ORAL PRESENTATIONS

BALANCING TOLERANCE AND AUTOIMMUNITY: CONTROLLING HARMFUL IMMUNE RESPONSES

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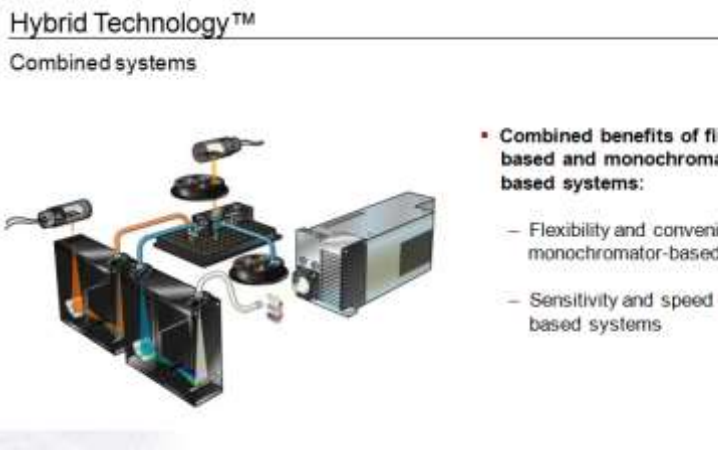
The immune system exists in an equilibrium, such that activation of the system to defend against pathogens is balanced by the mechanisms of tolerance, which prevent aberrant and harmful responses to self antigens. The most important mechanisms of T cell tolerance to self antigens are deletion of self-reactive T cells during their maturation in the thymus, inactivation of the cells by the engagement of inhibitory receptors of the CD28 family, mainly CTLA-4 and PD-1, and suppression of the response by regulatory T cells (Treg), which are generated in the thymus and peripheral tissues. Tregs respond to tissue antigens by developing an enhanced capacity to suppress immune responses and by migrating to tissue sites of inflammation. A fraction of these Tregs survive as long-lived memory Tregs, and are able to limit subsequent inflammation in the tissue. Elucidating the stimuli that generate and maintain functional Tregs in the periphery will likely be valuable for manipulating immune responses in inflammatory diseases and for optimal vaccination and cancer immunotherapy. We have used transgenic and knockout mouse models to address the mechanisms of the generation and activation of Tregs in tissues. Our studies indicate that antigen and cytokines are the major stimuli that induce peripheral Tregs and control the balance of effector and regulatory cells. In particular, the growth factor IL-2 is essential for the generation and maintenance of functional Tregs. These studies are leading to renewed attempts to exploit Tregs and IL-2 treatment to control harmful immune responses.

NEW AND UNIQUE TECHNIQUES IN LIFE SCIENCE USING MICROPLATE INSTRUMENTATION FROM BIOTEK INSTRUMENTS
DIFFERENT CELL-BASED ASSAYS USING THE PATENTED HYBRID TECHNOLOGY™ AND CELL IMAGING

Dr. Andreas Rieger
BioTek Instruments, Inc., Winooski, VT, USA

BioTek Instruments has been awarded US patent 8,218,141 covering the combination of two alternate paths for light within the same analytical instrument. Known as Hybrid Technology™, the patented functionality is available in BioTek's Synergy™ NEO HTS Multi-Mode Microplate Reader and in the Synergy H4 and H1 Hybrid Multi-Mode Microplate Readers.
Using Hybrid Technology™, light can be directed to and from the microplate samples through a filter-based optical system or through a monochromator-based optical system for wavelength selection. This two-optical-path system provides optimal performance and flexibility for a wide range of applications in fluorescence microplate assays. BioTek's Synergy readers are modular platforms that can include Fluorescence Intensity, UV-Vis Absorbance, Luminescence, TRF, TR-FRET and FP detection modes.
This presentation we will demonstrate the power of BioTek instrumentation which can perform a wide range of diverse applications in the biochemistry laboratory. These applications cover micro-volume nucleic acid quantification from 2 µL samples using the monochromators in absorbance mode; high sensitivity cellular signal transduction assays (AlphaScreen™) using spectral filters; live cell fluorescence assays using the monochromators in fluorescence mode and finally using the GloSensor® (Promega) for monitoring cAMP levels of GPCRs using a bioluminescent readouts or powerful Imaging to be used for phenotypic analysis or cell counting.



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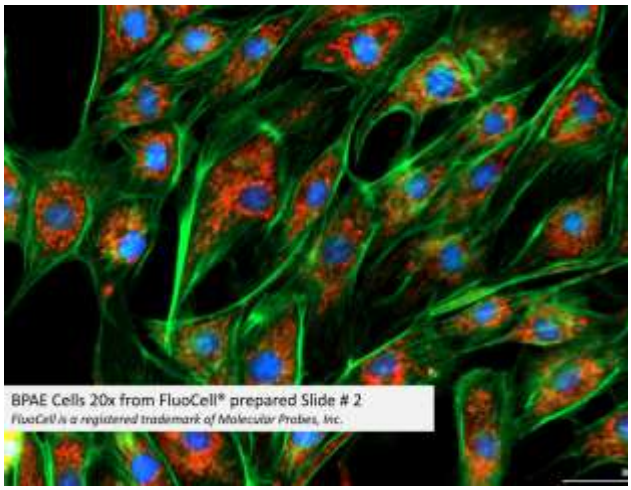

NEW KID ON THE BLOCK: UNEXPECTED ROLES OF 8-OXOGUANINE DNA GLYCOSYLASE-1 IN THE CELLULAR RESPONSES

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Introduction: 8-Oxo-7,8-dihydroguanine (8-oxoG) is one of the most abundant DNA base lesions induced by reactive oxygen species (ROS). Accumulation of 8-oxoG in the mammalian genome is considered a marker of oxidative stress, to be causally linked to inflammation, and is thought to contribute to aging processes and various aging-related diseases. 8-OxoG is excised from DNA by 8-oxoguanine DNA glycosylase-1 (OGG1) during DNA base excision repair (BER); the resulting exogenomic 8-oxoG base is thought to have no biological role, and is excreted from cells and organisms. Unexpectedly, mice that lack 8-oxoguanine DNA glycosylase-1 (OGG1) activity and accumulate 8-oxoG in their genome have a normal phenotype and longevity; in fact, they show increased resistance to both oxidative stress and inflammation.
Methods: Human diploid fibroblasts (MRC5), HeLa S3 cervical epithelial cells, A549 type II alveolar epithelial cells, U937 monocytic lymphoma cells, KG-1 myeloid leukemia cells expressing a temperature-sensitive mutant OGG1, mouse models of airway inflammation, siRNA ablation of gene expression, and a variety of molecular biological assays were utilized to define a link between OGG1-BER and cellular signaling.
Results: It has been demonstrated that OGG1 binds its repair product 8-oxoG base with high affinity at a site independent from its DNA lesion-recognizing catalytic site and the OGG1•8-oxoG complex physically interacts with GDP-bound Ras and Rac1 proteins. This interaction results in a rapid GDP→GTP, but not a GTP→GDP, exchange. Importantly, a rise in the intracellular 8-oxoG base levels increases the proportion of GTP-bound Rac1. Exogenously added 8-oxoG base is able to enter the cells and increase the proportion of both GTP-bound Ras and Rac1. In turn Rac1-GTP mediates an increase in ROS levels via nuclear membrane-associated NADPH oxidase type 4. Activation of Ras GTPase results in phosphorylation of the downstream Ras targets Raf1, MEK1,2 and ERK1,2. Ogg1 silencing in the airway epithelium decreases TNF-α-induced expression of chemokines/cytokines including Cxcl-2 and neutrophil recruitment. Silencing of OGG1 expression hampers TNF-α-induced association of transcription factors with promoter sequences and lowers Cxcl-2 expression. Furthermore, decreased Ogg1 expression in the airway epithelium conveys a lower inflammatory response after ragweed pollen challenge of sensitized mice.
Conclusions: These findings reveal novel mechanisms by which OGG1 in complex with 8-oxoG is linked to redox signaling and cellular responses. Results from *in vivo* studies indicate that a transient modulation of OGG1 expression/activity in airway epithelial cells could have clinical benefits.

VASCULAR PATTERNING AS A DETERMINANT OF IMMUNOLOGICAL COMPETENCE IN VISCERAL LYMPHOID TISSUES

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The capacity of mammalian organisms to mount efficient adaptive immune responses requires the establishment of highly ordered tissue architecture in peripheral lymphoid organs, ensuring continuous leukocyte influx and subsequent segregation. As a crucial component, local vasculature plays a key role in the tissue-specific recirculation of leukocytes. The patterning and differentiation of specialized vessels are closely linked to the embryonic development of peripheral lymphoid organs; however, details of endothelial commitment, morphogenic signals and communication pathways between hemopoietic cells, stromal cells and endothelial cells, and their impact in inflammatory processes are still largely unexplored. Recently Nkx2-3 homeodomain-containing transcription factor has emerged as a major regulator for splenic and intestinal lymphoid vascular commitment and a susceptibility trait associated with chronic inflammatory bowel diseases. Work in our laboratory has established that Nkx2-3 plays an important role in the local decision between lymphatic/blood endothelium within the spleen as well as commitment towards the high endothelial lineage within Peyer's patches. Here we report that, in addition to defining local vasculature, altered Nkx2-3 expression also influences intestinal IgA secretion and in the spleen the capacity for germinal center formation and plasma cell proliferation in a process that may involve red pulp megakaryocytes. Collectively, these observations indicate that the vascular commitment influenced by Nkx2-3 has far-reaching consequences beyond the structural evolution of peripheral lymphoid organs.
Supported by Broad Medical Research Program of The Eli and Edythe Broad Foundation and CABCOS II. HUHR/1001/2.1.3/0007 grants

HUMAN INTESTINAL DENDRITIC CELLS DICTATE INFLAMMATION AND T-CELL POLARIZATION

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*Introduction:*Enormous diversity of commensal bacteria determines individual functions acting on the development and activities of the human immune system. This complexity can directly be translated to T-cell polarization to support tolerance induction or inflammation. We have establisheda sensitive *in vitro* culture system for investigating the response of monocyte-derived dendritic cell (moDC) subsets to various gutbacteria by monitoring the expression of type I/II CD1 proteins, secretion of chemokines, pro-inflammatory and T-cell polarizing cytokinesin the context of the T-cell polarizing potential of these moDC subsets. Under physiological conditions the gut microenvironment is conditioned by all-transretinoic acid (ATRA) produced by gutepithelial cells and $CD103^+$ DCs. To consider the impact of this special microenvironment on moDC-induced T-cellresponses we compared the effects of selected microbes on DC and T-celldevelopment in the absence and presence of ATRA.
*Methods:*Monocytes were separated from human buffy coats and differentiated *in vitro*in the presence of IL-4 and GM-CSF with or without 1 nM ATRA for 2 days. Gram(-) (*Schaedler'sE. coli, E.coli 058, M. morganii)* and Gram(+) *(B.subtilis)*bacteria were grown in antibiotic-free LB medium and were added to the 2-day moDCs for 24 hrs.Activation of moDCwas monitored by the expression of membrane CD1a, CD1d and CD83 by FACS analysis. Culture supernatants of activated moDCwere collected on day 3 and cytokine concentrations were determined by ELISA. The number of IFNγ and IL-17 producing T-cells was measured by ELISPOT assay. Expression levels of selected NOD-like receptors and genes involved in ATRA synthesisweremeasured by qRT-PCR.
*Results:*Increased expression of CD83 revealed that all tested commensal bacteriawere able to activated moDCsfor pro- and anti-inflammatory cytokine secretion. ATRA had a significant impact on the differentiation, inflammatory response and T-cell polarizing activity of moDCs. It increased the cell surface expression of CD1a while increased that of CD1d, previously shown to be associated with a shift in moDC functionality. ATRA also enhancedIL-1ß secretion and upregulated the expression of genesinvolvedin ATRA synthesisandNLRP12mRNAlevels. Interestingly, theseATRA-inducedeffectscould be counterregulatedbythe tested microbe. The interaction of microbes resulted in IL-23 production supporting Th17 polarization of autologous T-cellsandincreased the number ofIFNγ producing T-cells however, these effects were down modulated by ATRA.
*Discussion:*In our culture system we identified two moDC subpopulations referred as DC-$SIGN^+CD11c^+CD14^{med}CD1a^+CD1d^-$ and DC-$SIGN^+CD11c^+CD14^+CD1a^-CD1d^+$ cells, which respond to and coordinateof stimuli from commensal bacteria differently to induce T-cell polarization and expansion. Our results also showed that the tested bacteria modulate the differentiation and activation of moDCs in a dose- and bacterial strain-dependent manner. Moreover, the interplay of moDC and commensals can be modified by the actual milieu of the cells such as ATRA.

THE ROLE OF ZAP-70 KINASE IN THE FINE-TUNING OF TCR SIGNALLING: IMPLICATIONS FOR IMMUNOPATHOLOGY AND –THERAPY

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ZAP-70 (zeta-chain associated 70 kDa) kinase is a key regulator of T cell receptor signaling. After ligand binding of the T cell receptor (TcR), Lck kinase phosphorylates tyrosine (Y) residues of the CD3 ζ chains and ZAP-70, which, in turn, phosphorylates a number of downstream target proteins (eg. LAT, SLP-76, PLCγ, Cbl).
ZAP-70 itself contains a number of Y residues, which can be phosphorylated. Using an array of mutant cell lines where targeted Y-Phenylalanine (F) mutations were introduced into ZAP-70, we were able to characterize the fine details of TcR signaling. Our data confirmed the function of earlier described activator (Y315, Y493) and inhibitory (Y292, Y492) residues; moreover, we described the regulatory role of previously less-known (Y069, Y126, Y178) positions.
Glucocorticoid treatment is widely used for suppressing the immune response, primarily through the inhibition of T cell functions. Our earlier work demonstrated, that ZAP-70 is also involved in non-genomic (rapid) GC signaling mechanisms. Using our Y-F mutant ZAP-70 expressing cell line array, we identified that Y315 and Y492 were phosphorylated upon short-term high dose GC analogue treatment. These results confirmed that ZAP-70 represents an important link between the non-genomic GC and TcR/CD3 signaling pathways.
Moreover, potential role of ZAP-70 kinase was implicated in chronic lymphoid leukemia (CLL) and autoimmune arthritis. It has been shown in a subgroup of patients with CLL that the malignant B-lymphocytes express ZAP-70 kinase, which was associated with inferior clinical outcome and prognosis. Using two ZAP-70 specific antibodies recognizing different epitopes in the kinase, we performed intracellular staining of malignant B cells from CLL patients. Based on our preliminary experiments, it seems possible that the ZAP-70 molecule expressed in the tumorous B-cells is structurally different from that found in normal T-cells, as some patients showed positivity with either one or the other antibody, while the normal T-cells were positive with both antibodies, just as expected.
A spontaneous single point mutation at 163 from Triptophane (W) to Cysteine (C) in the SH2 domain of ZAP-70 caused altered thymic selection and leads to the development of autoimmune arthritis in SKG mice. Another study has shown that targeted simultaneous mutation at positions Y315 and Y319 to Alanine led to similar defects in T cell development than in SKG mice, interestingly, however, these mice did not develop autoimmune arthritis despite the presence of rheuma factor in the sera, increased IL-17 production and impaired Treg development.
These data clearly show, how our understanding about ZAP-70 kinase has emerged from being exclusively a T cell specific signaling molecule to an important therapeutic target and potential regulator of pathologies like CLL or autoimmune arthritis.

EPIMUTAGÉN BAKTÉRIUMOK HATÁSA IMMUNGÉNEKRE

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Szegedi Tudományegyetem, TTIKar, Biokémiai és Molekuláris Biológiai Tanszék, Szeged

Epigénekrõl nem szoktunk beszélni, **epigenetika** azonban van és epigenetikainak tekintjük valamely jelleg öröklõdését akkor, ha annak továbbadása sejtrõl-sejtre, nemzedékrõl-nemzedékre nem nukleotid sorrendben rögzített. Ebben az értelmezésben az epigenetikai öröklõdést tekinthetjük génmûködési mintázatok megõrzésének a sejtosztódások során. Molekuláris alapját tekintve ez kromatinszerkezeti jellegzetességek fenntartása: a DNS és hozzá kapcsolódó fehérjék összecsomagolódásának megõrzése és továbbadása. A kromatinszerkezetet a DNS metilációs állapot, a hisztonok típusai és módosításai, számos transzlációra nem kerülõ RNS-féleség (ncRNS) és sok-sok DNS- és hiszton-kötõ fehérje együttesen határozza meg. Az ***epigenom*** ezeknek a molekuláknak és kapcsolataiknak az összessége, beleértve a nukleoszómák elrendezõdését, a DNS és a hisztonok módosításait, valamint a DNS szekvenciarészletekhez és a hisztonokhoz kapcsolódó RNS és fehérjefaktorokat és azok kölcsönhatásait is. Az elõzõekbõl következhet, hogy az epigenom megváltozásait elõidézõ ágenseket **epimutagének**. Számos patogén baktériumtörzs sorolható ebbe a kategóriába, azaz rendelkezik olyan képességekkel, amelyekkel az epigenomot módosítani tudja elõsegítve ezzel saját szaporodását és/vagy gyengítve a gazdaszervezet védekezõ rendszerét. *Bacillus*, *Campylobacter*, *Chlamydia*, *Helicobacter*, *Legionella*, *Mycobacterium, Shigella* és számos további baktérium csoport fajai képesek DNS és hiszton módosításokat, nukleoszóma átrendezést, ncRNS szintézist és érést úgy megváltoztatni, hogy annak eredményeként immunreakciókban szerepet játszó terméket kódoló gének kifejezõdése módosul. A megváltozott génmûködés hatással lehet az általános és specifikus immunválaszra, az immunmemóriára és autoimmun folyamatok kialakulására. Az epigenom módosításaira használt baktérium mechanizmusok megismerése ezért fontos adatokat szolgáltathat a bakteriális fertõzések és megbetegedések leküzdéséhez.

Az elõadás az elõbbiek szerint értelmezett epigenom legfontosabb jellegzetességeit és megváltozási lehetõségeinek típusait fogja bemutatni humán patogén baktériumok immungének expressziójára kifejtett hatásának példáival.

THE ROLE OF EXTRACELLULAR VESICLES IN IMMUNOREGULATION

Edit Buzás, Katalin Szabó-Taylor, Tamás Szabó G, Borbála Aradi, Xabier Osteikoetxea, Andrea Németh, Mária Szente-Pásztói, Barbara Sódar, Bence György and András Falus

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Research in the past one and a half decade has drawn attention to the multifaceted roles of extracellular vesicles in immunoregulation. Extracellular vesicles are generated in an evolutionarily conserved manner, and an increasing body of evidences supports the complex interactions of microbe-derived vesicles and the host organism. On the other hand, extracellular vesicles secreted by cells of our immune system interact with pathogens, and play a role in the antimicrobial defense.

Most data regarding the role of extracellular vesicles in immunoregulation stem from the field of tumor immunology. Tumor cell derived extracellular vesicles inhibit the cytotoxic activity of CD8 + T cells and NK cells, and carry on their surface among others TGF β1-et, NKG2D ligands, FASL and/or TRAIL. Extracellular vesicles exert their cellular effects in concert with soluble mediators. The combinatorial effect of extracellular vesicles and soluble mediators may be synergistic, additive or antagonistic. The effect of extracellular vesicles unites paracrine and juxtacrine regulations, and may significantly contribute to the functions of the regulatory networks of the immune system. Furthermore, extracellular vesicles may play a role in all phases of inflammation and in the regulation of lymphocyte migration.

Moreover, extracellular vesicles provide novel therapeutical tools for translational medicine opening new perspectives for the diagnosis, prevention and /or therapy of diseases with immune pathomechanism.

AZ ANCA-ASSZOCIÁLT VASCULITISEK BIOLÓGIAI TERÁPIÁJA

Czirják László

Granulomatosis with polyangiitis (GPA) is characterized by the granulomatous inflammation of the upper and lower respiratory tracts, necrotizing vasculitis of small and medium-sized blood vessels and necrotizing glomerulonephritis. Both cellular and humoral immune system are involved in the disease. The production of ANCA specific for the serine protease PR3 or for MPO. The cytokine-primed neutrophils and monocytes express PR3 and MPO on their cell surface membranes. ANCA binds to the cell surface and activates the neutrophils which release oxygen radicals, proteolytic enzymes, and inflammatory cytokines. Furthermore patients with active vasculitis have a lower proportion of Bm1 cells whereas patients in remission have higher proportions of CD25+ (the α-chain of the interleukin 2 receptor) and CD86+ (co-stimulatory molecule) B cells suggesting that B cells may play a regulatory role in the pathogenesis of GPA. ANCAs also induce the release of BLyS from activated neutrophils that support B cell survival in vitro. BLyS is detectable in the serum of patients with active disease suggesting that it plays a role in B cell activity and survival.

The standard glucocorticoid and cyclophosphamide treatment for GPA is often not satisfactory. Rituximab (RTX) is a chimeric monoclonal anti-CD20 antibody which causes a selective depletion of B lymphocytes. The rituximab for ANCA-associated vasculitis, which involved 197 ANCA-positive patients with GPA or microscopic polyangiitis, found that RTX therapy was not inferior to daily cyclophosphamid treatment in inducing remission and that it may be superior in relapsing disease.

THE ANTAGONISTIC FUNCTION OF COMPLEMENT RECEPTORS CR1 (CD35) AND CR2 (CD21) ON HUMAN B CELLS IN HEALTH AND AUTOIMMUNITY

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As shown earlier, CR1 (CD35) on human B cells mediates inhibition of various BCR-induced functions (Józsi et al, JI, 2002) - in contrast to the stimulatory role of CR2 (CD21). The reduced expression of CD35 and CD21 on the B cells of RA patients is known for long, however their exact role in B cell tolerance and autoimmunity is not fully understood. To analyze the possible mechanisms we studied the expression and function of CR1 and CR2 on various B cell subsets of healthy donors and RA patients at various stages of the disease. We found, that $CD19^{+}CD27^{-}$ naive B cells up-regulate the expression of the inhibitory CR1 during differentiation to $CD19^{+}CD27^{+}$ memory B cells both in healthy donors and in RA patients, while the expression of the activatory CR2 is down-regulated. We found that the inhibitory function of CD35 is maintained in RA patients, despite its significantly reduced expression compared to healthy individuals. Besides blocking BCR-induced proliferation, CR1 inhibits the differentiation of B cells to plasmablasts and Ig-production.
Our data show that the expression of CD35 and CD21, these two antagonistic complement receptors is regulated differentially during the development of human B cells, a phenomenon which may influence the maintenance of peripheral B cell tolerance and might be involved in the pathogenesis of autoimmune processes.

EPIGENETICS AND IMMUNE RESPONSE (a review)

Falus András

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From a biological point of view, the complex diseases are all multifactorial syndromes, which means that the susceptibility to the disease is determined by interactions between multiple genes, gene networks, but also involves important covalent and reversible modifications of DNA by non-genetic (epigenomic),environmental factors. Many proofs were collected, that complex physiological functions, such as immune regulation are also influenced by multiple epigenetic factors (i.e. DNA methylation, chromatin rearrangements, a set of small RNA entities and telomerase). In the last years immense amounts of genetic data were collected (e.g. GWAS/ENCODE results). Although we may know the DNA sequences and variants in a genome, the uncovering the way of ontogeny of immune system, the precise action of protein- and RNA-based regulatory factors in a cell resulting in genes turning on and off requires epigenetic studies, as well. One cannot avoid a further, provocative question whether which epigenetic modifications (a „cell memory") could even be transmitted to the next generation of an organism via meiotic proliferation? This last question is very important since it raises the point as to whether our lifestyle affecting the epigenetic modifications can influence the physiology (i.e. immune activity) of our children and grand-children. Tit seems rather convincing, that the conscious change of the lifestyle (e.g. diet, exercise, stress management, psycho-social elements, etc), may basically alter the outcome of the potential of the immune defense, complex diseases and life-span.

BLOOD CELL DIFFERENTIATION- COMPARTMENTS, REGULATION AND EVOLUTION

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Blood cell differentiation shows striking similarities among evolutionarily distant taxa. Hematopoiesis starts in the early embryo, and blood cells localize in separate hematopoietic compartments during ontogeny. Certain compartments function as classic hematopoietic niches that contain hematopoietic stem cells, which are able to proliferate and differentiate into functionally diverse effector cells. In spite of the significant differences in their immune systems, the functions of immune compartments and cells are similar in distantly related organisms, and blood cell differentiation is regulated by highly conserved transcription and epigenetic factors in the animal kingdom. The parallels in the function of blood cells, their organization in compartments and the regulation of their development indicates convergent evolution that underlines the importance of innate immunity in the defence against invaders. The comparison of our experimental research data on the hematopoiesis and immune functions of different *Drosophila* species with the knowledge gained so far on mammalian models allows us a better understanding of the most important features of innate immunity.

RESHAPING NEUROLOGY: THE EMERGING ROLE OF AUTOANTIBODIES

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The increasing significance of autoantibodies in a number of neurological diseases has been recognized during the last decades. Pathogenetic role of antibodies in old diseases have been unexpectedly found, which has entirely changed previous concepts of diagnosis and treatment; new autoantibodies have been discovered in antibody-mediated disorders; and novel disease entities have been established based on association of autoantibodies and previously unrecognized syndromes.

The discovery of importance of autoantibodies in neurological diseases, which have been traditionally regarded as neurodegenerative disorders changed not only diagnostic thinking and treatment strategies, but also resulted in developing new diagnostic assay systems and created novel research interest. Highly sensitive and specific cell-based assays using single or multiple transfectants (biochip) became commercially available and revolutionized diagnosis in neuroimmunology. In vitro cultures and in vivo models using systemic, intrathecal or intracerebral transfer of isolated IgG along with human complement proved the pathogenic role of such autoantibodies. Such experiments and pathological studies also highlighted basic differences among antibody-mediated neuroimunological diseases: complement activation or downregulation of antigens in the absence of inflammation result in severe residual symptoms or reversible, well-responding diseases if treated early, respectively.

PSORIASIS: FROM THE GENETICS TO THE THERAPY

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Psoriasis is a multifactorial, chronic inflammatory skin disease characterized by increased proliferation of keratinocytes, activation of immunecells and susceptibility to metabolic syndrome. Genetic predisposition and environmental factors are both important in disease etiology. Several genome-wide association studies have been carried out and until now 36 susceptibility loci have been identified. Hyperproliferation of the keratinocytes in the psoriatic plaques is triggered by infiltrating T-lymphocytes at the dermal–epidermal junction. Autoimmune basis for chronic inflammation is supposed, although no consistent autoantigen has been found. The keratinocytes of the uninvolved psoriatic epidermis are inherently over sensitive to proliferative signals, and this elevated sensitivity plays a crucial role in the development of psoriatic lesions. Thus, resident skin cells and infiltrating immune cells cooperate in the formation of psoriatic lesions, but the exact molecular mechanisms that regulate the interactions between these cells are still far from understood. In the present overview our data on the altered response of the clinically uninvoved skin of psoriaticpatients will be presented. Inaddition, using systems biology approach we could find novel important targets, that were previously not yet associated with psoriasis. Furthermore, analysis of chemical-protein interaction networks suggested many promising drug candidates for the treatment of thedisease.

SLAM-FAMILY RECEPTORS IN THE REGULATION OF CD40L-INDUCED DENDRITIC CELL RESPONSES

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Background and objectives:
Dendritic cells (DCs) regulate both adaptive and innate immune responses. Activation of immature DCs (IDCs) by Toll-like receptors (TLR) and/or interaction with CD40L cause the maturation of DCs. However, the diversity of DC-responses requires concomitant signaling of various co-receptor molecules including several members of the SLAM receptor (SLAMF) family. Similar to monocyte-derived DCs (mDCs), plasmacytoid dendritic cells (pDCs) are chief regulators of both innate and adaptive responses and offer high degree of flexibility in directing differentiation of effector T-cells depending on the maturation signals and co-receptor signaling. pDCs are capable of promoting the differentiation of Th1, Th2 Th17 or Treg cells based on environmental clues. Despite the abundance and the clearly strong immune modulatory function of SLAMF-receptors, their role in the regulation of DC functions is poorly understood. We have previously shown that SLAMF1 is inhibitory to CD40-induced cytokine responses in mDCs suggesting the existence of a feedback loop controlling inflammatory responses. Here we seek to identify the impact of SLAMF5 on mDC and pDC functions induced by CD40 signaling.

Methods and results:
We used human mDCs or the pDC-line Gen2.2 cell line that were stimulated by soluble or cell surface expressed CD40L alone or in combination with cell surface-expressed SLAMF1 or SLAMF5. Under these conditions both mDCs and Gen2.2 cells become potent antigen presenting cells expressing high levels of co-receptors (CD80, CD83, OX40L, ICOSL) and produce pro-inflammatory cytokines (IL-6, IL-8, IL12 and TNFα). In addition to CD40L Gen2.2 cells also received activation signals via TLR7 or TLR9. Gen2.2 cells activated by CD40L and Imiquimod or CpG-B upregulated CD83 and OX40L expression which was augmented by the presence of SLAMF5 while production of inflammatory cytokines was decreased. These effects were SLAMF5-dependent as they were reversed by silencing of SLAMF5 expression by specific siRNA. Interestingly, we also found that SLAMF5/SLAMF5 homoassociation increased the capacity of CD40L and TLR7L-activated Gen2.2 cells to support T-cell proliferation. The effect of SLAMF5 signaling on instructive signals driving differentiation of various T-cell subsets is underway. To date we have shown that phosphorylation of the p38 map-kinase is increased in the presence of SLAMF5 signaling.

Conclusions:
We propose that similar to SLAMF1, SLAMF5 is an inhibitory receptor in dendritic cells controlling exuberant inflammatory responses induced by both plasmacytoid and myeloid DCs and thus, may have significant influence on the regulation of tolerogenic versus immunogenic character of DCs.

TRANSGENIC APPROACHES IN THE INVESTIGATION OF INNATE IMMUNE CELL SIGNAL TRANSDUCTION

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Recognition is a fundamental characteristic of the immune system, which regulates the immune response through a multi-step mechanism that often leads to the elimination of the triggering agent. Recognition or function defects of the innate immune cells can lead to severe infections or can participate in the escalation of autoimmune disorders. For the proper control of these latter diseases, it is crucial to have a better understanding of the signaling cascades linking immune recognition to immune response.

Experimental genetic modifications (transgenic techniques) have unique roles in the identification of the signaling cascades of several innate immune cells such as monocytes/macrophages, neutrophils or mast cells. The modifications are made in the genome of an experimental animal resulting in a total block of gene transcription, the inactivation of a specific protein segment with enzyme activity or the addition of a new gene. The effects of these transgenic changes can easily be tested in in vitro or in vivo experimental setups. (Several inflammatory disorders have experimental mouse models, for example the TNF trangenic or the K/BxN (serum transfer) models serve as useful platforms for a better understanding of the pathomechanisms of human autoimmune arthritides.) The identified signaling participants can be potential targets of pharmacological inhibitors. Beyond clarifying the role of a gene/protein in an animal model, it can be an interesting question to identify the function of the molecule in separate cell-lineages, for which the Cre-lox cell-specific knockout technology is a useful method.

The ligand binding of several cell surface receptors (like Fc receptors, integrins or cytokine receptors) can lead to the activation of innate immune cells. During the signal transduction of these receptors (besides several other molecules that are going to be discussed during the presentation) tyrosine kinases often get activated and serve as potential targets of pharmacological intervention in immune-mediated disorders.

We can conclude that transgenic approaches are important in the investigation of signal transduction pathways of innate immune cells that can participate in several systemic autoimmune diseases. A better understanding of the signaling routes of these cells can reveal underlying disease mechanisms and can have beneficial therapeutical effects.

TRANSCRIPTOME ANALYSIS OF CD8+ RESIDENT MEMORY T CELLS REVEALS ORGAN-LEVEL ENVIRONMENTAL ADAPTATION AND FUNCTIONAL DIVERSITY IN T CELL MEMORY

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Recent research focusing on CD8+ memory T lymphocytes suggests the existence of several, highly specialized organ-resident memory T cell (Trm) subsets. CD8+ Trm cells are instructed to preferentially home to, subsequently become resident in, and rapidly respond upon recall antigen challenge within distinct organ environments.
Although there is consensus that all CD8+ Trm cell subsets are characterized by integrin alphaE (CD103) expression, they are also known to express unique markers in an organ-restricted fashion. Hence, it is possible that Trm cells of distinct organs adapt to the surrounding environment or even differ in functional terms. Nevertheless, the full spectrum of these markers is unknown, leaving room for speculation about possible differences in the characteristics and functions of CD8+ Trm cells in individual organs.
In this study we sought a better understanding of these questions using a hypothesis-free approach. We show that pure fractions of intact, viable murine CD8+ CD103+ Trm cells can be isolated from various organs using automated tissue processing followed by two-step MACS multisorting. Using circulating CD8+ CD62L- T effector memory (Tem) cells as reference, we also present initial findings obtained from whole-genome gene expression profiling describing common features of, and organ-specific differences discriminating between murine CD8+ Trm cell subsets of the small intestine, lung and liver.
Our preliminary findings suggest that individual CD8+ Trm subsets of given organs display clear differences in the usage of various genes potentially affecting homing, signal recognition and responsibility, T cell activation and effector functions. Validation of these findings by independent methodologies and functional assays is currently under way to test whether CD8+ T cell memory displays a previously unknown functional heterogeneity becoming apparent at the organ-level.

MOLECULAR RECOGNITION STRATEGIES

József Prechl

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Living things constantly monitor their integrity aiming for survival of the individual organism and of the species. This monitoring requires molecular recognition strategies to identify changes to self and changes in the environment. While the immune system of animals is involved in defense by definition, defense and resistance strategies of plants and simpler life forms may now be compared to immune responses thanks to our current deeper insight into the molecular machinery of innate immunity.

In this presentation I aim to overview how and why species interact, recognize each other and themselves from the immunological point of view, giving examples of immune defense on the level of recognition molecules and the responses triggerred by these molecules. In the light of the recent advances in various fields of immunology some concepts and nomenclature may need revision and re-definition.

THE ROLE OF INNATE IMMUNE MECHANISMS IN THE PATHOGENESIS OF THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background: The protease catalyzing the maturation of von-Willebrand factor (ADAMTS13) plays critical role in the pathogenesis of thrombotic thrombocytopenic purpura (TTP). Genetic (mutations of *ADAMTS13*) and autoimmune (inhibitory autoantibodies against ADAMTS13) risk factors contribute to the development of TTP but direct triggers are needed to exacerbate acute disease.
Aim: The aim of our recent studies was to identify innate immune mechanisms associated with acute TTP, therefore, complement activation and neutrophil activation were investigated in the setting of acute TTP.
Patients: Multiple EDTA-plasma and serum samples of 38 TTP patients were investigated together with samples of 20 healthy controls.
Method: ADAMTS13 activity and anti-ADAMTS13 inhibitory antibodies were measured by the VWF-FRET73 assay. Complement parameters (C3, Factors H, I, B and total alternative pathway activity) together with complement activation fragments (C3a) or complexes (C1rs-INH, C3bBbP, sC5b9) were measured by ELISA or RID. A stable complex of PMNE-proteinase-inhibitor was measured by ELISA (Calbiochem, Merck-Millipore, Darmstadt, Germany).
Results: Increased levels of C3a, and SC5b9 were observed in TTP during acute episodes, as compared to healthy controls. Decreased complement C3 levels indicative for complement consumption occurred in 15% of acute TTP patients. The sustained presence of anti-ADAMTS13 inhibitory antibodies in complete remission was associated with increased complement activation. Furthermore, acute TTP was also associated with increased PMNE levels, increased PMNE levels and deficient ADAMTS13 activity together characterized hematologically active disease. PMNE concentration inversely correlated to disease activity markers platelet count (r= -0.349, p=0.032) and hemoglobin levels (p= -0.382 p=0.018). There was positive correlation between PMNE levels and complement activation markers C3a and Bb.
Conclusions: Activation of two important arms of innate immunity, the complement and neutrophils, was shown in acute TTP, and there was positive correlation between the two. Our data support previous observations that neutrophil extracellular traps (NETs) may be released in acute TTP, NETs may activate complement and potentially contribute to the pathophysiology of this disease. These results support the ‘multiple hit’ model of the pathogenesis of TTP.

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FUNCTIONAL INTEGRITY OF THE HOST, THE IMMUNE SYSTEM AND THE GUT MICROBIOME

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All multicellular eukaryotic organisms live together with beneficial bacterial communities in mutualistic or commensal association. They evolved in the presence of pro-caryocytes and acquired mitochondria and chloroplasts to help their physiological functions. The human microbiome involves several hundreds of various species, which outnumbers human somatic cells (10x) and genes (100x). The highly variable microbial communities share few conserved species, only and can be considered as a functional unit acting as a tissue. The microbiome is in continous contact with the immune- and nervous systems, food components and pathogens and thus it has an impact on host's physiology and defense mechanisms during all stages of life. Although several new high-through-put metagenomic approchases have recently been explored for studying the composition and functional attributes of complex ecosystems, the mapping of the human metagenome is far from complete. Recent studies revealed that the interaction of the nuclear genome, the cytoplasmic organelles and the microbiome supports the origin an the survival of new species and also promotes the development of protective mechanisms (*Brucker RM, Bordenstein SR Science 341:667,2013*). Based on the intimate link between the host, the microbiome and the environment the *hologenome* was introduced as a new term. The complexity of the microbiome acting as a functional unit is examplified by the modulatory role of retinoic acid, the metabolite of food-derived vitamin A, which plays an indispensable role in modulating the differentiation and functional activity of gut mieloid cell types ($CX3CR1^+$ macrophages and $CD103^+$ dendritic cells) and also in the regulation of T-lymphocyte polarization. In the special environment of the gut cells regulating innate and adaptive immunity acquire unique functions that support the growth of beneficial bacteria, while inhibit colonization by pathogens, prevent and decrease inflammatory reactions. The diversity and flexibility of the healthy microbiome is a pre-requisite of the development of the immune system as well as the induction and the maintenance of immunological tolerance. Vitamins, fatty acids, carbohydrates and food components play determining roles in our health, while changes in the microbiome causing dysbiosis may associate with chronic inflammation such as metabolic and cardiovascular diseases, diabetes, allergy, autoimmunity, inflammatory bowel diseases, coeliac disease, autism). Uncovering the extreme diversity of beneficial bacteria in the context of their functional attributes offers novel approaches for modulating the immune system and identifying innovative therapeutic targets.

SIGNAL TRANSDUCTION IN THE IMMUNE RESPONSE

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In cells of the immune system, signaling leads to activation of cell-type specific immune activities. Ligand interaction with receptors on the surface of cells of the immune system triggers intracellular signal transduction directly or through association with assistant signal transduction molecules (CD3, IgαIgβ, etc.).
Regulation of immune cells function upon response to environmental stimuli and to pathogens is essential for the defense of the organism. The strenghts of the signal is a decisive factor in life or death of lymphocytes. Signals above a threshold activate the cell, while below the threshold the cells do not respond. During development of lymphocytes to strong signal may result in programmed cell death to avoid autoimmunity. Tonic signals mediated by the antigen receptors are responsible for keeping cells alive before encountering the antigen. Receptors of the adaptive and the innate immune system interact in regulating the immune response. Innate receptor may activate the cells irrespectively from the antigen, thus the crosstalk between the adaptive and innate receptors must be tightly controlled. We have recently characterized the communication between BCR, TLR9 and BAFF-R mediated signaling pathways in human B cells. The results suggest that these pathways interact at the level of TAK1, the kinase that connect extracellular signals to NFkB activation being responsible for activating the inhibitor κB kinase (IKK) complex. Research targeting TAK1 raises the potential for new therapeutic options for inflammatory disorders, including autoimmune diseases and cancer.

THE ROLE OF THE SKIN'S COMMENSAL MICROFLORA IN HEALTH AND DISEASE

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One of the most important features of the human skin is the formation of a complex barrier. For a long time it was believed that this is a passive function originating from the unique structural features and anatomical properties of this organ (mechanical barrier). However, in the last decades it become increasingly accepted that the different types of skin cells -among them the keratinocytes and sebocytes- possess important functions in generating a coordinated, active protection, thus forming a true first line of defense against the harmful impacts of the external environment. These cells have been shown to act as sentinels capable of the recognition of external pathogens through the expression and function of all sorts of pathogen recognition receptors (PRRs). Activation of these receptors by various pathogenic microbes leads to the initiation of active defense processes, and as a result, inflammatory and innate immune events are launched (immunological barrier). Last, but not least keratinocytes, as well as sebocytes, also actively secrete different factors exhibiting antimicrobial properties (including the small cationic molecules, called defensins) altogether contributing to the formation of another level of protection (chemical barrier).

The different surface areas of the human body that are constantly exposed to the effects of the external environment (skin, gastrointestinal tract, parts of the reproductive system) are heavily colonized by various microbes, altogether making up the so-called commensal flora. The exact composition and function of these at the above listed diverse anatomical locations are currently being investigated. Traditionally it was suggested, that these microbes have a relatively passive function, as they populate these niches and use up the available food sources. Currently, however, there is a paradigm shift in this research field, as more and more beneficial effects of these microbes are being identified.

One of the most important members of the skin's commensal flora is the bacterium called *Propionibacterium acnes* (*P. acnes*). Even though it resides in the pilosebaceous unit of the skin, under certain circumstances it may also play an important role in the pathogenesis of the most common inflammatory skin disease, acne vulgaris. How and when this commensal microbe turns pathogenic is currently not known, but heavily investigated by us and other laboratories.

In this current talk I will summarize what is known about the function of the skin's commensal microflora, how we analyze the complex interaction that lay within our skin cells and the *P. acnes* bacterium, and how this bacterium contributes to the pathogenesis of acne vulgaris.

THE BERMUDA TRIANGLE OF GENETICS, ENVIRONMENT AND AUTOIMMUNITY INT HE PATHOGENESIS OF RHEUMATOID ARTHRITIS

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It has been postulated that genetic susceptibility and environmental factors are involved int he pathogenesis of most autoimmune rheumatic diseases. Yet, mostly indirect proofs have become available in this respect. Rheumatoid arthritis (RA) is a prototype of these diseases as it is relatively common with a 1% prevalence, rather homogenous with respect to clinical course and numerous new targeted therapies have been tried in RA first. Both HLA and non-HLA genes have been implicated in genetic susceptibility to RA. In addition to the weel-known contribution of HLA-DR1 and DR4 alleles, also known as „shared epitopes", as confirmed by SNP and GWAS studies, more than 30 non-HLA alleles may also contribute to susceptibility to RA. Environmental factors, such as smoking induces protein citrullination in RA, especially in genetically susceptible individuals. Such citrullinated proteins drive the production of anti-citrullinated protein antibodies (ACPA) in these patients. According to our current knowledge ACPA seropositive and seronegative RA may be two rather distinct phenotypes. In addition to smoking, excessive caffeine consumption and intake of oral contraceptives may also increase the risk of RA. Ont he other hand, responsible alcohol consumption, especially red wine may somewhat decrease the risk and severity of the disease. Genes, lifestyle-related factors and ACPA autoimmunity form the Bermuda triangle of RA.

CYTOKINE-LIKE AND CELL CYCLE REGULATORY EFFECTS OF THE PROGESTERONE INDUCED BLOCKING FACTOR (PIBF)

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PIBF is a progesterone target gene, localized on chromosome 13 in the humans and chromosome 14 in the mouse. The full length molecule is a 90 kDa, however, several smaller molecular weight isoforms are produced by alternative splicing. Upon activation, the smaller molecular weight PIBF isoforms enter the secretory pathway and are transported into the surrounding microenvironment. The full length PIBF shows a peri-nuclear localization is associated with the centrosome and has been identified as a component of the pericenteolar satellite. PIBF plays a role in the maintenance of murine pregnancy. Increased resorption rates in mice, - due to high NK activity or to progesterone receptor block - are corrected by PIBF treatment, whereas PIBF depletion in pregnant mice results in altered cytokine pattern and fetal loss.

Both trophoblast and tumor cells express high levels of the PIBF and the expression of this molecule is inversely related to trophoblast invasiveness. Invasiveness is a common feature of trophoblast and tumors; however, while tumor invasion is uncontrolled, trophoblast invasion is strictly regulated both in space and time. PIBF differentially regulates invasion tumor and trophoblast. Silencing of PIBF increased invasiveness as well as MMP-2,-9 secretion of trophoblast-, and decreased those of tumor cells. In trophoblast cells PIBF induced fast, but transient Akt and ERK phosphorylation, whereas in tumor cells, PIBF triggered sustained Akt, ERK, and late STAT 3 activation. The late signaling events might be due to indirect action of PIBF. PIBF induced the expression of EGF and HB-EGF in HT-1080 cells. The STAT 3-activating effect of PIBF was reduced in HB-EGF-deficient HT-1080 cells, suggesting that PIBF-induced HB-EGF contributes to late STAT 3 activation. PIBF binds to the promoters of IL-6, EGF, and HB-EGF; however, the protein profile of the protein/DNA complex is different in the two cell lines. We conclude that in tumor cells, PIBF induces proteins, which activate invasion signaling, while—based on our previous data—PIBF might control trophoblast invasion by suppressing pro-invasive genes.

OF MIRTRONS AND 3' MIRNA ISOFORMS:MICRORNAS FORMED BY ALTERNATIVE PATHWAYS

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Introduction:microRNAs (miRNAs) are non-coding RNA molecules of 20-30 nucleotides in length. They form an extensive regulatory network similar to that of transcription factors.In animal cells, most miRNAs are believed to use the "canonical" pathway involving the Drosha/DGCR8 complex and Dicer. However, recent investigations revealed several alternative maturation routes that bypass either of the two cleavage steps of the canonical pathway. The most prominent Drosha-independent pathway is the mirtron pathway which was first described in invertebratesand relies on the splicing machinery. However, due to the long average intron length, it was not obvious whether this pathway existsin higher organisms. In addition to alternative maturation mechanisms, the alternative usage of miRNA arms and the diversity of the 5'/3' sequence of miRNAs can also increase the complexity of miRNA regulation. We are investigating the existence and the role of the mammalian mirtron pathway, as well as the 3' isomir diversity of human miRNAs.
Methods: By expressing natural and artificial miRNA constructs in mammalian cells, we measure the level of miRNAs by Northern blot and qRT-PCR. We use 3' isoform specific assays to detect different miRNA species from the same locus; we also apply luciferase assays to test the function of the mature miRNAs.
Results: We could prove that the mirtron pathway indeed exists in higher vertebrates, including humans. We showed that predicted mirtronic miRNAs are formed independently of the Drosha/DGCR8 complex, using the splicing apparatus of the cells. Moreover, the flanking exons do not influence functional mirtrons, provided that the sequences are splicing-competent. In addition, we provided evidence for the first time that functional miRNAs can be formed simultaneously from both arms of the hsa-miR-877 mirtron locus. Finally, we revealed that several miRNA species exist in various 3' isoforms which can severely influence detection accuracy by qRT-PCR and may represent different regulatory functions.
Conclusions: Our results indicate that the miRNA repertoire and variability in the cells are far more complex thanpreviously anticipated. Nevertheless, it has to be emphasized that although bioinformatic predictions are useful as investigative pre-screens, they must always be followed by experimental verifications.

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INHIBITION OF Kv1.3 AND IKCa1 LYMPHOCYTE POTASSIUM CHANNELS AS A POTENTIAL THERAPEUTIC TARGET IN AUTOIMMUNE DISORDERS

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Introduction: The transient increase of the cytoplasmic free calcium level is a key signal transduction mechanism in the process of lymphocyte activation. Voltage-sensitive Kv1.3 and calcium-dependent IKCa1 lymphocyte potassium channels have been implicated as important targets of selective immunomodulation in autoimmune disorders. The relationship between the influx of calcium through the cell membrane and the efflux of potassium makes the activation and cytokine production of T lymphocytes sensitive to pharmacological inhibition of Kv1.3 and IKCa1 channels. We aimed to characterize the effects of lymphocyte potassium channel inhibition on peripheral blood T lymphocyte activation in a number of immune-related disorders, such as rheumatoid arthritis, multiple sclerosis, type I diabetes and stroke induced immunosuppression compared to healthy individuals.
Methods: We determined calcium influx kinetics and its sensitivity to Kv1.3 and IKCa1 channel inhibition following PHA activation in CD4, Th1, Th2 and CD8 cells applying a novel flow cytometry approach.
Results: The time when the peak of calcium influx in T lymphocytes was reached decreased in autoimmune patients compared to healthy individuals, indicating that these cells are in a state of sustained reactivity due to the ongoing autoimmune reaction. In healthy controls the inhibition of the IKCa1 channel decreased calcium influx in Th2 and CD4 cells to a lower extent than in Th1 and CD8 cells. On the contrary, the inhibition of Kv1.3 channels resulted in a larger decrease of calcium entry in Th2 and CD4 than in Th1 and CD8 cells. In the investigated autoimmune patients a greater decrease of calcium influx upon the inhibition of the Kv1.3 channel than that of the IKCa1 channel was observed in Th1 cells. However, the selectivity of the investigated inhibitors was limited in our experiments. The inhibitory effect was present not only in disease-associated CD8 and Th1 cells, but also in the anti-inflammatory Th2 subset. The induced decrease in their function could lead to unwanted side-effects and in a setback of therapy in vivo.
Conclusions: Based on our results, a number of dominant features of T lymphocyte calcium influx and its sensitivity to the inhibition of potassium channels were identified that were present in the investigated autoimmune diseases. Further studies are needed on human samples and experimental models to judge the usefulness of this approach in the fight against autoreactive lymphocyte subsets and harmful cellular responses in autoimmune patients.

TOLL-LIKE RECEPTOR ENGAGEMENT CONVERTS INNATE DYSREGULATION INTO OVERT CYTOKINE STORM AND PROMOTES AUTOIMMUNITY IN MURINE MODEL OF LEAKY SCID

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Introduction. Recombination Activating Gene *(RAGs)* are key elements of early events in V(D)J recombination. Impairment of these enzymes results in severe restriction of T and B cell repertoire. The clinical phenotype among patients with primary immunodeficiency (PID) secondary to *RAG* mutations spans from early severe infections to late onset autoimmune manifestations. Susceptibility and high mortality with viral infections are contributed to the absence of proper infection-specific adaptive responses. The role of innate response in this process has not been fully investigated.

Objectives. To evaluate innate response and autoimmunity during acute and chronic viral infections in a murine model of rag deficiency.

Methods. We utilized homozygous *rag1*$^{S723C/S723C}$ (*mut/mut*) mouse model of leaky SCID. To recapitulate acute and chronic viral infections, we administered high dose intravenous or prolonged low dose intraperitoneal Poly(I:C), respectively. Cytokine and autoantibody levels were measured.

Results. High dose i.v. Poly(I:C) treatment within 10 hours was fatal in 100% of *mut/mut* mice. Serum TNFα and IL-6 remained highly elevated and did not decline with time, compared to control wild-type mice. Genearray of splenic dendritic cells from *mut/mut* mice revealed skewed activation of TLR3 associated pathways. Prolonged low dose i.p. stimulation augmented and broadened the spectrum of autoantibodies in *mut/mut* mice.

Conclusions. In our murine model high and low dose TLR3 stimulation resulted in cytokine storm and increased autoantibody production, respectively. Dysregulation of innate immune system after acute or chronic infection may contribute to the increased mortality and autoimmune phenotype of patients with RAG-dependent PID.