsiveness and suppressed markedly control DC-induced GAD65-specific T cell activation compared to poorly controlled patients. In both groups of patients, tDCs were able to induce T regulatory cells (Tregs). However, Tregs from well-controlled patients had better suppressive abilities. These results suggest that metabolic control of T1D affects the functional characteristics of tDCs and effector T cell responses. This may be relevant for refining inclusion criteria of future clinical trials. This project was supported by the Charles University in Prague, GAUK 132215.

AIRE-EXPRESSING LYMPH NODE CELLS IN PERIPHERAL IMMUNE TOLERANCE

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The discrimination between body own components (the self) and foreign intruders (the non-self) is the fundamental aspect of the immune system. Developing T-cells are restricted to recognize non-self during thymic process of negative selection by deletion of potentially self-reactive thymocytes or their conversion to T-regulatory cells. This is achieved by active presentation of antigens derived from self-proteins by various resident or migratory antigen presenting cells in the thymus. Among them, medullary thymic epithelial cells are endowed with the unique ability to mirror immune periphery by the expression of thousands of self-proteins (tissue restricted antigens). Autoimmune regulator (Aire) was shown to promote the expression of considerable fraction of these tissue restricted antigens. Loss of Aire function leads to the escape of self-reactive T-cells into the immune periphery and devastating autoimmune disease. While the role of Aire in the maintenance of central immune tolerance is well established, its function in the immune periphery remains controversial due to several conflicting reports. Here we describe MHC class II positive, lineage negative, bone marrow-derived cells expressing Aire protein, which reside in the lymph nodes. We also showed that these cells produce a set of tissue restricted antigens. In addition, Aire-expressing lymph node cells not only expressed a battery of costimulatory molecules, notably CD86, CD80, ICOS-L, PD-L1 and CD40 which are implicated in the regulation of T-cell function, but also were able to mediate efficient self‑reactive T-cells deletion as well as their conversion into T-regulatory cells. On the basis of this set of data we propose that Aire-expressing lymph node cells play impact peripheral immune tolerance induction.

LASERS IN DENTAL PRACTICE - NEW TREATMENT STRATEGY FOR ORAL LICHEN PLANUS PATIENTS

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Oral lichen planus (OLP) is a chronic autoinflammatory disease. Activation of the autoreactive T-lymphocytes leads to destruction of the basal epithelial cells and formation of the painful wounds. Current treatments are partially effective and new therapeutic approaches are required. The photodynamic laser therapy (PDLT) is considered as painless and promising ability for OLP patients. The aim of the study is to detect and compare the expression of the pro- and antiapoptotic markers in patient's biopsies before and after PDLT. 30 OLP patients and 15 healthy donors underwent PDLT with methylene blue (500μg/ml) and diode laser (810nm) with parameters 0,50 W, 30s, 1, 2J/cm2, 3 times weekly for a month. The biopsies were taken before and after therapy and analyzed immunohistochemically for expression of the p53 and bcl-2. Biotin-streptavidin peroxidase method was used. The expression intensity was measured using semiquantitative scale: (+++) >70% positive cells, (++) 30–70% positive cells, (+) <30% positive cells.

The results from immunohistochemistry showed that before therapy p53 expression in the basal keratinocytes was higher (+++) and decreased to (+) after PDLT. In contrast, bcl-2 positive reaction slightly increased from (+) to (++) after therapy.

The present study demonstrates that laser therapy is a new therapeutic strategy in dental practice because of its ability to maintain the fine balance between molecules that control cell death and surviving.

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OPPOSING EFFECTS OF ACTIN SIGNALING AND LFA-1 ON TCR AFFINITY THRESHOLD IN MICE

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CD8+ T cells use a narrow antigen affinity threshold to generate tissue-infiltrating cytotoxic effector T cells but the underlying molecular mechanisms are still poorly understood. Antigens with affinities above the threshold induce stable contacts with APCs, polarization and asymmetric T-cell division while antigens below threshold form only transient contacts with APC, kinapses. Previous reports suggest that LFA-1 inside-out signaling might be involved in establishing the antigen affinity threshold. In our work, we show that subthreshold antigens weakly activate all major distal TCR signaling pathways. Low-affinity antigens are more dependent on LFA-1 than suprathreshold antigens. Furthermore, augmenting the inside-out signaling by hyperactive Rap1 does not enhance responses to the subthreshold antigens. Thus, LFA-1 signaling does not contribute to the affinity-based antigen discrimination. T cell:APC conjugation is also accompanied by cytoskeleton rearrangement. However, we observed that subthreshold antigens do not induce actin rearrangement towards an APC, mediated by Rho-family GTPases, Cdc42 and Rac. Our data suggest that Rac and Cdc42 contribute to the establishment of the narrow antigen affinity threshold in CD8+ T cells by enhancing responses to high-affinity antigens, or by reducing the responses to low-affinity antigens.

IMMUNOLOGICAL METHODS TO INVESTIGATE ALLOIMMUNE HABITUAL ABORTION

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Recurrent spontaneous abortion (RSA) is the occurrence of pregnancy loss at 6-12th week of gestation despite normal implantation and normal embryonic development. In several cases where no anatomical, genetic, hematologic or endocrinologic cause or no infection can be found for RSA we speak about RSA of unknown cause. In these cases autoimmune/alloimmune conditions or immune regulation disorders can be the background. Clinical observations underline that immunotherapies used to treat RSA are effective only in those cases, where the immunological cause of RSA is accurately assessed. For this reason a national RSA committee was founded in 2013 at the 2nd Department of Obstetrics and Gynaecology. In cooperation with this committee we introduced a complex protocol for the investigation of immunological RSA that consists of the following measurements:

Mixed Leukocyte Culture (MLC) and blocking antibody analysis: By this method we can determine the cellular reactivity of the patient against the partner and we can measure also whether the patients' serum contains any factor that modifies this reactivity.

Determination of Th1-Th2 ratio: The determination of Th1 vs. Th2 cell ratio is based on measuring their characteristc cytokines. Th1 dominance points to elevated cellular immune response while Th2 dominance represents humoral immune response and suppressive immunregulation.

Analysis of natural killer (NK) cells: If elevated NK cell numbers and activity are detected in the blood and/or their reactivity is elevated means a general upregulated immunreactivity of the patient.

In the present work we summarize our experience with the 300 RSA patients and 9 healthy first trimester pregnant women investigated so far in our laboratory. We analyse the efficiency of the different measurements and emphasize the importance of the integrated evaluation of the results. Most informative results were concluded from the MLC measurement. Interestingly all healthy pregnant women had elevated reactivity against partner cells compared to RSA patients, where only 73%. Moreover 63% of RSA patients' serum contained factors that enhanced the reactivity, while only 33% of healthy pregnant women showed this reaction. In contrast, only 37% of RSA patients had components in their serum that blocked the reaction against the partner cells, while 67% of healthy pregnant women showed this blocking reaction.

THE BENEFICIAL EFFECT OF GLYBURIDE ON THE EVOKED BY MATERNAL DIABETES CHANGES IN THE OFFSPRING BRAIN

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Diabetes during pregnancy causes neurodevelopmental and neuroinflammatory abnormalities in offspring. Recently data suggest that effective action of anti-diabetes drugs may be amplified by their beneficial properties on the apoptotic and inflammatory processes. We investigated the impact of maternal diabetes on the viability/death parameters and NO realase in basal and LPS-stimulated conditions in the hippocampal organotypic cultures. Moreover, the effect of glyburide -an ATP-sensitive potassium channel blocker -was evaluated. Cultures were prepared from brains of 7-day-old rats- offspring of control and diabetic dams. Hippocampi were sectioned into slices and transferred onto membrane inserts. On the 7th day in vitro slices were pre-treated with different concentration of glyburide and stimulated for 24h with LPS. The cell death/viability parameters and nitric oxide realase using flow cytometry, LDH , MTT and Griess method were estimated.We observed that glyburide at 0,1-5μM did not evoke any changes in viability or in cell death in cultures obtainted from control and diabetic dams. Exposure to glyburide at 10μM resulted in a significant decrease in viable cells and simultaneously enhanced death processes. Addition of LPS caused a substantial decrease in viability, increase cell death and NO realase- more susceptible to damage were slices of pups born to diabetic dams. Nevertheless, in LPS-stimulated slices, glybyride reduced the number of dying cells, enhanced viability and diminished NO overproduction. In this study- we demonstrated for the first time that glyburide shows the protective properties in LPS-stimulated hippocampal cultures obtained from diabetes dams offspring. Supported by grant no. 2014/13/N/NZ7/00279 National Science Centre, Poland.

THYMIC MAST CELLS: A NEW FUNCTION OF AN OLD CELLS

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Thymus is acutely sensitive to stress resulting in T-cell apoptosis and thymic involution. In the present study, we examined the role of mast cells (MCs) in the thymic recovery after involution induced by hydrocortisone injection. The study was performed on C57Bl/6 mice (n=40). At the stage of cortico-medullary inversion (in 48 h after injection), the number of MCs was increased in 10 times in comparison with the control ($p<0.05$). Different stages of mast cell maturation were observed in the thymus during involution. This data suggest that mast cells may maturate directly into the thymus in pathology conditions. At the stage of cortico-medullary inversion MC degranulation index was also increased (in 2.5 times in comparison with the control ($p<0.0001$). We consider signals from the catecholaminergic nerve system as one of the mechanisms of the thymic MC activation in stress. In support of this hypothesis, we identified contacts between MCs and catecholaminergic terminals in the thymus. The most significant increase of both the numbers and the degranulation activity of MCs was observed in the thymic cortex. Identified mast cells contain mouse mast cell protease 4 (mMCP-4). This specific mast cell protease have been shown to be associated with extracellular matrix remodelling. We evaluated the extracellular matrix in both, normal and involuted thymus, and found that the density of connective tissue in the cortex was maximally increased in 48 h after injection, but it was extremely decreasing on later terms (in 72-96 h after injection). We suppose that mMCP-4 secreted by numerous thymic mast cells contributes to connective tissue destruction in the thymic cortex at the stage of cortico-medullary inversion; this allows T-cell progenitor migration and proliferation to occur on later stages of thymic recovery. The study was supported by RFBR grant №15-04-05093.

KNOCKDOWN OF P65 SUBUNIT OF NF-□ B TRANSCRIPTION FACTOR DOWN-REGULATES THE INDUCTION OF PRO-INFLAMMATORY CYTOKINES IN HUMAN LUNG EPITHELIAL CELLS INFECTED WITH INFLUENZA H9N2 VIRUS

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Inflammation is a hallmark feature of many influenza virus infections. To obtain insight into the inflammatory mechanisms involved in influenza H9N2 infection of human lung epithelial cells, A549 airway epithelial cells were infected with H9N2 virus and the expressions of pro-inflammatory cytokines (IL-1β, IL-6) and chemokine (IL-8) in these cells were examined by qPCR and ELISA. Moreover, the effect of silencing of p65 component of NF-κB in A549 cells infected with H9N2 virus on the expression and secretion of pro-inflammatory cytokines and chemokine and virus replication were evaluated by qPCR, ELISA, Immunocytochemistry and western blotting. H9N2 virus was able to cultivate in the human lung epithelial cell line (A549) and stimulate production of IL-1β, IL-6 and IL-8. Expressions of cytokine and chemokine genes were down-regulated to a significantly lower level (IL-1β after 24hours ($P < 0.1$) and 48hours($P < 0.01$), IL-6 after 24 hours ($p < 0.01$) and IL-8 after 24 hours($p < 0.05$)) in p65 knocked down A549 cells as compared with scramble shRNA cultured cells infected with H9N2 influenza virus. The amount of IL-6 and IL-1β proteins secreted into the culture medium was also decreased after silencing the p65 component of NF-κB in A549 cells infected with H9N2 influenza virus in different multiplicity of infections (MOI). The results presented in this study provide the mechanism by which H9N2 virus induce inflammation in human lung epithelial cells. These findings will broaden our understanding of host innate immune mechanisms and the pathogenesis of H9N2 influenza viruses in human respiratory epithelium.

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF THE MOUSE INHIBITORY C-TYPE LECTIN-LIKE RECEPTOR NKRP1B

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NK cells play an essential role in reproduction or organism's defense against viral infections and tumor growth. Besides the immune response is very early, healthy tissues are considered due to prevention of autoimmunity. These complex functions require intricate system of regulation ensured by many receptors on a cell surface. One way leading to understanding of NK cell biology is through the structure of the NK receptors thatcan reveal conditions of ligand binding.
This project addresses structure of the mouse inhibitory C-type lectin-like receptor Nkrp1b (Klrb1b) using several mass spectrometric techniques, as well as itsbinding capacity, which was examined on ligand-expressing murine cells derived from a bone marrowby fluorescence microscopy. Main interest is focused on the position of the loop and the stalk region in the context of whole protein structure and interaction with its binding partner.
Besides design of Nrp1b models, binding properties of several Nkrp1b forms differing in the presence of the stalk region and monomeric/homodimeric conformation were compared. These forms exhibited surprisingly distinct behavior. Based on the data obtained, our investigation will evolve towards question whether the receptor forms monomers or homodimers as it is reported in the literature without direct experimental evidence for over 25 years.

THE ROLE OF INTERACTION OF LCK WITH CD4 AND CD8 CORECEPTORS IN SHAPING THE SELF-REACTIVITY OF MATURE T CELLS

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The frequency of CD8 and CD4 coreceptor molecules coupled to Lck largely determines the sensitivity to suboptimal antigens and shapes repertoire of positively selected thymocytes. Because CD4 is more frequently coupled to Lck than CD8, MHCII-restricted thymocytes use lower antigen affinity threshold for negative selection than CD4 thymocytes. In this study, we observed that the coupling frequency of CD8-Lck, but not CD4-Lck, increases dramatically upon T cell maturation. Our mathematical model predicts that mature MHCI-restricted T cells have higher sensitivity to suboptimal antigens than thymocytes expressing the same TCR, whereas MHCII-restricted thymocytes and mature T cells are comparable in this respect. We addressed the predictions of the model experimentally using monoclonal OT-I and B3K508 T cell populations in vitro and in vivo. The results were in a very good agreement with the prediction of the model.
Based on our data, we hypothesized that the CD8+ T cells are on average more self-reactive than CD4+ T cells. Indeed, CD8+ T cells exhibit features of stronger homeostatic TCR signaling than CD4+ T cells. Moreover, CD8+ T cells undergo more rapid expansion during lymphopenia and experimental model of systemic autoimmunity than CD4+ T cells.

DISTINCT IMMUNE STATUS IN PATIENTS WITH ADENOCARCINOMA AND SQUAMOUS CELL CARCINOMA: IMPLICATION FOR IMMUNOTHERAPY OF NON-SMALL CELL LUNG CANCER

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In this study we compared immune cell populations, T cell responses and secreted cytokines in primary tumors and non-tumoral lung tissue from more than 30 adenocarcinomas (AC) and 30 squamous cell carcinomas (SCC) of non-small cell lung cancer (NSCLC) patients. We compared immune suppressive populations such as CD4+CD25+Foxp3+ T regulatory cells and myeloid-derived suppressor cells (MDSC) and the expression of immune checkpoint molecules PD-1, TIM3, LAG-3 and CTLA-4 in the blood of these patients. In both tumor subtypes we observed similarly higher infiltration of B cells, memory T cells, dendritic cells, monocytes/macrophages, mast cells and T regulatory cells compared to non-tumoral tissue. However, immune cells seemed to be suppressed functionally more in SCC than AC as we detected lower IFN-γ-positive T cells and production of proinflammatory cytokines after stimulation. PD-1, TIM3 and LAG-3 expression on T cells in blood and PD-1 on intratumoral CD8+ T cells of SSC patients were significantly elevated compared to AC patients. Similarly, the high number of MDSC, which correlated with Arginase 1 mRNA levels and downregulation of CD3ζ in T cells, was detected only in SCC. These results suggest that immune system of SSC patients might be subjected to a higher systemic and tumor–associated immune suppression than in AC patients. This should be taken into consideration in designing lung cancer immunotherapeutic approaches.

THE TRANSCRIPTION FACTOR C/EBPγ IS INVOLVED IN THE EXPANSION OF GRANULOCYTES DURING EARLY PHASES OF EMERGENCY GRANULOPOIESIS

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CCAAT/enhancer binding protein γ (C/EBPγ) is a member of the C/EBP family of myeloid transcription factors, which are known as master regulators of hematopoiesis. Recently, it has been shown that increased C/EBPγ levels contribute to the block of neutrophilic differentiation in specific cases of acute myeloid leukemias. Hovewer, the function of C/EBPγ remains largely unknown. This study investigate the role of C/EBPγ in steady-state and stress-induced hematopoiesis. We generated a C/EBPγ conditional knockout murine model, which allows excision of C/EBPγ in the hematopoietic system from the early embryonic stages. We employed C/EBPγ f/f Vav-1Cre- and C/EBPγ f/f Vav-1Cre+ mice, refered here as wt and C/EBPγ ko respectively. Similar hematopoietic system of wt and C/EBPγ ko mice suggests that hematopoietic-specific genetic ablation of C/EBPγ is not critical for normal hematopoiesis. In vivo administration of different stimuli, including lipopolysacharide (LPS), granulocyte colony-stimulating factor (G-CSF) and *Candida albicans*, was used to model bacteria- or candidemia-induced emergency granulopoiesis. Deletion of C/EBPγ did not compromise the ability of the hematopoietic system to respond to G-CSF or LPS. Interestingly, C/EBPγ ko mice showed reduction of blood neutrophils during early phases of candidemia. In addition, the distribution of developmental stages within the neutrophilic compartment in bone marrow was perturbed. In conclusion, our data unravel C/EBPγ as a player in the expansion of granulocytes during early phases of candidemia-induced emergency granulopoiesis.

PROTEIN AND TRANSCRIPTIONAL LEVEL OF LAMPs IN GLIAL TUMORS

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Cancer cells harbor several changes that help them to avoid spontaneous and therapy-induced apoptosis. The lysosomal cell death pathway is a novel concept in which lysosomal membrane permeabilization participates in the induction of cell death, using death pathways still functional in can¬cer cells. Lysosome-associated membrane glycoproteins (LAMP) 1 and 2 are highly conserved proteins with still undefined biological functions. There is evidence that they are implicated in autophagy, angiogenesis and tissue remodeling. The aim of the current investigation is to search a relationship between protein and transcriptional level of LAMPs in glial tumors. Nineteen patients newly diagnosed preoperatively as high-grade glioma and control samples from 5 forensic cases - individuals without any brain pathology were included in the study. LAMP-1 and LAMP-2 expression were determined by immunohistochemistry in tumor and control samples. Our data demonstrate that in 12 out of 19 analyzed patients, LAMP -2 was clearly down regulated with the lowest expression of -3,17 log2FC by using log2FC≥0,7 as a threshold. Unlike LAMP-2, LAMP-1 transcription levels were induced in tumor tissues vs healthy controls (6 out of 19 more than log2FC≥0,7 as a threshold) with the highest expression of 1,24 log2FC. Large areas of positive malignant cells were labeled in high-grade gliomas, where the most intensive immunohistochemical reaction was detected. LAMP-2 showed a more intensive staining compared to LAMP-1. In the normal brain both glycoproteins were presented in single glial cells with feeble reactivity. We suggest that LAMPs could be involved in tumor progression. These findings may provide new insights into their role in glial tumors.

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A HUMANIZED MOUSE MODEL TO STUDY SKIN IMMUNOLOGY

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Replacement therapies aim at introducing neo-antigens to a patient to compensate loss of function mutations that cause genetic disease. Protein or gene replacement introduces a novel (i.e. foreign) protein and thus poses the risk of a neo-antigen specific immune response that will likely diminish the success of any therapeutic approach. We hypothesize that the manipulation/amplification of the regulatory arms of the immune system will lead to the establishment of long lasting tolerance towards the new foreign antigen (i.e. during skin gene therapy).

Therefore we are currently establishing a humanized mouse model to study skin graft rejection and the antigen-specific immune response to a new skin antigen in vivo. In this model we mimic the gene therapy setting in vivo using immuno-deficient recipient mice that are adoptively transferred with peripheral blood mononuclear cells (hPBMC). Simultaneously these host mice are grafted with autologous skin equivalents (SE) containing a defined neo-antigen. We could already show, that the hPBMC engraft and stay functional within the recipient. Furthermore the PBMC maintain their skin homing properties and specifically migrate to the human SE, even though the SE lacks any adnexal structures. We are currently phenotyping the engrafted skin homing T cell population, in order to exploit or modulate their regulatory and effector activities respectively with the goal to induce long lasting tolerance to a neo skin antigen.

This model can serve as a potent tool to elucidate the mechanisms involved in the generation and maintenance of peripheral tolerance for neo-antigens. Specifically, it will be of importance for the investigation of the suppressive capacity and stability of in vivo generated neo-antigen specific regulatory T cells.

EXPRESSION OF TUMOR ANTIGENS ON PRIMARY OVARIAN CANCER CELLS COMPARED TO ESTABILISHED OVARIAN CANCER CELL LINES

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In order to develop efficient strategy for active serous epithelial ovarian cancer immunotherapy protocols based on the use of multiple tumor-associated antigens, it is crucial to describe the expression level of tumor antigens on primary cancer cells and consequently select a suitable combination of cancer cell lines as an appropriate source of antigens. We analyzed the expression level of twenty one tumor associated antigens (BIRC5, CA125, CEA, DDX43, EPCAM, FOLR1, Her-2/neu, MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A10, MAGE-A12, MUC-1, NY-ESO-1, PRAME, p53, TPBG, TRT, WT1) in established ovarian cancer cell lines and in primary tumor cells isolated from the high-grade serous epithelial ovarian cancer tissue. Both primary epithelial serous ovarian cancer samples and ovarian cancer cell lines show a significant variability in TAA expression. However, more than 90% of tumor samples expressed very high levels of CA125, FOLR1, EPCAM and MUC-1 and elevated levels of Her-2/neu, similarly to OVCAR-3 cell line. The combination of OV-90 and OVCAR-3 cell lines showed the highest overlap with patients' samples in the TAA expression profile.

SHARED T-CELL CLONES ARE FOUND IN SYNOVIAL FLUID OF PATIENTS WITH ANKYLOSING SPONDYLITIS

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It is believed that spondyloarthropathies, including ankylosing spondylitis (AS), are caused by a breakdown in self-tolerance that leads to expansion of (self-) antigen specific T-cell clones in the sites of inflammation. Detailed characterization of the pathogenic T-cell subsets involved in (self-) antigen recognition is critical for the understanding of the immunopathogenic principles underlying these diseases. Here we applied Next Generation Sequencing to investigate TCR repertoires of peripheral blood (PB) and synovial fluid (SF) from several patients with idiopathic AS. Original RNA based technology was used for TCRβ cDNA libraries preparation. For accurate T cell repertoire reconstruction and quantitative assessment of clones' concentrations unique molecular identifiers were introduced during cDNA synthesis allowing to mark each TCRβ mRNA molecule. Raw sequencing data processing and further analysis of individual repertoires were carried out with our MiTCR software and the tcR R-package. We observed that T-cell repertoire of both CD4+ and CD8+ SF fractions is highly oligoclonal. The most abundant PB clones are also found in SF, but mostly synovial T-cell repertoire is comprised of unique clones with different set of V-segments. Furthermore, we found that although SF clones are rather patient restricted, several clones with similar amino acid CDR3 sequences are shared between AS patients. Such clones was not detected in PB samples of HLA-B*27+ healthy donors in a separate study. Our findings support the possible role of T-cells in the pathophysiology of AS. The T-cell clones found in inflamed joints of AS patients could play a significant part in the disease onset and progression.

A LOCAL PRODUCTION OF CYTOKINES AFTER CORNEAL DAMAGE AND TREATMENT WITH STEM CELLS

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Corneal damage is one of the most common causes of impaired vision or even blindness in the world. It is estimated that 39 million of people are considered blind. Approximately 25 % of these cases are caused by the ocular surface injury or alterations. Corneal transplantation is the first choice of the treatment of these defects. If the damage is more extensive and includes the limbal region, where limbal stem cells (LSCs) reside, cornea cannot regenerate from the reason of LSC deficiency. The corneal damage is also associated with the break of immune privilege of the cornea, with a harmful inflammatory reaction and production of cytokines. The corneal transplantation is not sufficient treatment in the most cases. The only option of the treatment is transplantation of limbal tissue, LSCs or other types of stem cells (SCs). In this regard, mesenchymal stem cells (MSCs) turned out as a suitable source of autologous SCs in cases, when autologous LSCs are not available. MSCs have ability to suppress cytokine production and improve the healing process. We showed that LSCs and MSCs have the ability to inhibit production of IL-1, iNOS, TNF-α and VEGF in the damaged cornea and these inhibitory properties were confirmed in both in vitro and in vivo models. Results thus showed beneficial effects of SCs transplantation for corneal healing and for suppression of a local immune reaction which can impede the healing process.

ROLE OF SRC FAMILY KINASES IN IMMUNOLOGICAL SYNAPSEOF ANTIGEN PRESENTING CELLS

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Antigen presentation is a key process in the initiation of adaptive immunity and the role of Src Family Kinases (SFKs) in the antigen presenting cells (APCs)is not completely understood.The activity of SFKsis negatively regulated by phosphorylation of their inhibitory tyrosines by Csk kinase. This effect can be potentiated by artificially targeting Csk to the plasma membrane, resulting in strong inhibition of SFK activity. We decided to use constructs coding for membrane targeted Csk to investigate the role of SFKs in APCs. For targeting Csk to the plasma membrane, we generated inducible constructs with Csk fused to transmembrane adaptor proteinsLAT and SCIMPor their membrane-anchoring sequences. SCIMP brings Csk directly to the immunological synapse, while LATis targeting Csk to lipid rafts. These constructs allow us to study SFK activity in the dendritic cellsduring the process of antigen presentation to the T cell.Furthermore, we focused on the role of the transmembrane adaptor protein SCIMPin the signaling inDCsand on its contribution in the processes of antigen-presentation, fungal antigen recognition and ensuing signaling cascades.

ROLE OF CALCINEURIN NFAT SIGNALING IN MOUSE MYELOID CELLS AND HUMAN PERIPHERAL BLOOD MONOCYTES

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The calcineurin-NFAT pathway has been recently identified as an important player in the innate immune response and there is increasing evidence that transcription factor NFAT is functional in various subset of myeloid cells (Fricet al., 2012). Recently we have shown that calcineurin/NFAT signaling in myeloid cells is essential for better survival of mice with aspergillosis (Zelanteet al., 2016). Upon trigger with TLR4 and Dectin-1 ligands we identified number of calcineurin NFAT dependent genes driving direct innate responses and genes regulating renewal of myeloid compartment. These findings remain to be confirmed in human myeloid cells.

Monocytes from peripheral blood, which serve as a systemic reservoir of myeloid cells, were analyzed in order to understand the involvement of calcineurin NFAT signaling in pattern recognition receptors (PRRs) driven responses. Calcineurin NFAT pathway is activated through PRRs signaling during tissue damage or infection. To study the role of PRRs signaling in human myeloid cells, we used monocytes isolated from blood of healthy volunteers or patients with sepsis. We sorted out 3 monocyte subpopulations CD14hiCD16lo, CD14loCD16hi, and CD14hiCD16hi and stimulated them with broad range of PRRs ligands (LPS, zymozan, β-glucan) under selective inhibition of NFAT signaling by addition of immunosuppressive drug cyclosporine A or tacrolimus. Changes in gene expression upon the triggers were analysed using qPCR and this outcome was used to validate the results obtained in global gene analysis of mouse myeloid cells conditionally deficient in calcineurin.

Observed changes in calcineurin-NFAT dependent gene expression upon PRRs triggers in mouse CD11c+ myeloid cells and in human monocytes showed importance of NFAT pathway during innate immune response. Need of these results is especially pertinent for immunosuppressed patients treated with calcineurin/NFAT inhibitors where such data can explain part of patients' susceptibility to infections.

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ANTIMICROBIAL PEPTIDES AGAINST DENGUE VIRUS INFECTION

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Dengue virus is transmitted to humans through the bites of infected female Aedes mosquitoes. The skin is the first tissue in contact with the virus during infection, and the keratinocytes are the main component of the skin. These cells provide a chemical barrier thought of the production of antimicrobial peptides (HBD2, HBD3 and LL37). The AMPs are small proteins with a broad spectrum of antimicrobial activity and they are considered as part of the host innate immunity.

MATERIALS AND METHODS: Keratinocytes were infected with DENV-2 (10 MOI) and analyzed at 12, 24 and 48 hours post infection by flow cytometry and immunofluorescence. At the same time, to demonstrate a productive infection, the viral progeny was evaluated in the supernatant from infected cells and cells pretreated with different AMPs (HBD2, HBD3 and LL37) before of infection (10, 20 and 30 μg/mL of HBD2, 3 or LL-37) by plaques forming units (PFU in BHK-21cells).

RESULTS: The results showed that these cells are permissive to DENV-2 infection and positives for the expression of viral antigen E (7, 10 and 20% of expression respectively). By other hand, the viral progeny was decrease in cells pretreated with LL37, not being well with the others AMPs (HBD2, 3) or untreated cells.

CONCLUSION: This results suggesting that keratinocytes may be one of the early targets of DENV-2 and them maybe respond to this pathogen producing molecules of innate immunity such as antimicrobial peptides, and this AMPs have effect against DENV-2 infection.

TYPE III INTERFERONS: THE SURVIVING HEROES OF CELL DEFENSE

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During the primary infection, human gammaherpesviruses infect mostly the epithelial cells of the entry site, however, there is little information on innate antiviral response and virus-host interactions during this initial replication in the host. Therefore, we studied the interferon (IFN) signaling pathways, including type I and type III IFNs and their role in the first 6 hours of murid herpesvirus-4 (MuHV-4) replication in murine epithelial cells. MuHV-4 is genetically closely related to human gammaherpesviruses and is widely used as a model for in vitro and in vivo experiments. In our study we found that MuHV-4 induces degradation of type I IFN receptor in very early stages of infection (2-4 hpi), but not type III IFN receptor. In addition, type III IFNs are also present in infected epithelial cells early in infection. We can conclude that, in contrary to type I IFN signaling, which is strongly inhibited by MuHV-4 immediately after the infection, type III IFNs might play a key role in antiviral defense of epithelial cells in early stages of MuHV-4 replication. These findings help us to understand the role and the impact of innate immune system on ability of gammaherpesviruses to replicate after primary infection and establish life-long latency.

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SERUM AND SALIVA LEVELS OF CYTOKINES AND YKL-40 IN ORAL CANCER

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The modern concept of cancer reveals a clear link between inflammation and oncogenesis. The tumor microenvironment is essential for tumor development, progression, invasion, and metastasis. It is composed by many different cell types including immune inflammatory cells, serving as the main source of inflammatory cytokines. The later have pleiotropic effects on cells in the tumor microenvironment such as impaired apoptosis, increased proliferation, angiogenesis and invasion. From the other side, YKL-40, an extracellular matrix glycoprotein, has been established by our group as a stable marker in rheumatoid arthritis as well as a potential indicator of the invasiveness and malignancy in neoplastic processes. Oral squamous cell carcinoma (OSCC) is the eighth most common cancer worldwide, with a high rate of recurrence. The aim of the current analysis was to measure serum and saliva levels of several key cytokines and YKL-40 in oral cancer versus healthy controls by enzyme-linked immunosorbent assays (ELISA) for each separate analyte. Our results clearly showed altered levels of both cytokines and YKL-40 in oral cancer demonstrating an interaction between chronic inflammation and oral cancerogenesis.

DEVELOPMENT OF VIRTUAL MEMORY T CELLS

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Virtual memory T cells represent a subset of CD8+ T cells with features of memory cells, although they have not encountered their cognate antigens. The origin, functional characteristics, as well as the biological importance of virtual memory cells are still unclear. We addressed the development of virtual memory T cells in various genetically modified strains in SPF and germ-free mice.

We analyzed CD8+ T-cell compartment in CD8.4 knock-in mice, which express a chimeric CD8.4 coreceptor with higher CD8-Lck coupling than CD8WT. Virtual memory T cells were about twice as much frequent in the CD8.4 mice than in CD8WT mice in SPF and germ-free conditions. Monoclonal CD8.4 T cells expressing F5 transgenic TCR did not differentiate into virtual memory cells. In contrast, monoclonal CD8.4 T cells expressing OT-I transgenic TCR differentiated into virtual memory cells in the absence of any cognate stimulation. Because the OT-I TCR is more self-reactive than the F5 TCR, our results indicate that a combination of an intrinsic high level of self-reactivity of the OT-I TCR and CD8.4-mediated hypersensitivity to self-antigens induced the differentiation of CD8.4 OT-I cells into virtual memory T cells.

Overall, our data suggest that virtual memory T cells originate from peripheral T cells with a relatively high level of self-reactivity. Thus, the formation of virtual memory cells represents a novel cell-fate decision checkpoint occurring during homeostasis. Moreover, the CD8-Lck coupling frequency regulates the sensitivity of T cells to self-antigens and T-cell differentiation into virtual memory T cells. The level of CD8-Lck coupling frequency in peripheral CD8+ T cells determines the proportions of naïve and virtual memory T cell subsets.CD8.4 OT-I virtual memory T cells were hyperresponsive to stimulations with foreign antigens in vitro and in vivo. In this way, they phenotypically resembled virtual memory CD8+ T cells that accumulate in aged mice. It is tempting to speculate that impaired T cell responses in aged individuals are caused by the accumulation of relatively highly self-reactive T cells, their differentiation into virtual memory T cells, and their subsequent exhaustion as a result of long-lasting persistent stimulation.

IMMUNOGENIC AND SEROLOGIC EVALUATION OF SOME BIOMARKERS INVOLVED IN CUTANEOUS MELANOMA AND PROGNOSIS

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Cutaneous melanoma (CM) is the most severe skin neoplasia, the invasion and metastasis occurs rapidly, local phase being relatively short, so diagnosis is often established in advanced stages. Despite therapeutic developments, the prognosis of patients with CM remains unfavorable. Numerous studies have evaluated the prognostic relevance and diagnostic potential of a large number of serum biomarkers for melanoma. Cytokines and growth factors have biologic effects that could stimulate tumor growth, invasion and angiogenesis. Materials and Methods. Tumoral DNA was extracted from 8 paraffin processed tumor histologically diagnosed from patients with CM. Somatic mutations in BRAF, CDKN2A, CTNNB1 / beta-catenin, GNAQ, HRAS, KIT, KRAS, NR a S, PIK3CA and LKB1 / STK11 genes (Kit Somatic Mutation qBiomarker Human Melanoma - SA Bioscience/Qiagen) were detected by qPCR-array (System PCR Rotor-Gene 6000, Corbett). The products of the main genes with changes of expression in cutaneous melanoma were identified in serum by ELISA techniques (TECAN line): TP53, MITF, NRAS, GNAQ, CDKN2A/P16, BRAF, HRAS, KRAS (MyBioSource kits). MITF and TP53 concentrations were determined for 36 patients, HRAS, KRAS and BRAF in 21 patients, NRAS and GNAQ in 32 patients and concentrations CDKN2A/p16 for 43 cases. Results. In 5 out of 8 tested tumors were identified mutations of the following genes: BRAF (2 mutations), GNAQ, KIT, KRAS, NRAS (4 mutations), CDKN2A (2 mutations). The products of mutant genes had elevated as follows: 52% for BRAF, 9.5% for HRAS and 57% for KRAS. CDKN2A gene is ubiquitously over expressed in CM, while NRAS and GNAQ genes don't have a significant presence as a change of expression. Conclusions. Investigations reveal the possibility of accreditation of new biomarkers in melanoma, detectable by genomic or serological methods. The importance of these biomarkers is revealed in tumors in situ (markers of evolution) or to establish monitoring treatment in patients with known mutations in genes involved in the development and proliferation of malignant melanocytes.

THE IMMUNOMODULATING EFFECTS OF NOOTROPIC PREPARATIONS

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Inflammation plays an important role in the pathogenesis of ischemic stroke and other forms of ischemic brain injury. Normalization of vascularization, impaired due to hypoxia and recovery of the immune response are important components of ischemic disease treating. The aim of our work was to study of effects of nootropic preparations Cerebrolysine (Ebewe, Austria), Actovegin (Nycomed, Norway), Piracetam (Darnytsia, Ukraine) and Cerebral (a new trophinotropin agent)on immunocompetent cells.
Cultured cell lines MT-4 (human T-lymphoblastic leukemia), Raji (B-cell Burkitt's lymphoma), primary cultures of T- and B-lymphocytes obtained from peripheral blood of healthy donors and patients suffering the acute phase of hemorrhagic stroke were used. Cells were incubated in the medium RPMI under standard conditions. Cytotoxic/pro-proliferative effect on cultured cells was determined using cytofluorymetric analysis and MTT-test.
As a result of a study, it has been found that Cerebrolysine has a cytotoxic and proliferative effect, Actovegin and Piracetam have antiproliferative effect relative to cultured cells MT-4 and Raji. It is shown that Cerebrolysine increase the number of apoptotic cells in 2-3 times, Actovegin in 2 times compared with control. Cerebral is pro-proliferative factor for MT-4 and Raji cells and decrease the level of apoptosis in 2 times compared with control.Cerebrolysin, Piracetam and Actoveginincreased of apoptotic cells in a group of donors, whereas they not shown such effect in a group of patients.At the same time, Cerebral reduced the level of apoptosis in 13 times compared with control and normalized cell cycle of lymphocyte in a group of patients.
Therefore, a new trophinotropin agent - Cerebral can be quite effective to recovery of the immune response, which is important in post-stroke period at ischemic complication

MACROPHAGE-ASSOCIATED INFLUENCE OF TIECHOIC ACIDS FROM ST. AUREUS AND BIMETALLIC COMPLEX ON PRIMARY TUMOR CULTURE

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Ligands of Toll-like receptors are often used as adjuvants anticancer therapy. Such ligands are biopolymers from cell wall of gram-positive microorganisms Staphylococcus aureus – techoic acids (TAs). Our previous studies showed that TAs in joint application with bimetallic complex of copper and cadmium with ethylenediamine (PO244) increased antitumor effect of the last. In order to determine possible mechanisms of TAs+PO244 impact on tumor through immune cells we studied primary Lewis lung carcinoma (LLC) culture after the impact of macrophages from LLC-bearing mice at the last stage of carcinogenesis. Both TAs and PO244 were administrated on 8th day after tumor cell inoculation. After the therapy macrophages were contactless cocultivated with primary LLC culture during 48 h. Macrophages from peritoneal exudate of mice was obtained by standard Pietrangeli's procedure. Apoptotic index and cell distribution in phases of cell cycle were assessed by flow cytometry. Aforementioned combination revealed in 2-times increasing of LLC cells apoptotic level in comparison with primary LLC cells (without coculture) and LLC cells under condition of cocultivation with macrophages from mice without therapy. TAs+PO244 therapy decreased population of LLC cells in proliferative pool (G2/M+S phase) to 40%, whereas control rates were 65% and 60% in LLC cells without coculture and LLC cells with macrophage coculture from mice without therapy, respectively. Cytotoxic/cytostatic influence was defined after cocultivation of macrophages from LLC-bearing mice treated by TAs+PO244 with primary LLC culture. This effect can be one of the possible mechanisms of TAs+PO244 impact on the tumor.

STREM-1 AS A NEW DIAGNOSTIC AND PROGNOSTIC MARKER IN SEPSIS

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Introduction: The sepsis is extremely komplex pathological state caused by dysregulation of immunological mechanisms leading to metabolic failure during systemic infection. It's the most frequent cause of death in ICUs. Early diagnostics of sepsis is essential for reducing the mortality. TREM-1 (triggering receptor expressed on myeloid cells 1) is one of PRR (pattern recognition receptors), which binds PAMPs (pathogen associated molecular patterns) and supports production of proinflammatory cytokines and activation of neutrophils, monocytes and macrophages. Its expression on myeloid cells and plasma level of its soluble form raises during infection. **Materials and Methods:** The plasma level of sTREM-1 was measured by ELISA test in 74 samples from 12 patients in sepsis or with suspicion of sepsis and 39 samples from healthy individuals. We used flow cytometry to study expression of TREM-1 and TREM-2 on myeloid cells in peripheral blood of patients. The differential blood count and level of procalcitonin in plasma was measured too. **Results**: The statistical Spearman analysis revealed correlation between sTREM-1 level and neutrophil-lymphocyte ratio (Neu/Ly; p=0,0285). Mann-Whitney test showed that plasma level of sTREM-1 was significantly higher in patients (96,407pg/ml) than in healthy controls (25,7pg/ml; $p<0.01$). Non-parametric Spearman test indicated modest correlation between sTREM-1 and procalcitonin level in plasma(p=0,0560). **Discussion**: Correlation between sTREM-1 level and neutrophil-lymphocyte ratio indicates it could be used as a potential prognostic marker and improve the detection of patients in higher risk. Higher level of sTREM-1 in patients also suggests its role as a potential marker in early diagnostics of sepsis.

DEVELOPMENT OF IMMUNOMONITORING ASSAYS FOR DENDRITIC CELL-BASED LUNG CANCER IMMUNOTHERAPY

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Allogeneic cancer cell lines might serve as a universal source of tumor antigens in the development of dendritic cell-based cancer vaccines. We showed that selected antigenic profile of lung cancer cell lines overlaps with antigenic profile of primary non/small cell lung cancer (NSCLC) tumors. However, it is unclear if T cells responses to all of these antigens can be detected in blood of NSCLC patients. Therefore peripheral blood mononuclear cells (PBMCs) of NSCLC patients were stimulated and re-stimulated by commercially available mixes of antigenic peptides derived from these antigens over the course of 10 days. Tumor antigen-specific CD8+ and CD4+ T cells were characterized by IFN-γ production, granzyme B, perforin, CD137 or CD154 expression by flow cytometry. In addition, the expression of inhibitory molecules TIM3, CTLA-4, PD-1 and LAG-3 were evaluated on CD8+ and CD4+ T cells from PBMC of NSCLC patients. We further analysed 6 populations of myeloid-derived suppressor cells (MDCS) by a multicolor flow cytometry and their possible functional suppression by qPCR analysis of ARG1, iNOS, IDO expression in PBMC and downregulation of CD3ζ in T cells from patient's PBMCs compared to T cells from PBMCs of healthy donors. These data will allow us to develop a protocol for immunomonitoring studies of the effectivity of dendritic cell-based lung cancer immunotherapy in ongoing phase I lung cancer clinical trial (NCT02470468).

ASSESSING THE QUANTITATIVE IMPACT OF NON-RECEPTOR PROTEIN TYROSINE PHOSPHATASES SHP-1 and SHP-2 ON TCR SIGNALLING THRESHOLDS

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A fine balance between the opposing actions of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) governs decision-making processes throughout the life of a T lymphocyte. Acute changes in this balance can trigger genuine signalling cascades, as proposed in the kinetic segregation model of TCR signal initiation. Aberrant or misguided spatio-temporal patterns of Tyr-phosphorylation lead to inappropriate T-cell activation and differentiation culminating in autoimmunity. Not surprisingly, several PTPs are involved in human autoimmune disorders.

Non-receptor protein tyrosine phosphatases (NRPTPs) are a class of cytosolic PTPs modulating TCR signalling cascades at many levels. Human T cells express 14 out of the 17 NRPTPs encoded in the genome. SHP-1 and SHP-2 are prototypic T-cell expressed NRPTPs with a partially overlapping substrate range. Structurally, the SHPs consist of two N-terminal SH2-domains followed by the catalytic domain and a C-terminal tail containing regulatory Tyr-residues. Despite a wealth of literature, no clear consensus has been reached on the role of the SHP enzymes within the TCR signalosome. Available data cannot be reliably compared due to differences in cellular systems and experimental conditions used. Additionally, we lack a comprehensive picture of bona-fide substrates and interactors in T human cells. Recent data on a T cell specific deletion of SHP-1 in mice have raised doubts about an essential, non-redundant role for SHP-1 in TCR signaling. Hence, there is a need to re-assess the function (and functional redundancy) of these phosphatases in the TCR cascade under precisely controlled stimulatory conditions.

Here we report the use of a fluorescent NFkB transcriptional reporter Jurkat line expressing the 1G4 TCR, specific for the A2-restricted NY-ESO-1 melanoma peptide SLLMWITQC. A set of altered peptide ligands of varying affinities, presented by engineered antigen presenting cells, allows graded and physiologic TCR stimulation conditions. Differences in ligand affinity and pharmacological perturbation of proximal signaling are faithfully converted into quantitatively different fluorescence readouts by our reporter system. Employing an inducible shRNA system, we are studying the effect of acute SHP-1 and SHP-2 knockdown on TCR signaling thresholds under different affinity stimulations.

CD222 POSITIVE EXTRACELLULAR VESICLES RELEASED FROM T CELLS

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Extracellular vesicles (EVs) are heterogeneous membranous structures released from the cells. They are tools for the intercellular communication to transfer lipids, proteins and RNA. EVs are generated either by shedding from plasma membrane (microvesicles) or within endosomal structures (exosomes). In our work, we focus on the CD222-positive vesicles released from both resting and activated T cells. CD222 - the mannose 6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2R) - is one of the a major endosomal protein transporters in the cell. We have recently shown that, first, CD222 transports the primary kinase of T cell activation, Lck, to the plasma membrane and thereby is crucially implicated in T cell activation; and second, CD222 is upregulated on activated T cells. Here, we study how is this novel role of CD222 in T cells associated with CD222-positive EVs.

EPSTEIN-BARR VIRUS PNEUMONIA SUCCESSFULLY TREATED WITH INHALED INTERFERON-ALPHA

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A 22-Year-Old Caucasian woman with chronic active Epstein-Barr virus disease, diagnosed 1.5 years earlier, was admitted to the Immunology Clinic due to a 4-week history of productive cough, fever and general weakness. Previously, the patient has been receiving antibiotics for two weeks in an outpatient setting, but no significant improvement was observed. The patient has been diagnosed with infectious mononucleosis at the age of 17 with resultant significant deterioration of her status lasting for 6 months. Long-term treatment with subcutaneous interferon-alpha (IFN-alpha) was implemented in our patient (3 million units 3 times a week) before she was discharged home with recommendation to continue the therapy. However, patient's condition deteriorated three months later: she presented with fever, persistent cough and was in generally poor physical and mental status. The dose of IFN-alpha was escalated to 6 million units 3 times a week, but still with no significant clinical improvement. Cultures of blood, urine and sputum were negative, but 9 million/ml of EBV copies were found in the sputum. Disseminated maculate infiltrative areas in both lungs, more prevalent on the right side, were observed on a CT chest scan Diagnosis: Interstitial pneumonia in chronic active Epstein-Barr virus disease (CAEBV). 180 million/ml EBV DNA copies were found in bronchoalveolar lavage fluid. Owing poor response to previous treatment, inhaled IFN-alpha (1.5 million units 3 times a day) was implemented three months later. To the best of our knowledge, this was the first documented use of inhaled IFN-alpha in a patient with CAEBV and concomitant IP. Patient's status has finally improved after 10 days of treatment, as manifested by a decrease in peripheral blood viral load. Furthermore, partial regression of pulmonary changes was observed on a CT scan obtained one month later. The patient was qualified to bone marrow transplantation with reduced conditioning, which was carried out 6 months later. Currently, the patient feels well, no EBV

GC-RICH DNA AS AN INDUCTOR OF VICIOUS CIRCLE OF INFLAMMATION IN RHEUMATIC ARTHRITIS

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It has been established that cell-free DNA (cfDNA) circulates throughout the blood stream affecting cells. The characteristics of cfDNA depend on the physiological state of the organism. Diseases can cause GC-enrichment of the cfDNA pool (cerebral atherosclerosis, heart attack, rheumatic arthritis, cancer; N=760).We have demonstrated that GC-rich DNAcan induce a vicious circle of inflammation in patients with rheumatic arthritis (RA).CfDNA of RA patients stimulates expression of TLR9 and MyD88 in lymphocytes. Moreover, GC-rich DNA activates NFkB signaling pathway: genes of NFkB signaling pathway: *MAP3K1, MAP4K4, NFKB1A, REL, IKBKB, RelA (p65), NFRKB, NFKB1* and *NFKB2* increase 2 – 5-fold. Expression of NFkB target proinflammatory cytokines genes *TNFa*, *IL1B*, *IL8*, *IL6*, *TNFRSF1A* increases 2 – 4-fold. These events area ccompanied by increase of concentration of proinflammatory cytokinesIL6 and TNFα in the medium.We are proposing the following scheme that explains potential role of GC-rich cfDNA in induction and maintaining of chronic RA.Damaging factors like traumas, stress, infection cause accumulation of GC-rich cfDNA fragments in the blood plasma of RA patients due to dying cells.GC-richcfDNA activates expression of genes of TLR9-MyD88-NFkB-signaling pathways leading to expression of anti-inflammatory cytokines IL6 and TNFα in lymphocytes. This proccess leads to the death of a new fraction of cells causing additional ejection of cfDNA into blood.

REDUCED FUNCTIONAL RESERVE PERITONEAL MACROPHAGES AT THE STAGE OF AVASCULAR METASTASIS AFTER LEWIS LUNG CARCINOMA TRANSPLANTATION

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An index that characterizes the integrated level of the functional state of macrophages in the tumor progression is spontaneous and induced activity in NBT-test. The level of metabolic activity of peritoneal macrophages (respiratory burst) was determined by their ability to restore nitroblue tetrazol. This test is an indicator of a degree of macrophage activation in different organs (spontaneous activity) and the availability of reserves cell activation by various factors (induced activity). In determining the spontaneous and induced phagocytic activity in NBT - test, it was found that the level of induced forbol-merystat-acetate phagocytic activity of peritoneal macrophages of mice with Lewis lung carcinoma decreases over time as compared with intact animals. This indicates exhaustion of the immune system of animals, which in turn is reducing the number of control tumor cells by it. As a result, studies have found that the level of metabolic activity of peritoneal macrophages in mice depending on the day after tumor transplantation is different. Thus, as a result of the research found that the rate of spontaneous respiratory activity of peritoneal macrophages of mice with transplanted tumor different depending on the time after tumor transplantation, and on the 25th day after it increased significantly relative to the same indicator of intact animals. Studied the activity levels induced in mice on different day's tumor growth. As a result of investigations it was found that the level of induced respiratory activity of mice with tumors decreased from 7th to 25th day after transplantation.

RAPID MULTIPLEX ANALYSIS OF LIPID RAFT SIGNALING COMPONENTS WITH SINGLE LYMPHOCYTE RESOLUTION

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Lipid rafts, a distinct class of highly dynamic cell membrane microdomains, are integral to cell homeostasis, differentiation and signaling. Raft-association of important lymphocyte receptor signaling molecules - e.g. Src family kinases - and stimulation-induced variations in raft composition was demonstrated as well as a clear correlation of their raft-association with signaling function. However, biochemical quantitative analysis of lipid raft components involves laborious and time-consuming cell lysate fractionation via sucrose density gradient ultracentrifugation. Further, this method needs a large number of input cells and single cell information is completely lost. This complicates or even precludes the examination of rare cells, developmentally heterogeneous cell populations or weakly raft-associated factors. We established a fast and reliable method that is based on the low g centrifugation of cells through a detergent gradient, requiring little starting material and effort. Our widely applicable protocol enables multidimensional and sensitive flow cytometric quantitation of raft-associated proteins with single cell resolution. It allows easy and precise assessment of endogenously and ectopically expressed membrane components from a few cells in complex isolates as well as their dynamics due to cell differentiation, signaling and mutation. In conclusion, our approach is well suited to elucidate the role of lipid rafts in regulation of factors that govern proximal signaling thresholds of crucial leukocyte receptors, including the T cell antigen receptor.

HUMAN DENDRITIC CELLS PULSED WITH HIGH HYDROSTATIC PRESSURE-INACTIVATED PROSTATE CANCER CELLS AND MATURED WITH POLY(I:C) INDUCE AUTOLOGOUS LYMPHOCYTES TO EX VIVO RECOGNIZE AND KILL PROSTATE CANCER CELLS

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Human dendritic cells (DCs) pulsed with high hydrostatic pressure (HHP)-inactivated prostate cancer cell line LNCaP was found to induce proliferation of autologous lymphocytes. These lymphocytes then readily responded to a re-challenge with the DCs. However, what impact these lymphocytes might have on living LNCaP cells is not known. Here we show that monocyte-derived immature DCs from healthy donors that were pulsed with HHP-inactivated LNCaP cells and stimulated with TLR3 agonist poly(I:C) have an enhanced surface upregulation of DC maturation markers CD80, CD83, CD86 and HLA-DR, and a capacity to induce a strong expansion of autologous lymphocytes. Such the expanded lymphocytes were found to elicit a cytotoxic response once exposed to living LNCaP cells but not to a control ovarian cancer cell line SKOV-3. Importantly, autologous lymphocytes that were not previously induced by the DCs did not show a cytotoxic response to either of the tested lines. Our data indicate that DCs loaded with HHP-inactivated prostate cancer cells and matured with poly(I:C) may induce immune response that leads to expansion of lymphocytes that are able to recognize and kill prostate cancer cells.

PROTUMORIGENIC EFFECTS OF SENESCENT CANCER CELLS

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Cellular senescence is considered to be the irreversible growth arrest in which cells remain metabolically active and display characteristic senescence-associated secretory phenotype. Cellular senescence can be induced by antitumor therapy, such as chemotherapy or irradiation and, under certain circumstances, also by cytokines (IFNγ and TNFα). In this study, we assessed the impact of docetaxel treatment on process of senescent induction, using TC-1, TRAMP-C2, and B16-F10 tumor cell lines. Docetaxel-induced TC-1 senescent cells have phenotypical changes, including enlarged flattened morphology, high activity of senescence-associated β-galactosidase, decreased cell proliferation and changes in their secretory phenotype. Increased expression of GROα, IL-6, IL-8, TGFβ and p21 was found. Docetaxel-induced
TC-1 senescent cells lose their growth ability in vivo in syngeneic mice. However, they accelerated growth of tumour transplants when inoculated in admixture with parental proliferating tumour cells. The same effect we observed when parental cells were admixed with unrelated docetaxel-induced TRAMP-C2 (murine prostate cancer cell line) senescent cells. Further, we have demonstrated in vitro that Th1 cytokines IFNγ and TNFα were able to induce cellular senescence in the B16-F10 melanoma cells but not in TC-1 tumour cells. Induction of this cytokine-induced senescence was associated with ROS production and NOX4 expression in B16-F10 cells. Collectively, our findings could be relevant for optimization of chemoimunotherapeutic protocols in cancer treatment. This work was supported by grant No. 15-24769S/ Czech Science Foundation, by NT 14461/ Grant Agency of the Ministry of Health of the Czech Republic.

EXPRESSION, INTERACTION AND ANTITUMOR ACTIVITIES OF BIGLYCAN IN HER-2/NEUMEDIATED CARCINOGENESIS

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The level of HER-2/neuovexpression in breast cancer is often inversely correlated withMHCclass I surface expression leading to escape from T cell-mediated immune surveillance. Biglycan(BGN), a leucine rich proteoglycan, is involved in autophagy and immunological responses thereby suppressing or exacerbate pathological conditions like tumorigenesis and angiogenesis.In HER-2/neu-transformed murine models systems BGN expression is downregulated, which is associated with neoplastic properties of these cells. However, a link between BGN expression and the HER-2/neu-mediated downregulation of MHC class I antigen expression has not yet been shown.Over-expression of BGN in HER-2/neu-transformed cells enhanced both classical (H2-Lq) and non-classical (H2-M3) MHC class I alleles, which might be due to aBGN-induced upregulation of components of the MHC class I antigen processing machinery (APM). Indeed qPCR and promoter assay displayed higher transcription of some members of APM, such as TAP1 and TAP2 in BGN transfectants versus mock controls .Furthermore, this effect was even more pronounced upon IFN-γ treatment. Interestingly, HER-2/neu+ down-regulated not only the expression of BGN in comparison with HER-2/neu-, but also affected the expression of other proteoglycans, namely decorin, osteoglycin, lumican, fibromodulin, PRELP and asporin. Interestingly, increased BGN expression was accompanied by an enhanced decorin and fibromodulin expression. In contrast, osteoglycin expression was reduced in BGN transfectants. Our data provides highly novel and unexpected proteoglycan signatures as adirect function of systemic administration of BGN protein and reveals a fundamental basis of action for BGN to modulate the MHC class Iantigen expression as a biological mechanism for the described anti-tumorigenic properties.

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DYNAMICS OF HUMAN T CELL REPERTOIRE IN COURSE OF INFLUENZA VACCINATION AND SUBSEQUENT REVACCINATION WITHIN A YEAR

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Annual vaccination is considered as an effective tool to prevent flu epidemics. However, efficiency of vaccines is limited by the high level of viral variability. To elucidate this issue we performed a deep quantitative analysis of TCR repertoire of three individuals in course of vaccination against influenza twice within a year. To analyze the dynamics of the immune response during vaccination at each of 5 timepoints per one vaccination we isolated two full fractions (biological replicas), CD4+, CD8+ and memory T lymphocytes fractions of the PBMC. cDNA libraries were prepared from isolated RNA and sequenced by NGS. For all timepoints TCR repertoires were reconstructed. All T cell clones were characterized by their frequency, phenotype and gene segments. Statistical model was developed and responded T cell clones were defined as clones that statistically significant changed their concentration between different timepoints. Among three individuals all responded clones were unique. Furthermore, within one individual the majority of the responded clones that were found during revaccination were not observed in the first vaccination. Also we received evidences of new memory T cell generation after first and second vaccinations. This work was supported by grants RSF 15-15-00178 and RFBR 14-04-01823

CD222 IN THE HUMAN URINE: ORIGIN AND DIAGNOSTIC VALUE

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The mannose 6-phosphate/insulin like growth factor II receptor (CD222, M6P/IGFIIR) is a multifunctional receptor, mostly localized intracellularly, less on the cell surface of all types of mammalian cells. It is involved in the processes like the transport of acid hydrolases containing mannose 6-phosphate moieties in its structures into lysosomes, or in binding and internalization of extracellular ligands like IGFII or plasminogen. This is related to functions of CD222 in regulation of cell proliferation, migration and apoptosis. Recently, it has been shown that soluble CD222 (sCD222) can be proteolyticaly released from the surface of human endothelial cells by the tumour necrosis factor-α-converting enzyme (TACE). sCD222 was also detected in the human blood serum. In this study, we, first, show that CD222 is present in the urine of oncological patients; second, scrutinize its origin; and finally, question its diagnostic value.

Keywords: CD222; cancer; diagnostics; human body fluids; exosomes

TOLL-LIKE RECEPTOR SIGNALING IN THYMIC EPITHELIAL CELLS

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Tolerance to "self" is the fundamental property of the immune system and its breakdown can lead to autoimmunity. To eliminate self-reactive T-cells during thymic development, Aire promotes the expression of several thousands of tissue restricted antigens in the medullary thymic epithelial cells (mTECs). Based on several reports which demonstrated the importance of non-canonical NF-κB signaling in development and function of these cells, we assessedthe expression profile and potential roles of Toll-like receptors (TLRs)in the physiology of thymic epithelial cells (TECs).Our data show that similar to the thymic dendritic cells, both cTECs and mTECs expressTLR2, 3, 4, 5 and 9 on mRNA as well as on protein level. In addition, mTECs (MHCIIhighCD80high) stimulated with ODN1826, the TLR9ligand, significantly upregulated the production of proinflammatory IL-6, IL-12, TNF-α, and tolerogenic IL-10cytokines. This upregulation was dependent on MyD88 adaptor protein. Strikingly, the expression of IL-6 seems toalso depend on the presence of AIRE. To mimic more physiological conditions, deoxyguanosine-treated fetal thymic organ culturesstimulated with ODN1826 exhibited upregulated MHCII and PDL-1 expression. These results characterize MyD88-dependent immunomodulatory role of TLRs in mTECs.Future experiments utilizing several mouse models with deficiency in MyD88 and Aire genes will shed a more comprehensive insight into the role of TLR signaling in negative thymic selection.

FEATURES OF LACTOFERRIN EXPRESSION IN MALIGNANT AND NON-MALIGNANT PROSTATE TUMORS

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Background. One of the priority tasks of fundamental oncology is an in-depth study of the tumor cell biology to search for new diagnostic and prognostic markers of prostate cancer (PCa). Prostate gland is one of the few organs that produce lactoferrin (LF), but its role in pathogenesis and clinical course of PCa in not completly understood today
Aim: to evaluate clinico-pathological characteristics and features of LF expression in malignant and non-malignant prostate cancer tumor cells.
Materials and methods: We stutied 45 patients with stage II and III PCa and 18 with benign prostatic hyperplasia (BPH). Morphological and immunohistochemical studies of LF expression were performed according to standard protocols. Results of immunohistochemical study of 27 samples of prostate gland tissue without any visual changes were used as a control. Results were evaluated by H-Score method and was performed using STATISTICA 6.0 software.
Results. Indices of LF expression in PCa cells were 86.2±7.8 (with individual variations from 23 to 135 points) and in BPH cells – 149.4±14.0 points (with individual variations from 79 to 167 points). We identified an inverse correlation between levels of LF expression with age of patients with BPH ($r=-0,48$, $p<0.05$). Also we observed that LF expression depended on clinico-pathological features of the PCa: inverse correlation with tumor size ($r=-0.37$, $p<0.05$) and presence of metastases in regional lymph nodes ($r=-0.42$, $p<0.05$). High rates of prostate specific antigen in serum of PCa patients were associated with decrease in LF expression level in tumor cells ($r=-0.51$, $p<0.05$).
Conclusions. Different expression of LF in BPH and PCa cells associated with clinical and pathological features of PCa indicate the need of further studies, aimed at defining the role of LF in PCa pathogenesis.

THE TRANSMEMBRANE PROTEIN EVI2B IS A C/EBPα TARGET GENE REQUIRED FOR GRANULOCYTIC DIFFERENTIATION

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Transcription factor C/EBPα plays a crucial role in the formation of myeloid lineage. Alterations in C/EBPα expression cause impaired myeloid differentiation and are related to the development of acute myeloid leukemia (AML). Recently we discovered a novel C/EBPα target gene *EVI2B*, coding for a transmembrane protein abundantly expressed in hematopoietic system. Analysis of murine and human primary cells revealed the highest levels of EVI2B in granulocytes. Consistently, we observed that EVI2B expression is upregulated during granulocytic differentiation in vitro. Downregulation of EVI2B in murine primary hematopoietic cells and 32D/G-CSF-R cell line using specific shRNAs impairs myeloid lineage development. In addition, we observed decreased cell proliferation and increased apoptosis of EVI2B-silenced primary hematopoietic cells under myeloid differentiation conditions. To further investigate the role of EVI2B in myelopoiesis, we generated EVI2B knockout mice (EVI2B KO). No defects in steady state hematopoiesis was observed in the EVI2B KO animals, however KO cells demonstrated impaired G-CSF dependent myeloid colony formation *in vitro*. In addition to the role of EVI2B in myeloid differentiation, we observed reduced *EVI2B* levels in AML patient samples with defects in C/EBPα compared to other types of AML. This observation is consistent with the data showing that *EVI2B* is a direct target of C/EBPα and stresses the importance of further investigation of EVI2B function.

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INFLAMMATION-ASSOCIATED MARKERS IN SALIVA OF PATIENTS WITH SJÖGREN'S SYNDROME

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Sjögren's syndrome (SS)is a chronic autoimmune disease affecting exocrine glands including salivary glands. Biomarkers are neededfor early diagnosis and monitoring progression of this disease. The aim of this study was to analyse selected inflammatory markers in whole mouth saliva of patients with Sjögren's syndrome, patients with a complaint of dry mouth and healthy controls. A further aim was to clarify the source of inflammation. Twenty SS patients, 10 with xerostomia and 26 healthy individuals were recruited.Whole mouth saliva flow rate was reduced in the SS and dry mouth groups compared to healthy controls whilst mean total salivary protein concentration was increased in the SS group. Lactoferrin and myeloperoxidasewere analysed by ELISA and western blot. The mean lactoferrin concentration was significantly higher in the Sjögren's syndrome group compared to the other groups. Myeloperoxidase concentration was not significantly different between the groups when assayed by ELISA.These results suggest that the inflammation detected by these biomarkers is derived from salivary glands. Lactoferrin is potentially a useful biomarker for SS induced dry mouth. In the future, it is necessary to confirm this finding by further analysis of saliva from individual salivary glands.