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Postacím: 1245 Budapest 5, Pf. 1012
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Tartalom

Tisztelt Kolléganők és Kollégák, kedves Olvasóink!

Már hozzászokhattak ahhoz, hogy minden évben lapunk 3. számát a Magyar Immunológiai Társaság vándorgyűlésének szenteljük. A vezetőség köszöntéséhez csatlakozva, a lap nagy részét az ezúttal angol nyelven benyújtott kivonatoknak szenteljük. Emellett három közlemény is színesíti a lapszámot. Szabó Krisztina és Zeher Margit professzornő (Debrecen) a follicularis T-sejtek immunológiai és klinikai jelentőségéről, Sipka Sándor professzor (Debrecen) az adenozin immunológiájáról, míg Medgyasszay Balázs (Farkasgyepű) a tuberculosis mai felfogásáról és modern diagnosztikájáról írt összefoglalót.

A MIT kongresszus résztvevőinek szakmai feltöltődést, minden olvasónknak élvezetes lapozgatást kívánunk!

Dr. Szekanecz Zoltán
főszerkesztő

Kedves Kollégák!

Megtiszteltetésnek vesszük, hogy a Magyar Immunológiai Társaság 42. Vándorgyűlését Pécsen rendezhetjük az új Kodály Központban 2013. október 16–18. között, amire nagy szeretettel és érdeklődéssel várjuk a tudomány művelőit.

Örömteli esemény, hogy rendezvényünkön részt vesz az immunológia egyik meghatározó szaktekintélye, Abul K. Abbas professzor, a University of California, San Francisco, Department of Pathology intézetvezetője. A kongresszus díszvendégeként ő tartja a tudományos nyitó előadást. Ezen kívül október 18-án az immunológia és immunpatológia legújabb eredményeit bemutató egész napos továbbképzést tart a kongresszus résztvevőinek, amely hallgatók, rezidensek és szakorvosok számára pontszerző kurzus is.

A tematika és a kongresszus szerkezete a megszokottól némiképp eltérő lesz. Az első napon angol nyelven mutatják be eredményeiket a hazai immunológiai műhelyek képviselői. A második napon tematikus szekciókban felkért referátumokat követően hangzanak el a témákhoz csatlakozó előadások. Ugyanezen a napon kerül sor a MIT vezetőségválasztó közgyűlésére is.

A záró pénteki napon a továbbképző előadások szüneteiben tartjuk a poszterszekciókat.

A tudomány mellett élvezetes társasági programok is kikapcsolódást kínálnak, így a Kodály Központban tartandó fogadás, a 2010-es Európai Kulturális Főváros projekt keretében megújított történelmi belvárosban a kongresszusi vacsora, vagy a Zsolnay-negyed megtekintése.

Reményeink szerint a program méltó lesz mind a MIT hagyományosan magas színvonalához, mind a másfél ezer éves város gazdag szellemi és művészeti örökségéhez, amit a megújult Pécs nyújthat.

Mindenkit szeretettel várunk Pécsre!

Baráti üdvözlettel:

Kemény Lajos, Széll Márta
MIT elnök, MIT főtitkár

és Balogh Péter, Berki Tímea,
Czirják László, Németh Péter
helyi szervezők

A Magyar Immunológiai Társaság 42. Vándorgyűlésének összefoglalói

Az előadások és poszterek absztraktjait az első szerző vezetékneve alapján ábécérendben mutatjuk be.

ORAL PRESENTATIONS

BALANCING TOLERANCE AND AUTOIMMUNITY: CONTROLLING HARMFUL IMMUNE RESPONSES

Abul K. Abbas, MBBS

University of California San Francisco

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

Attila Bácsi

Department of Immunology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary

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

Péter Balogh, Árpád Lábadi and Zoltán Kellermayer

Department of Immunology and Biotechnology, University of Pécs

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.

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HUMAN INTESTINAL DENDRITIC CELLS DICTATE INFLAMMATION AND T-CELL POLARIZATION

Krisztián Bene[1], Nadiya Boyko[2]; Éva Rajnavölgyi[1]

[1]Department of Immunology, Medical & Health Science Centre, University of Debrecen,
[2]Department of Microbiology, Immunology, Virology and Aetiology of Infectious Diseases, Medical Faculty, Uzhhorod National University, Uzhhorod, Ukraine

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's E. 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 moDC was monitored by the expression of membrane CD1a, CD1d and CD83 by FACS analysis. Culture supernatants of activated moDC were 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 synthesis were measured by qRT-PCR.

Results: Increased expression of CD83 revealed that all tested commensal bacteria were able to activated moDCs for 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 enhanced IL-1ß secretion and upregulated the expression of genesinvolvedin ATRA synthesis and NLRP12 mRNA levels. Interestingly, these ATRA-induced effectscould be counterregulatedbythe tested microbe. The interaction of microbes resulted in IL-23 production supporting Th17 polarization of autologous T-cells and increased the number of IFNγ 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

Ferenc Boldizsár, Mariann Szabó, Zoltán Kohl, Lilla Prenek, Réka Kugyelka, Péter Németh, Tímea Berki

University of Pécs, Department of Immunology and Biotechnology

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

Imre Miklós Boros

Szegedi Tudományegyetem, TTI Kar, Biokémiai és Molekuláris Biológiai Tanszék, Szeged

Epigénekről nem szoktunk beszélni, **epigenetika** azonban van és epigenetikainak tekintjük valamely jelleg öröklődését akkor, ha annak továbbadása sejtről sejtre, nemzedékről nemzedékre nem nukleotid-sorrendben rögzített. Ebben az értelmezésben az epigenetikai öröklődést tekinthetjük génműködési mintázatok megőrzésének a sejtosztódások során. Molekuláris alapját tekintve ez kromatinszerkezeti jellegzetességek fenntartása: a DNS és hozzá kapcsolódó fehérjék összecsomagolódásának megőrzése és továbbadása. A kromatinszerkezetet a DNS-metilációs állapot, a hisztonok típusai és módosításai, számos transzlációra nem kerülő RNS-féleség (ncRNS) és sok-sok DNS- és hisztonkötő fehérje együttesen határozza meg. Az epigenom ezeknek a molekuláknak és kapcsolataiknak az összessége, beleértve a nukleoszómák elrendeződését, a DNS és a hisztonok módosításait, valamint a DNS szekvenciarészletekhez és a hisztonokhoz kapcsolódó RNS és fehérjefaktorokat és azok kölcsönhatásait is. Az előzőekből következhet, hogy az ***epigenom*** megváltozásait előidéző ágenseket **epimutagének**. Számos patogén baktériumtörzs sorolható ebbe a kategóriába, azaz rendelkezik olyan képességekkel, amelyekkel az epigenomot módosítani tudja elősegítve ezzel saját szaporodását és/vagy gyengítve a gazdaszervezet védekezőrendszerét. Bacillus, Campylobacter, Chlamydia, Helicobacter, Legionella, Mycobacterium, Shigella és számos további baktériumcsoport fajai képesek DNS- és hisztonmódosításokat, nukleoszómaátrendezést, ncRNS-szintézist és -érést úgy megváltoztatni, hogy annak eredményeként immunreakciókban szerepet játszó terméket kódoló gének kifejeződése módosul. A megváltozott génműködés hatással lehet az általános és specifikus immunválaszra, az immunmemóriára és autoimmun folyamatok kialakulására. Az epigenom módosításaira használt baktériummechanizmusok megismerése ezért fontos adatokat szolgáltathat a bakteriális fertőzések és megbetegedések leküzdéséhez.

Az előadás az előbbiek szerint értelmezett epigenom legfontosabb jellegzetességeit és megváltozási lehetőségeinek 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, András Falus

Department of Genetics, Cell- and Immunobiology, Budapest

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 b1-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.

BIOLOGICAL THERAPY OF ANCA-ASSOCIATED VASCULITIDES

László Czirják

Department of Rheumatology and Immunology,
University of Pécs

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

Anna Erdei[1, 2], Mariann Kremlitzka[2], Bernadett Valent-Mácsik[1], Anna Polgár[3], Emese Kiss[3], Gyula Poór[3]

[1]Department of Immunology, Eötvös L. University,
[2]Research Group of the Hungarian Academy of Sciences at Eötvös Loránd University,
[3]Institute of Rheumatology and Physiotherapy, Budapest, Hungary

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 pheno-

menon 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)

András Falus

Genetikai, Sejt- és Immunbiológiai Intézet,
Semmelweis Egyetem, Budapest

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

Viktor Honti

Immunology Unit, Institute of Genetics, Biological Research Centre of the Hungarian Academy of Sciences, Szeged

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

Zsolt Illes

University of Southern Denmark

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

Lajos Kemény

Department of Dermatology and Allergology,
University of Szeged, Hungary

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

Árpád Lányi, Zsófia Agod, Mónika Baráth

Institute of Immunology, University of Debrecen Medical and Health Science Center, Debrecen, Hungary

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.

TYROSINE KINASES IN AUTOIMMUNE DISEASES

Attila mócsai

Department of Physiology, Semmelweis University School of Medicine, Budapest, Hungary

Myeloid leukocytes such as neutrophils or macrophages are critical components of innate immunity but their improper activation may also lead to tissue damage during autoimmune inflammation. We have previously shown that certain neutrophil responses require Src-family kinases, Syk and PLCγ2. Therefore, we tested the role of tyrosine phosphorylation pathways in in vivo inflammatory reactions. Src-family kinases, Syk and PLCγ2 were all found to be required for autoantibody-induced inflammatory reactions such as the K/BxN serum-transfer arthritis or autoantibody-

induced skin blistering disease in experimental mice. The genetic deficiency of those signaling molecules also prevented accumulation of myeloid cells at the site of inflammation. Given the role of tyrosine kinases in β2 integrin-mediated leukocyte activation, we hypothesized that Src-family kinases, Syk and PLCγ2 are also required for β2 integrin-mediated leukocyte migration. Surprisingly, neutrophil migration in a conventional Transwell assay did not require Src-family kinases, Syk or PLCγ2 even though it was strongly reduced by the genetic deficiency of the β2 integrin-chain CD18. In addition, the Src-family kinase inhibitor dasatinib did not affect in vitro neutrophil migration. In vivo competitive migration assays (in which wild type and knockout cells are allowed to migrate to the site of inflammation within the same animal) also revealed that Src-family kinases, Syk and PLCγ2 were not required for neutrophil or monocyte migration in sterile peritonitis or autoantibody-induced arthritis models. On the other hand, tyrosine kinases were required for immune complex-induced cytokine production by neutrophils and macrophages. Taken together, Src-family kinases, Syk and PLCγ2 are required for neutrophil activation and cytokine production but do not play any direct role in CD18-mediated migration of myeloid cells to the site of inflammation.

TRANSGENIC APPROACHES IN THE INVESTIGATION OF INNATE IMMUNE CELL SIGNAL TRANSDUCTION

Tamás Németh, Attila Mócsai

Semmelweis University, Department of Physiology, Budapest, Hungary

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

Zoltan Pos[1, 2], Barbara Molnar-Ersek[1], Nikolett Lupsa[1, 2], Tunde Simon[2], Anett Toth[1, 2], Bence Bagita[1, 2], Andras Falus[2], Edit Buzas[2]

[1]„Lendület" Experimental and Translational Immunomics Research Group, Hungarian Academy of Sciences – Semmelweis University, Budapest,
[2]Department of Genetics, Cell and Immunobiology, Semmelweis University, Budapest

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 pu-

re 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

MTA-ELTE Immunology Research Group

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

Zoltán Prohászka

Semmelweis University, IIIrd Department of Medicine, Research Laboratory

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

Éva Rajnavölgyi

University of Debrecen Medical and Health Science Center, Debrecen

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 se-

veral hundreds of various species, which outnumbers human somatic cells (10×) and genes (100×). 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.

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

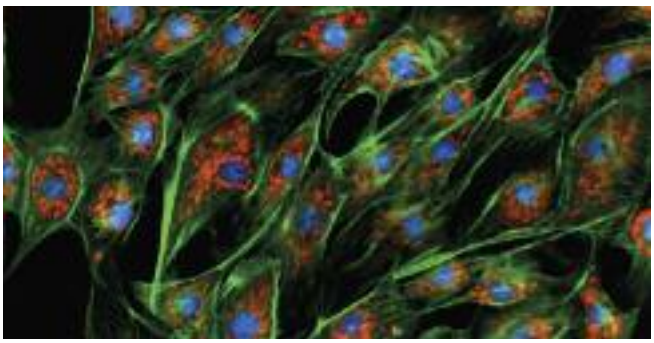
Andreas Rieger

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.



SIGNAL TRANSDUCTION IN THE IMMUNE RESPONSE

Gabriella Sármay

Deparment of Immunology, ELTE

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 kB 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

Kornélia Szabó

MTA-SZTE Dermatological Research Group, Szeged, Hungary

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 IN THE PATHOGENESIS OF RHEUMATOID ARTHRITIS

Zoltán Szekanecz

University of Debrecen Medical and Health Sciences Center, Department of Rheumatology, Debrecen

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 rat-

her 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)

Szekeres-Bartho J, Barakonyi A, Halasz M, Kozma N, Miko E, Polgar B, Palkovics T. Szereday L,

Department of Medical Microbiology and Immunology, Medical School, Pecs University

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

Tamás I. Orbán[1, 2]

[1]Institute of Molecular Pharmacology, RCNS, HAS, Budapest, Hungary,

[2]Chemical Technology Transfer Ltd., Budapest, Hungary

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.

This work was supported by the KMR_12-1-2012-0112 grant (TransRat).

GLOBAL PROTEOME ANALYSIS OF THE HUMAN PLASMA REVEALS NEW BIOLOGICAL FEATURES: A REVIEW OF EVOLUTIONARY IMPLICATIONS

Laszlo Takacs

Department of Human Genetics, University of Debrecen, Health Science Center

Coding capacity of the genome determines primary amino acid sequence of proteins. However, the human proteome space, is not limited to the primary peptide sequence coded by the approximately 25 000 protein coding genes, complexity is estimated to be at least 102 – 103 fold higher. This, "added" proteome complexity is at least partly due to the variability introduced by splice variation and other genetically coded differences. Genetically non coded variability, like protein-protein interactions, post translational modifications, and folding state heterogeneity also influence protein function. Thus, while deciphering primary peptide sequence and its' variability is important, it is insufficient for understanding protein function, especially in the case of complex protein mixes like the human blood plasma, or i.e. the hemolymph of drosophilidae. Popular global, mass spectrometry based proteome analysis tools do not provide sufficient insight into protein complexity.

Similarly to domains carrying functional activity, antigenic epitops of proteins are also influenced by secondary and tertiary structure, splice variation, genetic variation and genetically non-coded variability (protein interactions, folding variation). In order to test whether the quasi global analysis of medium and highly abundant human plasma proteome by non redundant (at the epitome level) mAb libraries would detect at least some of addressed protein features, we performed monoclonal antibody proteomics profiling of the human plasma proteome. Initial results indicate that our approach may shed light to novel aspects of proteome heterogeneity with considerable biological relevance from human to insects.

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

Gergely Toldi

First Dept of Pediatrics, Semmelweis University, Budapest

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

J. E. Walter[1, 2], M. Recher[3], K. Kis-Toth[4], H. Mattoo[9], D. Matthew[2], S. Volpi[2], F. Rucci[2], A. Szabo[5], O. Walter[6], E. Csizmadia[7], F. Alt[8], G. C. Tsokos[4], L.D. Notarangelo[2]

[1]Section of Pediatric Allergy/Immunology, Massachusetts General Hospital for Children, Harvard Medical School,
[2]Department of Pediatrics, Division of Immunology, Children's Hospital Boston and Harvard Medical School, Boston, MA, USA,
[3]Department of Internal Medicine, University Hospital Basel, Basel, Switzerland,

[4]Division of Rheumatology, Beth Israel Deaconess Medical Center,
[5]Department of Molecular Biology, Massachusetts General Hospital, Boston,
[6]Department of Pathology, University of Massachusetts, Worchester,
[7]Beth Israel Deaconess Medical Center,
[8]Immune Disease Institute, Harvard Medical School, Boston, MA, USA,
[9]Cancer Center, Massachusetts General Hospital, Boston, MA

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.

POSTERS

ANALYTICAL EVALUATION OF THE QUANTIPLASMA 300 (QP300) MONO-CLONAL ANTIBODY CHIP

Péter Antal-Szalmás[1], József Lázár[2], Róza Földesi[1], Zoltán Steiber[1], Stuart McGregor[3], John V. Lamont[3], S. Peter Fitzgerald[3], I stván Kurucz[2], László Takács[2], János Kappelmayer[1]

[1]University of Debrecen, Medical and Health Science Center, Department of Laboratory Medicine, Debrecen, Hungary;
[2]Biosystems International Ltd, Debrecen, Hungary;
[3]Randox Laboratories Ltd., Crumlin, United Kingdom

Introduction: Plasma proteome profiling with monoclonal antibody (mAb) library based protein chips is a promising new opportunity in biomarker discovery that can be used to identify novel plasma markers in a wide variety of diseases. An example of the mAb libraries used on protein chips of Randox Ltd. (UK) is the QuantiPlasma™ (Biosystems International Kft, Hungary) system that could help the identification of novel biomarkers in different types of cancer. After the feasibility biochip containing 69 mAb-s (QP69), recently a "discovery" version of this system – the QP300 kit – has been introduced that covers 290 different human plasma protein epitopes in one sample. The mAbs – recognizing the different protein epitopes – are immobilized on 9x9 mm ceramic chips and a biotinylated plasma protein tracer is competing with plasma proteins in the tested sample for mAb binding. The amount of the bound tracer is determined by a streptavidin-peroxidase conjugate and a chemiluminescence substrate. We aimed to evaluate the analytical properties of the new QP300 system.

Methods: The performance of QP300 system was tested on the Evidence Investigator analyzer platform of Randox Ltd.. In the case of each mAb the interassay variability of the maximal relative light unit measured in the presence of the tracer alone (RLUmax) was determined first. Then aliquots of one plasma sample – diluted 300.times – were tested in ten subsequent experimental days and an interassay variability of the measured RLU and RLU/RLUmax rates were calculated. Finally an interoperator variability was also determined as the 10 replicates were prepared by 2 operators.

Results and Conclusions: The RLUmax values ranged from 45 to 102,500 and were below 1,500 only in the case of 30 mAbs, resulting in slightly higher interassay %CVs. The interassay and interoperator %CVs were typically <15%. The RLU values of the tested plasma sample were lower (50 to 71,175), and the number of mAbs with RLU <1,500 was somewhat higher (55) but the interassay and interoperator RLU %CVs were also typically <15%. The RLU/RLUmax rate was within the optimal inhibition range (20% to 80%) in the case of 214 mAbs, meaning that both increase and decrease in the plasma protein concentration can be measured. The inte-

rassay and interoperator %CVs were typically <15% in the case of this parameter, too.

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PRESENCE OF IGA CLASS ANTI-NEUTROPHIL CYTOPLASMIC ANTIBODIES (ANCA) IN CIRRHOSIS – POSSIBLE HINT TOWARDS THE INVOLVEMENT OF GUT MUCOSAL IMMUNE SYSTEM?

Peter Antal-Szalmas[1], Nora Sipeki[2], Zsuzsanna Vitalis[2], Tamás Tornai[2], Istvan Altorjay[2], Istvan Tornai[2], Miklos Udvardy[3], Kai Fechner[4], Silvia Jacobsen[4], Bianca Teegen[4], Andrea Sumegi[5], Gabor Veres[6], Peter Laszlo Lakatos[7], Janos Kappelmayer[1], Maria Papp[2]

[1]Department of Laboratory Medicine,
[2]2nd Department of Medicine, Division of Gastroenterology and
[3]2nd Department of Medicine, Division of Hematology, University of Debrecen, Debrecen, Hungary;
[4]EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany;
[5]Vascular Biology, Thrombosis and Hemostasis Research Group, Hungarian Academy of Sciences, Debrecen, Hungary;
[6]1st Department of Pediatrics and
[7]1st Department of Medicine, Semmelweis University, Budapest, Hungary

Background: Anti-neutrophil cytoplasmic antibodies (ANCA) are a non-uniform family of antibodies recognizing diverse components of neutrophil granulocytes. ANCA formation might be induced by protracted bacterial infections or probably reflect an abnormal immune response to commensal microorganisms. Bacterial infections are common complications in cirrhosis with high incidence of episodes caused by enteric organisms, therefore, we sought to study the presence and clinical importance of ANCA in cirrhosis.

Methods: Sera of 385 patients with cirrhosis of different etiologies were assayed for ANCA of IgG, IgA, IgA1, IgA2 and secretory IgA subtypes by indirect immunofluorescence and ELISAs. Control group comprised of 202 patients with chronic liver diseases without cirrhosis and 100 healthy subjects. In cirrhosis, a 2-year follow-up, observational study was conducted to assess possible association between presence of ANCA and clinically significant bacterial infections.

Results: Prevalence of ANCA IgA was significantly higher in cirrhosis (52.2%) compared to chronic liver diseases (18.6%) or healthy controls (0%, $p<0.001$ for both). ANCA IgA subtyping assays revealed marked increase in the proportion of IgA2 subtype (46% of total ANCA IgA) and presence of the secretory component concurrently. Presence of ANCA IgA was associated to disease-specific clinical characteristics (Child-Pugh stage and presence of ascites, $p<0.001$). During a 2-year follow-up period, risk of infections was higher among patients with ANCA IgA compared to those without (41.8% vs. 23.4%, $p<0.001$). ANCA IgA positivity was associated with a shorter time to the first infectious complication (pLog-Rank<0.001) in Kaplan–Meier analysis and was identified as an independent predictor in multivariate Cox-regression analysis (HR:1.74, 95%CI:1.18–2.56, $p=0.006$).

Conclusions: Presence of IgA type ANCA is common in cirrhosis. Involvement of gut mucosal immune system is in center of the formation and probably reflects sustained exposure to bacterial constituents.

UNDERSTANDING BINDING PROPERTIES OF MONOCLONAL ANTI-CHOLESTEROL ANTIBODIES

Andrea Balogh*, Eva Szenes, Eszter Angela Toth, Janos Matko

Eotvos Lorand University, Insitute of Biology, Department of Immunology, H-1117 Budapest (Hungary)

Monoclonal anti-cholesterol antibodies (ACHA, IgG clones AC1 and AC8 and IgM clone AC9), made in our laboratory, reacted with clustered cholesterol or structurally closely related sterols, but not with other lipids, assayed by ELISA. They bound to lipoproteins, but only IgG clones bound to locally clustered cholesterol (in lipid rafts and caveolas) in the cell membrane of various intact immunocytes (1, 2). Based on these results we aimed to test the reactivity of our antibodies using large scale multiplex analysis, microarray. Cholesterol, cholesterol analogues and lipoproteins were printed onto nitrocellulose membrane and binding of AC1, AC8 and AC9 was visualized by fluorescent secondary antibody. Furthermore, sequences of variable regions of ACHA clones' heavy and light chain were determined to support interpretation of microarray data. We found that all ACHA clones, with different affinities, were capable of binding to cholesterol and lipoproteins printed onto membranes. They also showed different extent of cross-reactivity to DNA, reflecting that cholesterol and DNA share structurally common epitopes for anti-cholesterol antibodies. Sequence analysis showed that AC1, AC8 (IgG3) and AC9 (IgM) mAbs differ from each other and use gene segments in germ-line configuration for their antigen-binding portion. In the future, we plan to develop microarray-based high throughput raft-analytical method for the detection of total cellular or membrane cholesterol that might be both perspectivic in direction of rapid and reliable diagnostics and therapy of lipid raft-related diseases.

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References:
(1) A. Biro, et al., J lipid Res., 48 (2007) 19.
(2) E. Izsepi, et al. Immunol Lett., 143 (2012) 106.

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IN VITRO DIFFERENTIATION OF HUMAN TH17 CELLS

Eszter Baricza[1], Barbara Molnár-Érsek[1], Edit Buzás[1], György Nagy[1, 2]

[1]Department of Genetics-, Cell- and Immunbiology, Semmelweis University, Budapest,
[2]Department of Rheumatology, Semmelweis University, Budapest

Background: Th17 cells represent a subset of T helper lymphocytes that produce several inflammatory cytokines, including interleukin-17A, -17F, -21, -22, and tumor necrosis factor. Increased Th17 cell differentiation and IL-17 production have been observed in rheumatoid arthritis (RA) and in several other autoimmune diseases. IL-17 contributes to development of inflammation and promotes osteoclast differentiation in RA. We have studied the differentiation of Th17 cells.

Methods: CD4 positive T cells were separated by magnetic method from peripheral blood mononuclear cells (PBMC) of healthy volunteers. The cells were treated for 5–10 days with anti-CD3 and anti-CD28 antibodies and with TGFβ (2,5 ng/ml), IL-6 (25 ng/ml) and IL-1 (10 ng/ml) cytokines, and with anti-IL-4 (10 μg/ml) and anti-IFNγ (10 μg/ml) blocking antibodies. The IL-17 production was measured by ELISPOT and ELISA, the RORc expression was measured by real-time PCR and by western blot methods, cell viability was monitored by Trypan blue staining and by Annexin V binding.

Results: Anti-CD3/CD28 treatment increased the IL-17 production, but did not alter the RORc expression. The anti-CD3/CD28, IL-1, IL-6 and TGFβ induced RORc expression was further increased by the anti-IL-4 and anti-IFNγ antibody treatment, without affecting cell viability.

Conclusion: Our data suggests that IL-4 and IFNγ blockade promote the T-cell activation and cytokine treatment induced Th17 cell differentiation.

NLRP3 INFLAMMASOME-MEDIATED IL-1β PRODUCTION BY LPS-PRIMED GM-MFs IS INDEPENDENT OF P2X7 RECEPTOR

Ágnes Becsei[1], Marietta Margit Budai[1], Judit Danis[1], László Csernoch[1], József Tőzsér[2], Szilvia Benkő[1]

Medical and Health Science Center, University of Debrecen,
[1]Department of Physiology,
[2]Department of Biochemistry and Molecular Biology

Introduction: IL-1β is one of the main inflammatory cytokines that regulates immune responses. The main source of this cytokine is the activated macrophages. IL-1β is synthetised as a proprotein, which is proteolytically processed to its active form by an intracellular protein complex, called NLRP3 inflammasome. Activation of NLRP3 inflammasome requires two signals: the first signal leads to the synthesis of pro-IL-1β and the components of the inflammasome, the second signal, like the extracellular ATP, results in the assembly of the NLRP3 inflammasome and the cleavage of pro-IL-1β. Under metabolically stressful conditions like inflammation and cell damage ATP is released into the extracellular space via pannexin channels. The extracellular ATP is sensed by P2X7 receptor and the activation of the P2X7 leads to K^+ efflux.

Methods: Human monocytes were separeted from human peripheral blood and were cultured for five days in presence of GMCSF to become inflammatory macrophages (GM-MFs). The macrophages were activated by LPS. The cytokine production was measured by ELISA.

Results: Our results show that GM-MFs can secrete substantial amounts of mature IL-1β upon stimulation with LPS in the absence of ATP stimulation. Previous studies have shown that human monocytes can release mature IL-1β with LPS stimulation alone, which is dependent on autocrine stimulation by ATP. LPS-primed GM-MFs can also release ATP, but the addition of apyrase, an enzyme that hydrolyzes extracellular ATP, do not affect IL-1β secretion, which is consistent with the lack of requirement for P2X7 receptor or pannexin inhibitor as well as the high extracellular K^+ concentration.

Conclusion: To summarize our results we can determine that the accepted two-signal model necessary for activation of the NLRP3 inflammasome in response to TLR ligands does not apply to GM-MFs and other mechanisms may play role in the secretion of IL-1β by LPS primed GM-MFs in the absence of extracellular ATP.

THE EXPRESSION PROFILE OF TAM AND NLR RECEPTORS UPON IMMUNE CHALLENGE AND CHRONIC INFLAMMATION

Éva Boros[1], Gerda Strifler[1], Andrea Vörös[1], András Szász[1, 2], Lajos Kemény[3], Csaba Varga[4], Vilmos Tubak[5], István Nagy[1]
[1]Hungarian Academy of Sciences, BRC, Szeged,
[2]University of Szeged, JuhászGyula Teacher Training Faculty, Szeged,
[3]University of Szeged,Department of Dermatology and Allergology, Szeged,
[4]University of Szeged, Faculty of Science and Informatics, Szeged,
[5]Creative Laboratory Ltd., Szeged

Pattern Recognition Receptors (PRRs), which recognize distinct pathogen-associated molecular patterns have a pivotal role in the efficient defence against invading microorganisms. For example, activation of different Toll-like receptors induces the secretion of proinflammatory mediators resulting in local inflammatory properties. However, innate immunity must be properly controlled, as its continuous activation leads to the development of chronic inflammation.

The TAM family (TYRO3/AXL/MER) of receptor tyrosine kinases and their ligands GAS6 and protein S regulate, among others, erythropoesis, development of oligodendrocytes, phagocytosis of apoptotic cellsand immune system.Apart functioning as intracellular PRRs, members of theNLR (NOD-like receptor) receptor family also play important roles in the formation of inflammasomes (eg. NLRP3, NLRC4), regulation of the expression of major histocompatibility complexes (eg. CIITA, NLRC5) and negative regulation of immune response (eg. NLRC3, NLRC5, NLRP6). Perturbation of signallingthrough either TAM or NLR receptors leads tocontinuous and increased expression of proinflammatory effector molecules,a hallmark of autoimmune diseases, such as psoriasis.Thus, the aim of this study was to determine how the expression of TAM and NLR receptors and proinflammatory molecules correlates upon innate immune response. To this end, epithelial cells (eg. keratinocytes and vaginal epithelial cells) and monocytes were treated with distinct microbial agents (such as PGN, poly I:C and LPS) and the gene-, and protein expression profile of TAM and NLR receptors and proinflammatory cytokines/chemokines were investigated by QPCR, Western-blot and immunofluorescence labelling. As expected, microbial agents induced the expression of proinflammatory molecules, in contrast, the expression of TAM and NLR receptors is mostly down-regulated.

Since the continuous expression of proinflammatory molecules characterizes pathologic conditions such as Inflammatory Dowel Disease (IBD) and psoriasis, we next sought to determine the expression profile of TAM and NLR receptors in vivoin 2,4,6-trinitrobenzenesulfonic acid-induced rat model of IBD, imiquimod-induced mouse model of psoriasis and in psoriatic patients. Our results show that in parallel with the markedly upregulated expression of proinflammatory effector molecules, the expression of negative regulatory molecules is also altered, predominantly decreased.

Taken together, our results strongly suggest that the down-regulation of TAM and NLR receptor expression modifies the expression profile of proinflammatory molecules thereby inducing chronic inflammation.

NLRP3 INFLAMMASOME-MEDIATED IL-1β PRODUCTION BY LPS IN DIFFERENT PHENOTYPES OF HUMAN MACROPHAGES

Marietta Margit Budai[1], Judit Danis[1], Aliz Varga[1], László Csernoch[1], József Tőzsér[2], Szilvia Benkő[1]
Medical and Health Science Center, University of Debrecen,
[1]Department of Physiology,
[2]Department of Biochemistry and Molecular Biology

Introduction: IL-1β is a "master" cytokine that has an indispensable role in orchestrating effective innate and adaptive immune responses. Due to its critical function, NLRP3 inflammasome-mediated IL-1β production requires distinct signals. Some of these signals induce the expression of the inactive pro-IL-1β through the activation of signaling pathways. Other signals, such as the activation of ATP-sensing P2X7 receptor trigger the processing of pro- IL-1β to mature IL-1β. Among the most important sources of the IL-1β are the activated macrophages (MFs). However depending on the tissue environment, monocytes differentiate into alternative MF subpopulations and it is clear that the actual IL-1β production by a particular cell is strongly depends on the cell type and its characteristic intra- and extracellular modulators. We aimed to study the molecular mechanisms of IL-1β production and secretion by LPS-activated human MFs which were polarized in different ways.

Methods: Macrophages were generated from human peripheral blood in the presence of granulocyte-macrophage colony stimulating factor (GM–CSF) or macrophage colony stimulating factor (M-CSF) which indicate the immuno-stimulatory (GM-MF) or the tissue repair (M-MF) functions of the cells.

Results: Our results show that though both types of LPS-activated MFs secrete IL-1β in the presence of ATP, in the case of M-MFs IL-1β is released rapidly and only for a short time period, while IL-1β secretion by GM-MFs is sustained. The IL-1β secretion in the presence of ATP depends on the P2X7 receptor, which is expressed in both MF types. The differential ability of M-MFs to release mature IL-1β is associated with early increased expression of NLRP3 and pro-IL-1β as well as enhanced LPS-induced early activation of the key signal transduction pathways. Using IL-10 neutralizing antibody we show that the notable amounts of IL-10 anti-inflamma-

tory cytokine produced by M-MF has substantial role in the decrease of IL-1β.

Summary: Our results indicate that while LPS-activated GM-MFs secrete robust IL-1β contributing to activate certain immune responses against microbial stimuli, the "anti-inflammatory" M-MFs also release substantial amounts of mature IL-1β, but only in the early phase of stimulation and it is rapidly down-regulated which may correlate with their anti-inflammatory characteristics.

NF-κB INDUCES OVEREXPRESSION OF BOVINE FCRN: A NOVEL MECHANISM THAT FURTHER CONTRIBUTES TO THE ENHANCED IMMUNE RESPONSE IN GENETICALLY MODIFIED ANIMALS CARRYING EXTRA COPIES OF FCRN

Judit Cervenak[1], M. Doleschall[2], B. Bender[1], B. Mayer[3] Z. Schneider[4], Z. Doleschall[5], Y. Zhao[6], Zs. Bősz[7], L. Hammarström[8], W. Oster[1], I. Kacskovics[1, 4]

[1]ImmunoGenes Kft, Budakeszi, Hungary;
[2]Mol. Med. Res. Group, Hungarian Academy of Sciences and Semmelweis Univ., Budapest, Hungary;
[3]Dep. of Dermatology, Venereology and Dermatooncology, Semmelweis Univ., Budapest, Hungary;
[4]Dep. of Immunology, Eötvös Loránd Univ., Budapest, Hungary;
[5]Dep. of Pathogenetics, Nat. Inst. of Oncology, Budapest, Hungary;
[6]State Key Lab. of Agrobiotech., China Agricultural Univ., Beijing, China;
[7]Agricult. Biotech. Center, Gödöllő, Hungary;
[8]Div. of Clin. Immunology, Karolinska Inst., Stockholm, Sweden

Among many functions of the neonatal Fc receptor (FcRn) for IgG, it binds to IgG-opsonized antigen (Ag) complexes and propagates their traffic into lysosomes where Ag processing occurs. We previously reported that transgenic (Tg) mice that express multiple copies of bovine (b)FcRn have augmented humoral immune response. Among the mechanisms that contribute to the boosted immune response, the augmented Ag-IgG immune complex presentation via professional Ag presenting cells (APCs) such as dendritic cells that express bFcRn seems to be especially important. Nuclear Factor-kappa B (NF-κB) is a critical molecule in the signaling cascade in the immune response. NF-κB induces human FcRn expression and our previous in silico analysis suggested NF-κB binding sites in the promoter region (PR) of the bFcRn α-chain gene (FCGRT).

This study was undertaken to analyze additional mechanisms that contribute to the immune capabilities observed in the bFcRn Tg mice. We investigated NF-κB binding sites in the PR of bFCGRT using luciferase reporter gene technology and electromobility shift assays. NF-κB mediated bFcRn regulation was studied in lipopolysaccharide (LPS)-treated primary bovine endothelial cells (BAECs) by quantitative PCR; in the spleen of LPS-injected bFcRn Tg mice by Northern blot analysis; and at protein level in macrophages isolated from the bFcRn Tg mice using flow cytometry.

We identified three functional NF-κB binding sites in the PR of bFCGRT. Stimulation of BAECs with LPS, which mediates its effect via NF-κB, resulted in rapid upregulation of the bFcRn expression, which was also observed in the spleen of bFcRn Tg mice treated with LPS. NF-κB mediated bFcRn upregulation was confirmed in macrophages isolated from the bFcRn Tg mice with a newly developed FcRn specific monoclonal antibody that does not cross-react with the mouse FcRn.

We concluded that NF-κB regulates bFcRn expression and thus optimizes its functions, e.g., in the professional APCs, and contributes to the much augmented humoral immune response in the bFcRn Tg mice.

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EFFECT OF INHOMOGENEOUS STATIC MAGNETIC FIELD (iSMF) ON POLLEN-INDUCED ALLERGIC INFLAMMATION

Anikó Csillag[1], Brahma V. Kumar[1], Krisztina Szabó[1], Kitti Pázmándi[1], Éva Rajnavölgyi[1], János László[2], Attila Bácsi[1]

[1]Department of Immunology and
[2]Department of Computer Science, University of Debrecen, Debrecen

Background: Allergic airway inflammation triggered by ragweed pollen is closely associated with oxidative stress. Pollen NAD(P)H oxidases generate reactive oxygen species (ROS) immediately upon exposure inducing oxidative stress in the airways independent of adaptive immune responses. Subsequent oxidative stress derives from ROS released by inflammatory cells recruited into the airways several hours after pollen exposure. Our goal was to define the effects of iSMF on pollen-induced allergic airway inflammation since several lines of evidence suggest that iSMF is able to trigger biological responses at least partly through free radical reactions.

Methods: Moderate strength iSMF was generated with an apparatus optimized to small experimental animals. Balb/c mice were sensitized by i.p. injection of ragweed pollen extract (RWE) on day 0 and 4 and challenged with RWE intranasally (day 11). Inflammation was evaluated (day 14) by determining inflammatory cell counts and mucin levels in the bronchoalveolar lavage fluid, as well as by histological analysis of the lungs. Studying the mechanisms of iSMF action, ex-

periments on RWE in cell-free environment and on human A549 airway epithelial cells were carried out using a redox sensitive fluorescent dye (H2DCF-DA) for detection of changes in ROS levels.

Results: Exposure to iSMF during the sensitization phase did not affect the allergic responses. However, even a single 30-min iSMF-exposure immediately following intranasal RWE challenge significantly reduced the airway inflammation. In addition, prolonged exposure to iSMF (for 30 or 60 min on 3 consecutive days) after RWE challenge decreased more effectively the severity of allergic inflammation. In cell-free experiments exposure to various intensity of iSMF for 30 min did not alter ROS production by RWE, while the same exposure of cultured epithelial cells to iSMF diminished the RWE-induced increase in the intracellular ROS levels. Moreover, in mice exposed to iSMF for 30 min immediately after challenge, RWE treatment induced a significantly lower increase in the total antioxidant capacity of the airways, than in those exposed to sham field.

Conclusions: These data indicate that iSMF is able to reduce airway inflammation in the elicitation phase of the allergic reaction in an experimental model of pollen allergy. This beneficial effect of iSMF presumably is due to cellular ROS eliminating mechanisms rather than direct modulation of ROS production by pollen NAD(P)H oxidases.

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REGULATING RESILIENCE: THE PLASTICITY AND DIFFERENTIATION POTENTIAL OF *DROSOPHILA* HEMOCYTES

Gábor Csordás*, Viktor Honti*, B.I. Gergely Varga, Éva Kurucz, Beáta Kari, István Andó

*These authors contributed equally to this work.
Immunology Unit, Institute of Genetics, Biological Research Centre of the Hungarian Academy of Sciences, Szeged

The immune defense of Drosophila melanogaster relies on the cooperation of humoral and cell-mediated components. The cellular immune reactions are carried out by specialized immune cells, the hemocytes. The differentiation of immune cells begins in early embryonic stages: two mesodermal segments give rise to two independent hemocyte lineages. These lineages form the larval hematopoietic compartments: the circulation, the sessile tissue and the lymph gland.

The cellular arm of the Drosophila immune response comprises morphologically and functionally distinct hemocyte classes. Plasmatocytes are phagocytic cells, which are mainly responsible for engulfing invading microbes. Crystal cells contain crystallized proenzymes, which, upon induction, are released into the hemolymph and through toxic intermediers result in melanization. The lamellocytes appear upon immune induction and form capsules around large foreign particles, which include tumors and eggs of parasitic wasps. Our earlier studies revealed that efficient encapsulation and the subsequent melanization require the mobilization of every hemocyte compartment. Also, we recently discovered that a portion of phagocytic plasmatocytes is capable of transforming into encapsulating lamellocytes during this process.

Our goal was to further understand the plasticity of the hemocyte lineages in the Drosophila larva. To investigate this phenomenon, we used a combination of in vivo lineage tracing transgenes and our molecular marker panel.

We demonstrated that although plasmatocytes can differentiate into lamellocytes following immune induction, crystal cells (marked by lozenge lineage tracing) are unable to do so. However, the overexpression of certain activating factors by a lineage tracing transgene in the crystal cell lineage led to both lineage autonomous and non-lineage autonomous lamellocyte differentiation.

Our experiments revealed that the regulation of lamellocyte differentiation consists of at least two levels: an upstream, non-cell autonomous induction and a downstream (cell autonomous) response. Our results also suggest that the ability of hemocytes to transform into lamellocytes differs in the plasmatocytes and crystal cell lineage. We theorize that this is possibly due to the lack of certain upstream factors in the crystal cells, which is also underlined by our finding that circumventing these factors by activated forms of downstream elements triggers the differentiation of lamellocytes from this cell type as well.

ACTIVATION OF THE FICOLIN-LECTIN PATHWAY DURING ATTACKS OF HEREDITARY ANGIOEDEMA

Dorottya Csuka[1, 2], Lea Munthe-Fog[2], Estrid Hein[2], Zsuzsanna Zotter[1], Lilian Varga[1], Zoltán Prohászka[1], Peter Garred[2], Henriette Farkas[1]

[1]3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary
[2]Laboratory of Molecular Medicine, Department of Clinical Immunology, Rigshospitalet, Faculty of Health Sciences, University of Copenhagen, Denmark

Introduction: The activation of the plasma enzyme systems is insufficiently controlled in hereditary angioedema caused by the deficiency of functional C1-INH (HAE-C1-INH), a disorder characterized by recurrent subcutaneous and/or submucosal edematous attacks. Recently, a few studies suggested that it is not the MBL-lectin pathway, but the ficolin-lectin pathway (ficolin-LP), which might play a role in the pathomechanism of HAE-C1-INH. As the role of ficolin-LP in the development of edematous attacks is still enigmatic, we analyzed its activity during such episodes.

Methods: Thirty-five HAE patients, who have experienced severe edematous attacks on 112 occasions, were enrolled. We analyzed blood samples drawn during attacks, and 39 samples obtained from the same patients during symptom-free periods. The serum concentrations of ficolin-3, ficolin-3/MASP-2 complex, antigenic C1-INH, C4, as well as the extent of ficolin-3 mediated activation of the lectin pathway (F3-TCC) were measured using in-house methods. Commercially available kits were used to quantify C1-INH activity, as well as C4d, and C3a levels.

Results: Levels of functional C1-INH and ficolin-3/MASP-2 complex were elevated (p=0.0009 and p=0.0224), whereas F3-TCC was lower (p=0.0002) during attacks, compared with the symptom-free period of the same patients. During symptom-free periods, the extent of F3-TCC significantly correlated to the concentrations of antigenic C1-INH (R=0.3152, p=0.0006) and C4 (R=0.5307, p<0.0001), whereas the ficolin-3/MASP-2 complex level correlated significantly with the C4d (R=0.8571, p=0.0107) concentration. During attacks, the level of the ficolin-3/MASP-2 complex correlated with ficolin-3 (R=0.5319, p=0.0025), functional C1-INH (R=0.5391, p=0.0066), and C3a (R=-0.4981, p=0.0096) levels. Interestingly, an inverse relationship was found between the ficolin-3, MASP-2, as well as MAP-1 levels, and the time from the onset of the attack to blood sampling. The levels of ficolin-2, ficolin-3 and MAP-1 were slightly elevated during submucosal attacks, compared to the subcutaneous location.

Conclusions: The strong association between the level of the ficolin-3/MASP-2 complex and C1-INH activity suggests that the ficolin-LP undergoes activation during edematous attacks in HAE-C1-INH patients. We presume that the ficolin-3 mediated activation of LP may contribute to the consumption of the small reserve of functional C1-INH and thus, it can lead to uncontrolled activation of the plasma cascade systems, and thereby to edema formation.

THE ROLE OF MANNOSE BINDING LECTIN IN INFECTIOUS COMPLICATIONS OF HEMATO-ONCOLOGIC DISEASES

Marianna Dobi[1, 2], Ágnes Szilágyi[1], Dorottya Csuka[1], Lilian Varga[1], Zoltán Prohászka[1], Gábor Kovács[2], Ferenc Fekete[3]

[1]Semmelweis University, III. Department of Internal Medicine, Research Laboratory, Budapest, Hungary,
[2]Semmelweis University, II. Department of Pediatrics, Department of Heamatology, Budapest, Hungary,
[3]Heim Pál Children's Hospital, Madarász Street Building, Department of Hematology, Budapest, Hungary

Introduction: The appropriate function of the complement system is essential for protection against infections in oncologic patients; partly because of the developing neutropenia due to the malignant disease and partly because of the chemotherapy induced immunosuppression. The key element in the activation of the complement system via the lectin pathway is the appropriate functioning of mannose-binding lectin (MBL) and mannose-binding lectin-associated serine protease 2 (MASP2) complex. One of the limiting factors of this activation may be the low serum concentration of MBL.

The aim of our study was to find association between polymorphisms resulting in low MBL level and activation of the MBL-MASP2 complex, and find connection between these abnormalities and the frequency and severity of febrile neutropenic episodes in children suffering from hemato-oncological diseases.

Methods: 97 children with hemato-oncological diseases (76 ALL, 10 AML, 11 NHL) were enrolled and followed from the beginning of the therapy for 8 months and several characteristics of febrile neutropenic episodes were recorded. Genotypes of 4 MBL polymorphisms (–221C/G, R52C, G54D, G57E) were determined by real-time PCR. Activation of the MBL-MASP2 complex was evaluated by ELISA from samples obtained at the time of diagnosis and during an infection.

Results: The number of febrile neutropenic episodes was lower and the time until the first episode was longer in patients with normal MBL level coding genotypes, than in patients with low MBL level coding genotypes (p<0.01). Patients with wild type genotypes have higher chance for a longer period without febrile neutropenia according to the Kaplan-Meier survival analysis (p=0.01). A correlation between the MBL-MASP2 complex activation and the MBL genotype was found, moreover activation level decreased significantly during infections (p=0.004) in patients with low MBL level coding genotypes.

Conclusion: Our results suggest that infections after immunosuppression therapy in children suffering from hemato-oncological diseases are associated with the MBL genotype. Changes of MBL-MASP2 activation confirm the important role of the lectin pathway in infections. Our results may contribute to the estimation of risk for infections in the future, that may modify therapeutic options for individuals.

SILVER NANOPARTICLES INDUCED CELL-DEATH AND MITOCHONDRIAL DAMAGE IN EARTHWORM COELOMOCYTES

Péter Engelmann[1], Yuya Hayashi[2, 3]

[1]Department of Immunology and Biotechnology, Clinical Center, University of Pécs,
[2]iNANO Interdisciplinary Nanoscience Center, Aarhus University, Aarhus, Denmark,
[3]Department of Biosciences-Terrestrial Ecology, Aarhus University, Silkeborg, Denmark

Nanotechnology is producing large amounts of nanoparticles (NPs) that are applied in industry, daily life and health care.

NPs are considered as a particle that has one or more external dimensions in the size range of 1 - 100 nm. One of the most widely used nanomaterials is the nano-silver (in clothing, wound-dressing and surgical instruments), mainly because of its wide antimicrobial activity. Because of the variety of nanotechnology applications, NPs can enter into the environment through many routes. Most toxicity and risk assessment studies of NPs focused on the human health and the environmental effects of nanoparticles are largely unexplored. It is known that silver nanoparticles (AgNPs) can interact with pathogens and immune cells. For instance silver nanoparticles are readily taken up by phagocytic cells (macrophages) of innate immunity that can lead to inflammatory processes. The relative simplicity of invertebrate immune functions offers a potentially sensitive and accessible means of monitoring nanoparticle effects and complex interactions which ultimately affect host resistance. Our current understanding of the potential impact of nanomaterials on invertebrate immunity is limited to only a handful of initial studies including those on earthworms as an ecological indicator organism.

Recently, we reported the cytotoxicity and accumulation of silver nanoparticles in immune cells (so called coelomocytes) of Eisenia fetida earthworms in vitro.

Hereby, we assessed the coelomocyte survival upon Ag^+ and AgNP challenge using flow cytometry based assays. Applying Annexin V/propidium iodide staining we observed that Ag^+ ions (EC20: 0.20 mg/mL; EC50: 0.60 mg/mL) and AgNPs (EC20: 1.90 mg/mL; EC50: 6.40 mg/mL) caused late apoptosis/necrosis of coelomocytes. Nanoparticles induced ROS formation lead to mitochondrial damage and cell death. Loss of mitochondrial membrane potential (demonstrated by JC-1 staining) -representing the mitochondrial damage- was observed at higher concentration of Ag^+ (EC20: 0.64 mg/mL) and AgNP (EC20: 3.20 mg/mL, EC50: 7.8 mg/mL) compared to the Annexin V assays. Moreover, ionomycin evoked calcium influx of coelomocytes after AgNP treatments (EC20, EC50) were biased at early time points.

In these experiments, we demonstrated subcellular toxicological implications arising from selective uptake of nanomaterials in the coelomocytes that might lead to inter-cellular communications of various coelomocyte subpopulations during the inflammatory process.

IDENTIFICATION OF NEGATIVE REGULATORY ELEMENTS COUNTERACTING THE *PROPIONIBACTERIUM ACNES*-INDUCED SIGNALING PATHWAYS IN *IN VITRO* CULTURED IMMORTALIZED KERATONICYTES

Lilla Erdei[1], Gábor Tax[1], Szilvia Beáta Bolla[1], Edit Urbán[3], Lajos Kemény[1, 2], Kornélia Szabó[2]

[1]Department of Dermatology and Allergology, Faculty of Medicine, University of Szeged, Hungary,
[2]MTA-SZTE Dermatological Research Group, Szeged, Hungary,
[3]Institute of Clinical Microbiology, University of Szeged, Hungary

Introduction: Acne is the most common dermatological disease affecting a large percentage of the adolescent population. Under special circumstances the otherwise skin commensal Propionibacterium acnes (P. acnes) bacterium plays a key role in lesion development. The bacterium has been shown to induce immune and inflammatory events by the activation of pathogen recognition receptors (e.g Toll-like receptors 2 and 4; TLR2-4) in human epidermal keratinocytes. Little is known, however, about the negative regulatory mechanism that counteracts TLR activation, thus protects the host from the prolonged, often destructive, uncontrolled inflammation.

Methods: In order to identify and analyze factors playing a key role in the attenuation of the P. acnes–induced TLR activation processes, we analyzed the mRNA expression of selected wellknown negative regulators of these signaling events (SIGIRR, TOLLIP, TNFAIP3, TNIP1). Cultured human immortalized keratinocytes (HPV-KER) were treated with P. acnes 889 strain and the gene expression changes were followed by real time RT-PCR.

Results: Our results show that all the investigated negative regulators are expressed in HPV-KER cells. Moreover, the TNFAIP3 and TNIP1 mRNS expression exhibited transient changes, reaching a maximum at 6–12 hours after the bacterial treatment. These processes seem to be dose-dependent, as parallel with the increase of the applied P. acnes dose, the mRNA expressions of TNFAIP3 and TNIP1 also increased, which may be the result of a growing rate of NF-kB activation.

Conclusions: Our study suggests that in our in vitro model system P. acnes causes the dose-dependent activation of the TLR signaling processes. Special negative regulators do exist, which can control these events, and can be important for the maintenance of epidermal homeostasis.

Based on our results we propose that the net ratio of positive and negative regulatory processes can be important determinants of the intensity of P. acnes driven innate, and inflammatory events, and thus also the severity of induced acne symptoms.

IMMUNOLOGICAL METHODS TO INVESTIGATE ALLOIMMUNE HABITUAL ABORTION

D. Fekete[1], E. Ujhelyi[1], Gy. Petrányi[1], Á. Galamb[2], A. Széll[3], A. Pajor[Z], I. Vályi-Nagy[1]

[1]Egyesített Szent István és Szent László Kórház-rendelőintézet, Budapest,
[2]Semmelweis Egyetem II. Sz. Szülészeti és Nőgyógyászati Klinika, Budapest,
[3]MikroMikoMed G1 Labor, Budapest

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. The identification of these immunological reasons and their pathologic role is in the focus of several investigations.Clinical observations underlie 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 committeewas 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:

Flowcytometrycrossmatch (between the RSA patient and her partner): Detection of IgG molecules in the patient serum that react with the T or B lymphocytes of the partner showing HLA sensitization or immunregulation.

Mixed Leukocyte Culture (MLC) and blocking antibody analysis: Describes the amount of cellular reactivity between two donors. 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 T_h1-T_h2 ratio: The determination of T_h1 vs. T_h2 cell ratio is based on measuring their signature cytokines. T_h1 dominance points to elevated cellular immune response while T_h2 dominance represents humoral immune response and suppressiveimmunregulation.

Analysis of natural killer (NK) cells: The natural killer cells are responsible for the elimination of foreign or altered self cells (e.g. virus infected or tumour cells) from the body. Ifelevated NK cell numbers are detected in the blood and/or their reactivity is elevated means a general upregulatedimmunreactivity of the patient.

In the present work we summarize our experience with the 91 RSA patients 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.We found that elevated NK cell numbers and Th1 dominancy are common in patients.Most informative results were concluded from the MLC measurement, where 32% of the patients showed hyperreactivity against the partner and 77% of patients' serum contained factors that enhanced the reactivity. In contrast, only 21 patients had components in their serum that blocked the reaction against the partner cells.The flowcytometrycrossmatch was only informative when evaluated together with the other results.

THE ROLE OF ABL FAMILY KINASES IN AUTOIMMUNE ARTHRITIS

K. Futosi[1], Zs. Szatmári[1], Anthony J. Koleske[2], A. Mócsai[1]

[1]Semmelweis University School of Medicine, Department of Physiology, Budapest,
[2]Molecular Biophysics & Biochemistry, Yale School of Medicine, Yale University, New Haven, Connecticut, USA

Background: The non-receptor tyrosine kinase c-Abl plays a role in various cell processes. It has an oncogenic counterpart, the Bcr-Abl fusion protein which causes certain human leukemias. Previous studies suggested, that the Abl tyrosine kinases play a role in the functions of mature myeloid cells. In this present study, we examined the function of Abl and its redundant protein Arg (Abl related gene) in a myeloid cell mediated autoimmune arthritis.

Materials and methods: The abl null mutation results in perinatal lethality, therefore to attain conditional deletion of Abl, mice carrying an Abl allele with flanked loxP sites(Abl^{flox}) were crossed with mice expressing the Cre recombinase from the myeloid-specific lysosyme M promoter ($LysM^{cre}$). By this crossing we generated $LysM^{cre}$/$creAbl^{flox/flox}$ ($Abl^{\Delta myeloid}$) mice with Abl deficiency in the myeloid compartment. We also tested Arg-deficient ($Arg^{-/-}$) mice and Abl and Arg dual deficiency. Development of autoantibody-induced arthritis was induced using the K/BxN serum. The expression of Abl and Arg protein in various myeloid compartments (neutrophils, macrophages) was tested by immunoblotting.

Results: The genetic mutations ($Abl^{\Delta myeloid}$ and $Arg^{-/-}$) dramatically decreased the expression levels of these kinases in myeloid cells (neutrophils and macrophages). Both $Abl^{\Delta myeloid}$ and the Arg-deficient mice showed the same macroscopic signs of autoantibody-induced arthritis and the same arthritis-induced loss of articular function compared to wild type mice. In addition, the double mutation ($Abl^{\Delta myeloid}Arg^{-/-}$) also did not affect the diseases course.

Conclusions: Our results indicate that the Abl family kinases in myeloid cells (e.g. neutrophils) are not indispensable for the development of autoantibody-induced arthritis in experimental mice.

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MOLECULAR AND FUNCTIONAL HETEROGENEITY OF HONEYBEE HEMOCYTES

Erika Gábor[1], Tibor Török[2], Gábor Csordás[1], Viktor Honti[1], Éva Kurucz[1], István Andó[1]

[1]Institute of Genetics, BRC HAS, Szeged,
[2]Department of Genetics, University of Szeged, Szeged

Honeybee is an eusocial insect with cosmopolitan distribution; it belongs to the Hymenoptera ordo. It has a substantial impact in pollination, thus in the development of biodiversity, as well as, they are vital in producing honey. In honeybee colonies, among other set of symptoms, Colony Collapse Disorder has become common worldwide in the past few years causing major economic losses. Immunity has a special role in the defense against the microbial and parasitic damages which honeybees are exposed to. In order to reduce this economic damage it is necessary to understand the host-pathogen interactions, mainly, through the investigation and understanding the structure and the machinery of honeybee immunity. Like other insects, honeybee has an effective immune system with a cellular- and a humoral module. Moreover, being a social species, honeybee uses alternative strategies, like hygienic behavior and hive-fever, thus its immune response may has contain special elements as compared to well known model-organisms. Humoral components of the honeybee's immune response were identified, however, our understanding of the cell-mediated immune-response is negligible. Circulating cells, so called hemocytes, have been detected in the hemolymph, however, their function and the differentiation of various blood cell populations and the existence of hematopoietic tissue/s have not been revealed yet.

The aim of our research is to develop a toolkit for the characterization and classification of the honeybee's hemocytes. We have identified clustered immunological markers on the basis of their expression patterns on hemocytes of the larva and of the adult. Furthermore, a correlation between the expression-pattern of the markers and the function of the blood cells was revealed.

These hemocyte specific markers offer means to study the cell mediated immunity of the honey bee and a tool for the identification of hematopoietic tissues and hemocyte lineages with characteristic functional properties in this economically important species.

PRESENCE OF CIRCULATING AUTOANTIBODIES AGAINST INTEGRIN ALPHA-6 IN PSORIASIS VULGARIS

Brigitta Gál[1], Mária Kiss[1], Lajos Kemény[1, 2], Zsuzsanna Bata-Csörgő[1, 2]

[1]Department of Dermatology and Allergology, University of Szeged, Szeged,
[2]MTA-SZTE Dermatological Research Group, University of Szeged, Szeged

Introduction: Psoriasis is a chronic inflammatory disease affecting the skin and the joints. We hypothesized that the abnormal laminin integrity described in psoriatic uninvolved skin may result in insufficient binding of integrin α-6 to its ligand (laminin) resulting in autoantibody production. The primary function of the $\alpha 6\beta 4$ integrin heterodimer is to anchor the basal epithelial cells to the basement membrane zone. Antibodies against integrin α-6 can disturb the dermal-epidermal association *in vitro* that may contribute to the sustained chronic inflammation seen in psoriasis.

Methods: Serum samples of 62 patients with psoriasis vulgaris and 36 patients with psoriatic arthritis and 20 healthy persons as contols were collected. Four different antigenic epitopes of integrin α-6 were defined with the use of PeptideStructure and PlotStructure softwares. The presence of anti- integrin α-6 antibodies in the serum was determined by using ELISA methodology. Ten control samples were always used per microplate, in order to determine the optimal cut off value on the basis of their optical densities.

Results: Circulating antibodies against at least one recombinant epitope of integrin α-6 protein were found in 54.8% and 55.6% of patients with psoriasis and psoriatic arthritis, respectively. Eighteen psoriatic patients and 13 patients with psoriatic arthritis presented autoantibodies to more than one antigenic sites of integrin α-6. Sixty-four percent of the patients received some form of systemic treatment. However, there was no correlation between the occurrence of these autoantibodies and the ongoing treatment.

Discussion: Our study provides evidence for the presence of anti- integrin α-6 antibodies in psoriasis vulgaris and psoriatic arthritis that may cause structural abnormalities, therefore in the skin contribute to micro-wounds and the characteristic wound-healing phenotype in psoriasis.

EXPRESSION OF CARD18/ICEBERG IN HUMAN KERATINOCYTES

Anikó Göblös[1], Kornélia Szabó[2], Attila Bebes[1], Zsuzsanna Bata-Csörgő[1, 2], Lajos Kemény[1, 2], Márta Széll[2, 3]

[1]Department of Dermatology and Allergology, University of Szeged, Hungary,
[2]Dermatological Research Group of the Hungarian Academy of Sciences, Szeged, Hungary,
[3]Department of Medical Genetics, University of Szeged, Hungary

Introduction: Our recent large scale gene expression study has revealed CARD18/ICEBERG as a differentially expressed transcript in psoriatic uninvolved epidermis compared to healthy epidermis. CARD18/ICEBERG is a negative regulator of inflammasome activation, thus IL-1β maturation. It is also well-known that keratinocyte-derived IL-1β plays an important role in the pathogenesis of psoriasis. Therefore, we aimed to study the expression of CARD18/ICEBERG in differentiating human keratinocytes and in response to various psoriasis-related stress factors.

Methods: The expression of CARD18/ICEBERG mRNA was followed in spontaneously differentiating normal human keratinocytes, in keratinocytes transfected with a synthetic DNA analogue (polydA/dT) and in organotypic skin cultures incubated with T-cell lymphokines (GM-CSF, INFγ, IL-3).

Results: The CARD18/ICEBERG mRNA was expressed at low levels in proliferating keratinocytes and its expression was induced by the differentiation of the cells. Transfection of keratinocytes with a synthetic DNA analogue slightly elevated the expression of CARD18/ICEBERG mRNA. The inducibility of CARD18/ICEBERG upon T-cell lymphokine induction showed differences in psoriatic uninvolved epidermis compared to normal human epidermis. The basal expression levels were relatively high, and could not be further induced in the psoriatic uninvolved epidermis in response to T-cell lymphokines. This was in contrast to what was found in the healthy skin, where lower basal expression levels were detected, but these were inducible in response to the same treatment

Conclusions: Our results demonstrated that the mRNA expression of CARD18/ICEBERG can be induced by various psoriasis-related stress factors in human keratinocytes and we hypothesize that its high-level but uninducible expression in the uninvolved psoriatic epidermis contributes to the susceptibility to the disease.

ANALYSIS OF GRANZYME B EXPRESSION OF PERIPHERAL B CELLS IN SJÖGREN'S SYNDROME

Edit Gyimesi, Gábor Papp, Zoltán Kerékgyártó, Krisztina Szabó, Éva Zöld, Margit Zeher

University of Debrecen, Medical and Health Science Center, Department of Medicine, Division of Clinical Immunology, Debrecen

Introduction: B cell hyperactivity, altered B cell subset composition and elevated serum levels of soluble interleukin-21 (IL-21) was published in primary Sjögren's syndrome (pSS). The serin protease Granzyme B (GrzmB), furthermore interaction of invariant NKT (iNKT) cells with B cells may also have potential significance in autoimmune processes. B cell GrzmB secretion is inducible by IL-21 and B-cell receptor (BCR) engagement. Our goal was to investigate the expression of GrzmB in peripheral B cells and $CD5^+$ B cell subsets of patients with pSS, the level of B cell IL-21 receptor (IL-21R) expression and the contribution of iNKT cells to IL-21 production in pSS.

Patients and methods: Twenty pSS patients and 12 healthy controls were included. B cell intracellular GrzmB expression was determined after stimulation of peripheral blood mononuclear cells (PBMC) with recombinant IL-21 and anti-BCR antibody for 20 hours. In the last four hours Brefeldin A was added. IL-21R (CD360) expression was determined in unstimulated PBMC. In both cases the cells were surface labelled with CD5-FITC and CD19-PC5, and intracellular staining was performed with anti- GrzmB –PE or CD360-PE.

iNKT cell IL-21 expression was analysed after 6 hour stimulation with PMA/Ionomycin in the presence of Brefeldin A and incubating the cells with 6B11-PE, CD3-PC7 and then IL-21-APC. Coulter Fix and Perm kit was used for intracellular staining. Cells were analysed on FACSCalibur or Coulter FC500 flow cytometer.

Results: The $CD5^+$ B cells, but not all $CD19^+$ B cells of patients with pSS showed an elevated baseline GrzmB expression compared to control samples ($P<0.05$; Mann-Whitney test).

In stimulated samples, the specific GrzmB expression (stimulated – unstimulated) on CD19+ B cells enhanced, but not significantly, and increased significantly on $CD5^+CD19^+$ B cells of pSS patients ($P<0.05$; Mann-Whitney test).

The intracytoplasmic IL-21 expression of iNKT cells elevated significantly in pSS patients group ($P<0.05$; Student t-test). There was no difference in the expression level of IL-21R on $CD19^+$B and $CD5^+CD19^+$ B cell subsets in pSS patients.

Conclusions: Endogeneous IL-21 may able to stimulate in vivo the $CD5^+$ B cell GrzmB expression in pSS patients. On the basis of our result the IL-21 may play a role in the pathogenesis of Sjögren's syndrome by increasing GrzmB in B cells and can induce the autoregulation of the $CD5^+$ B cells. Our da-

ta suggest that in pSS the IL-21R expression of B cells probably have no effect, but the production of IL-21 by iNKT cells can contribute to the regulation of B cell GrzmB expression.

CHARACTERIZATION OF AN INDUCED MURINE MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS

Ákos Hornung[1, 2], Roberta Fajka-Boja[2], Éva Kriston-Pál[2], László Kovács[1], Éva Monostori[2]

[1]University of Szeged, Faculty of Medicine, Rheumatology Clinic, Szeged,
[2]MTA Biology Research Centre, Institute of Genetics, Szeged

Introduction: Systemic Lupus Erythematosus (SLE) is a chronic, multi-organ autoimmune syndrome characterised by B-cell hyperactivity, autoreactive antibody probuction and aberrant T-cell function and apoptosis. Though changes in immune function are partly understood, the root causes and molecular mechanisms of disease are still to be elucidated. To establish a better understanding of this disease, our team uses an induced murine model that is primed with a single intraperitoneal injection of pristane (a mineral oil). The used mouse strain is not genetically prone to autoimmunity, but after pristane injection, exhibit an assortment of symptoms characteristic of SLE. Our goal is to establish a better understanding of this poorly defined method and assess its usefulness as a model of Lupus.

Methods: Two month old C57BL/6 (B6) female mice were injected peritoneally with either 0,5ml pristane oil or a saline solution. The mice were sacrificed eight weeks later and we determined the ratios of mononuclear cells (T-cell, B-cell and macrophage) in the lymph nodes, peritoneal lavage and spleen using cytofluorimetry.

Results: There were no significant differences between main lymphoid cell populations in the lymph nodes of control and pristane injected mice, except that the ratio of $CD4^+FoxP3^+$ cells was considerably higher in pristane treated mice. Also, the ratio of $CD11b^+$ macrophages rose significantly in response to pristane injection. The macrophage population showed dramatic growth in the peritoneal cavity as well, while B-cells, $CD4^+$ and $CD8^+$ T-lymphocytes decreased compared to those of controls. Spleens of pristane injected animals were significantly larger than controls, their T-cell content decreased slightly but significantly after ConA stimulus, while LPS stimulation resulted in appearance of a $CD11b^+$ population which was not present in LPS stimulated spleens of control animals.

Discussion: An increased $FoxP3^+$ population in peripheral blood of SLE patients is a well documented phenomenon. A similar change in lymph nodes of pristane injected animals suggests a similar mechanism, as the cells might differentiate before entering the periphery. Causes of the increased $CD11b^+$ cell population in the lymph nodes are unclear, macrophages might migrate here from the peritoneum to present antigen. An increase in $CD11b^+$ cells is well documented in the pristane model. These are mostly $Ly6C^+$ macrophages that serve a role in phagocytosis and insulation of foreign material. In mice, these cells – not plasmacytoid dendritic cells – secrete INF-α, which is an important factor in SLE pathogenesis. The reason for the decrease in T- and B-cell populations in the peritoneum is unclear, though they may migrate to the spleen after pristine treatment. The increased spleen size is probably caused by a strong inflammation stemming from the pristane injection, which is substantiated by the presence of macrophages.

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T-BET EXPRESSION IN REGULATORY B CELLS

Krisztina Huber, Gabriella Sármay, Dorottya Kövesdi

Department of Immunology, Eötvös Loránd University, Budapest, Hungary

T-bet (Tbx21) was originally described as a T cell specific transcription factor that plays a central role in Th1 development. It was recently discovered that T-bet has an important function in B cells too; T-bet regulates immunoglobulin class switching and contributes to the production of pathologic IgG2a. From our results, we have learned that in B cells, T-bet is regulated by signals mediated through the B cell antigen receptor and Toll-like receptor 9, the same receptor-mediated cascades that have a well known impact on the function of so-called regulatory B cells. Regulatory B cells are an inducible B cell population, characterized by cell surface phenotypes and IL-10 production and by the suppressive role they show during the remission phase of autoimmune diseases.

Based on these knowledge, we were very interested in to characterize the population of regulatory B cells and find out more about their suppressive function on autoimmune processes. For this purpose we generated the CIA mice (Collage-Induced-Arthritis in mice; an inducible animal model of human rheumatoid arthritis) and followed the changes in regulatory B cell numbers during the disease progression. Isolated regulatory B cells from the acute and remission phases of CIA were analysed for T-bet and IL-10 expressions in order to find out if there was any connection between T-bet expression and the suppressive function of IL-10 on isotype switching and autoimmune IgG2a antibody production.

For the induction of CIA, female DBA/1J mice at 10 weeks of age were immunized with bovine type II collagen in complete Freund's adjuvant. During the disease progression mice were observed and scored weekly for clinical signs of arthritis. Spleens were removed during the acute and remission

phases of arthritis and regulatory B cells were sorted by their cell surface characteristics. For the analysis of T-bet and IL-10 mRNA expressions RNA was isolated from sorted cells, converted to cDNA and analysed by real-time RT-PCR.

From our experiments we found that in correlation with the elevated serum IgG2a levels the absolute number of regulatory B cells increased during the disease progression, moreover gene expression experiments showed inducible upregulation of IL-10 expression by signals important for regulatory B cells.

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A DESIGNED MINI-FACTOR H TO INHIBIT ANTI-FACTOR H AUTOANTIBODIES

Mihály Józsi[1, 6], Mario Hebecker[1], María Alba-Domínguez[2], Lubka T. Roumenina[3], Stefanie Reuterv, Satu Hyvärinen[4], Marie-Agnès Dragon-Durey[3, 5], T. Sakari Jokiranta[4], Pilar Sánchez-Corral[2]

[1]Junior Research Group for Cellular Immunobiology, Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany;
[2]Research Unit, Hospital Universitario La Paz – IdiPAZ, and CIBER de Enfermedades Raras, Madrid, Spain;
[3]Cordeliers Research Center, INSERM; Université Pierre et Marie Curie; Université Paris Descartes, Sorbonne Paris Cité, France;
[4]Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Finland;
[5]Hopital Europeen Georges-Pompidou, Service d'Immunologie Biologique, APHP, Paris, France;
[6]MTA-ELTE „Lendület" Complement Research Group, Department of Immunology, Eötvös Loránd University, Budapest, Hungary

Introduction: Autoantibodies to complement factor H (FH) are associated with the kidney diseases atypical hemolytic uremic syndrome and C3 glomerulopathies. Restoring FH function could be a treatment option for such diseases. Therefore, we designed a minimized human FH (mini-FH) construct that directly combines the two major functional regions of FH, namely the N-terminal complement regulatory domains and the C-terminal surface recognition domains. The aim of this study was the comprehensive functional characterization of mini-FH.

Materials and methods: Recombinant mini-FH was expressed in insect cells. Interaction with C3b and C3d was analyzed by surface plasmon resonance. Binding to pentraxins, malondialdehyde epitopes and extracellular matrix was analyzed by ELISA. Cofactor activity of bound mini-FH was measured by analyzing C3b cleavage with Western blot. Cell protective activity was analyzed by flow cytometry using HUVEC. Sheep erythrocytes were used to measure complement-mediated cell lysis. Plasma samples of patients were collected after informed consent.

Results: Mini-FH bound to C3b and had complement regulatory functions similar to those of full-length FH. Mini-FH bound to C3d with higher affinity compared to FH. Mini-FH also bound to the FH-ligands pentraxin 3, C-reactive protein and malondialdehyde epitopes. Mini-FH was functionally active when bound to pentraxins, extracellular matrix and endothelial cells in vitro and inhibited C3 deposition on the cells. Disease-associated autoantibodies recognized mini-FH. Furthermore, mini-FH efficiently inhibited complement-mediated lysis of host-like cells in patients' plasma caused by anti-FH autoantibodies that bind to the N- or the C-terminal domains of FH. Notably, mini-FH was more efficient inhibitor in the cellular assays than FH.

Conclusion: These data suggest that mini-FH, in addition to blocking anti-FH autoantibodies, could be potentially used as a complement inhibitor targeting host surfaces and to replace dysfunctional FH.

THE IMMUNOMODULATORY ROLE OF THE EXTRACELLULAR VESICLES IN EXPERIMENTAL MODELS OF AUTOIMMUNE ARTHRITIS

Anikó Juhász, Borbála Aradi, Krisztina Pálóczi, András Falus, Edit Buzás

Semmelweis University, Genetics, Cell- and Immunobiology

Background: The recent discovery of extracellular vesicles is one of the most significant findings in cell biology of the past decades. The universally and evolutionarily conserved secretion of extracellular vesicles plays an important role in intercellular communication. Nowadays extracellular vesicles attract substantial attention because of their potential use as diagnostic and/or therapeutic tools.

Goals: To investigate the immunomodulatory effect of murine thymus and spleen extracellular vesicles in experimental models of autoimmune arthritis.

Methods: Extracellular vesicles were isolated by differential centrifugation and gravity driven size filtration from 24h supernatants of thymocytes and splenocytes isolated from BALB/c and DBA1 mice. The amount of the extracellular vesicles was standardized to protein content by a microBCA assay. BALB/c mice were immunized intraperitoneally by the emulsion of human fetal aggrecan (partially deglycolysated by chondoitinase ABC digestion) and DDA. Furthermore, in DBA1 mice arthritis was induced by a peptide of glucose-6-

phosphate isomerase (GPI) and CFA. At the time of antigen injection, some groups of mice were co-injected intravenously with extracellular vesicles secreted by either syngeneic thymus or spleen cells. The course of clinical arthritis was characterized by cumulative acute arthritis scores. Total and antigen-specific IgG and IgM levels were determined by ELISA at different stages of arthritis induction.

Results: We observed the development of characteristic symptoms of arthritis after immunization with aggrecan or GPI peptide. According to our results, co-injection with thymus extracellular vesicles partially prevented the development of the arthritis. The clinical symptoms were slightly reduced (the cumulative arthritis scores were lower), and the aggrecan-specific and total IgM values were lower in the group co-injected with extracellular vesicles. In the arthritis model induced by GPI peptide we could almost fully prevent the development of the inflammatory symptoms by co-injection of extracellular vesicles secreted by splenocytes.

Conclusion: Our data suggest that extracellular vesicles may modulate immune responses and clinical symptoms in arthritis.

IDENTIFICATION AND CHARACTERIZATION OF NOVEL HEMOCYTE-SPECIFIC MOLECULES IN *DROSOPHILA MELANOGASTER*

Beáta Kari, Gábor Csordás, Viktor Honti, Katalin Medzihradszky Fölkl, Zsuzsanna Darula, István Andó, Éva Kurucz

Biological Research Centre of the Hungarian Academy of Sciences, Institute of Genetics, Laboratory of Immunology, Szeged, Hungary

Insects are armed with an evolutionarily conserved cell mediated immune-defense mechanism, which may serves as a prototype of innate immunity in all phyla of the animal kingdom. The fruit fly, Drosophila melanogaster, with its unique genetic system, is one of the most significant model organisms to study regulation of cell mediated immune defense. The effector cells of the cellular immune response in the fruit fly are the hemocytes (Honti et al., Dev. Comp. Immunol. 2013). The subsets of hemocytes exert a concerted action in the cell mediated immune response. The plasmatocytes engulf microorganisms, the crystal cells are involved in melanization and the lamellocytes participate in the encapsulation of large foreign particles. Lamellocytes, the encapsulating cells, may develop from phagocytic plasmatocytes upon immune induction. In the course of plasmatocyte-lamellocyte transition the morphological and functional changes can be monitored by the expression pattern of hemocyte-specific marker molecules (Kurucz et al., Acta Biol. Hung. 2007); the plasmatocyte-specific markers become silenced and markers, defining subsets of lamellocytes, are expressed sequentially (Honti et al. Mol. Immunol. 2010).

In addition to the already identified hemocyte-specific markers we defined two novel markers on subpopulations of plasmatocytes and lamellocytes, H18 and 3A5. The H18 molecule is restricted to a subpopulation of plasmatocytes in naïve animals, however, after immune induction the proportion of the H18 positive plasmatocytes is increased and the molecule is also expressed on differentiated lamellocytes. The expression of H18 is related to blood cell differentiation after immune induction. The 3A5 molecule is expressed in the cytoplasm of a subset of plasmatocytes and it is present in the hemolymph too. Mass spectrometric analysis of immunoprecipitates identified specific sequence derived from specific Drosophila ORFs. The H18 molecule is encoded by the Drosophila homolog of the human Tetraspanin genes, which are involved in human in signal transduction, immune cell proliferation and activation. The 3A5 molecule is encoded by the CG2233 gene, which has no human homolog, however, it is well conserved among Drosophilidae and is most likely a novel clotting factor.

Further analysis of the functions of these molecules will provide additional information on the molecular events of blood cell differentiation and the cell mediated immune response.

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CLINICAL MANIFESTATIONS AND THERAPEUTIC CHALLENGES OF HYPOCOMPLEMENTEMIC URTICARIAL VASCULITIS SYNDROME

Ágnes Kinyó[1], Lajos V. Kemény[2], Erika Varga[1], Éva Kemény[3], Lajos Kemény[1], Zsuzsanna Bata-Csörgő[1]

[1]Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary
[2]University of Szeged, Szeged, Hungary
[3]Department of Pathology, University of Szeged, Szeged, Hungary

Hypocomplementemic urticarial vasculitis syndrome (HUVS) is a distinct type of urticarial vasculitis with multiorgan involvement, whose etiology and link with SLE and other immune diseases are still unknown. The major manifestations of HUVS are chronic urticarial vasculitic lesions, angioedema, laryngeal edema, ocular inflammation, arthritis, arthralgia, obstructive lung disease, recurrent abdominal pain and glomerulonephritis. We present 4 patients who showed the typical signs of hypocomplementemic urticarial vasculitis syndrome, including urticarial lesions persisting more than 24 hours, recurrent angioedema, ocular inflammation, arthritis and arthralgia. Two of them had progressive obstructive lung disease,

the third patient had glomerulonephritis, and the fourth patient did not show any further systemic involvement. In the autoimmune laboratory results we found hypocomplementemia and rheuma factor positivity in all cases, in one case ANA positivity with SS-A, SS-B positivity, in one case borderline ds-DNA positivity, in other one dsDNA and ANA borderline positivity, while the last patient had only a mild ENA positivity. The skin histological findings were leukocytoclastic urticarial vasculitis in all cases with immune complex deposits contains IgG, IgM and c3, and in 2 cases we stained for c1q and found strong positivity at the basal membrane. The therapeutic outcome with usual immunosuppressive treatment was insufficient in every case, skin flares and angioedema recurred irrespectively of therapy. HUVS is considered by some to be a SLE-associated immunological disease, whereas many others consider it a distinct disease entity. Among our patients, 3 fulfilled the diagnostic criteria for SLE, but 1 patient did not. The fact that not all HUVS patients have SLE, and only a minor fraction of SLE patients develop HUVS, indicates a distinct pathomechanism for developing HUVS.

SYK IS INDISPENSABLE FOR CpG-INDUCED ACTIVATION OF HUMAN B CELLS

Mariann Kremlitzka[1, 2], Bernadett Valent[1], Anna Erdei[1, 2]

[1]Department of Immunology, Eötvös Loránd University,
[2]Research Group of the Hungarian Academy of Sciences, Budapest, Hungary

Background: B cells are efficiently activated by CpG oligodeoxynucleotides (ODNs) resulting in pro-inflammatory cytokine and antibody production mainly via Toll-like receptor 9 (TLR9) and the adaptor molecule myeloid differentiation marker 88 (MyD88). Here we identified a novel, spleen tyrosine kinase (Syk) dependent pathway which is indispensable for CpG induced activation of human B cells.

Methods: Resting tonsillar B cells were stimulated with CpG. Effect of Syk inhibition on various B cells' functions (proliferation, cytokine- and antibody production) were assessed. Activation of Syk dependent pathways was investigated with Western Blot.

Results: Stimulation of B cells resulted in time- and dose-dependent Syk and src kinase phosphorylation, proliferation, cytokine and antibody production. Notably, all these functions were abrogated in the presence of Syk and Src inhibitors. Syk was induced both via TLR9-dependent and –independent manners. Uptake of CpG ODNs was not reduced in the presence of Syk inhibitor, however co-localization of CpG and TLR9 was clearly reduced after it. Expression of TLR9 was significantly elevated after CpG stimulation, which was again abrogated by Syk inhibitors.

Conclusions: These data indicate a new and alternative pathway of CpG induced B cell stimulation through cell surface pattern recognition molecules. CpG-induced Syk activation is a prerequisite for optimal delivery of CpG into TLR9-containing endolysosomes and for induction of its receptor, allowing efficient propagation of TLR9-mediated signaling in human B cells.

AN AUTOCRINE REGULATORY LOOP IN FIBROBLASTS BETWEEN THE KERATINOCYTE GROWTH FACTOR (KGF) AND THE EXTRADOMAIN-A FIBRONECTIN (EDA$^+$FN) MAY CONTRIBUTE TO PSORIASIS PATHOMECHANISM

Barbara Gubán Korczné[1], Krisztina Vas[1], Attila Bebes[1], Márta Széll[2, 3], Lajos Kemény[1, 2], Zsuzsanna Bata-Csörgő[1, 2]

[1]University of Szeged, Department of Dermatology and Allergology, Szeged, Hungary,
[2]University of Szeged, Dermatological Research Group of the Hungarian Academy of Sciences, Szeged, Hungary,
[3]University of Szeged, Department of Medical Genetics, Szeged, Hungary

Introduction: In our previous work we showed that the fibronectin splice variant EDA$^+$FN, its receptor the alpha5-integrin, the keratinocyte growth factor (KGF) and its receptor (KGFR) are overexpressed in psoriatic uninvolved skin compared to normal skin. EDA$^+$FN and KGF both stimulate keratinocyte proliferation, moreover KGF is also known to induce alpha5-integrin expression in keratinocytes. In the present work we aimed to examine the regulatory mechanism between KGF, KGFR, EDA$^+$FN and FN1 in human fibroblasts.

Methods: We silenced FN1 with gene specific trilencer-27 siRNA in human fibroblast. We carried out real-time RT-PCR, immunocytochemistry and flow cytometry analysis of EDA$^+$FN and KGFR 24 hours after gene specific silencing of FN1. Secreted KGF protein levels were determined in FN1 silenced human fibroblast samples by ELISA.

Results: FN1 mRNA (n=4) and protein (n=4) expressions decreased by 80% in FN1 silenced human fibroblasts. The EDA$^+$FN splice variant mRNA (n=4) and protein (n=4) expressions were also reduced 24 hours after gene specific silencing of FN1. Knockdown of the FN1 gene in normal human fibroblasts resulted in significantly increased KGFR (FGFR-2 IIIb receptor variant) protein expressions (n=4), however no changes in the mRNA levels were observed (n=4). The amounts of secreted KGF protein (n=4) were significantly higher in human fibroblasts 24 hours after gene specific silencing of FN1.

Conclusion: These data indicate the existence of a previously unknown autocrine regulatory network in fibroblasts between KGF, KGFR EDA$^+$FN and FN1 that may be relevant to psoriasis pathomechanism.

DECREASED GALECTIN-1 EXPRESSION AND APOPTOTIC ACTIVITY IN SYSTEMIC LUPUS ERYTHEMATOSUS

László Kovács[1], Ákos Hornung[1, 2], Magdolna Deák[1], Enikő Szabó[2], Julianna Novák[2], Ágnes Czibula[2], Roberta Fajka-Boja[2], Éva Monostori[2]

[1]University of Szeged, Faculty of Medicine, Department of Rheumatology,
[2]Hungarian Academy of Sciences, Biological Research Centre, Institute of Genetics, Lymphocyte Signal Transduction Laboratory, Szeged

Introduction: Galectin-1 (Gal-1) is a lectin with immunomodulant activities. It has been suggested to contribute to the T and B-lymphocyte dysfunction observed in animal models of systemic lupus erythematosus (SLE). As the effects of Gal-1 have not been investigated on human samples, the aim of the authors was to compare the expression of Gal-1, and the apoptotic response to exogenous Gal-1 between T-cells of SLE patients and healthy subjects, and to determine the Gal-1 binding ability of activated T-cells, as an altered binding pattern may contribute to an impaired response to apoptotic signals.

Methods: T-cells were separated from peripheral blood of 16 SLE patients, and were activated with PHA. The results were compared with those on samples from the same patient taken in (treatment-induced) remission (n=9), and from healthy controls (n=17). Intracellular Gal-1 expression at the mRNA level was measured with quantitative RT-PCR, and at the protein level with extra- and intracellular cytofluorimetry. In order to determine the response to exogenous Gal-1, activated T-cells were co-cultured with Gal-1-expressing and non-expressing HeLa tumour cells. After 16 hours of co-culture, the apoptosis rate of T-cells was assessed with fluorescent Annexin V-labelling by means of fluorescent microscopy. The cell surface binding of fluorescent-labelled Gal-1 was examined with flow cytometry.

Results: Gal-1 mRNA exhibited significantly lower expression in SLE activated T-cells than int he controls (0.25 vs 0.38, p=0.02). After successful therapy, the amount of Gal-1 protein significantly increased as compared with that in the active disease state (3.17 vs 2.25, p=0.015). The presence of exogenous Gal-1 significantly increased the apoptotic rate of the healthy T-cells, whereas the apoptotic cell death rate of lupus T-cells was significantly lower (relative apoptotic rate: 12.2 vs 3.03, p=0.01). Gal-1 displayed different cell surface binding patterns in the two groups.

Conclusions: T-lymphocytes from SLE patients produce less Gal-1 during active disease, and, in parallel, are resistant to the apoptotic effects of exogenous Gal-1. The reduced production and impaired regulatory activity of the immunosuppressant protein Gal-1 may play role in the pathogenesis of SLE. These results obtained with the use of T-cells from SLE patients corroborate the authors'observations on Jurkat cells which indicate that the emergence of Gal-1 intracellularly may sensitize the T-cells to the apoptotic effects of exogenous Gal-1.

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WNT4 PROMOTES TISSUE DESTRUCTION DURING LUNG AGING VIA INHIBITING PPARΓ EXPRESSION

Tamás Kovács, Veronika Csöngei, Dávid Ernszt, Diána Feller, Judit Pongrácz

PTE ÁOK Gyógyszerészi Biotechnológia Tanszék

The continuous increase of elderly population will put an enormous pressure on social and medical care in the near future. Therefore expanding the healthy lifespan or decreasing the occurrence of the age related diseases are the biggest challenges for developed countries.

While the aging of other organs are studied widely, the molecular background of lung senescence is hardly known. In the aging lung, the lung capacity decreasing and the formation of new alveoli slow down even in the absence of diseases.

For the lung regeneration ATII cells are one of the most important facultative progenitor cells. They are supported by the lipofibroblasts. The normal function is a PPARγ dependent mechanism. The loss of the number of lipofibroblast will cause destruction of ATII cell network, which will lead to different age related diseases such as COPD or IPF.

Recent studies suggest the role of Wnt molecules in aging. It was already proven that Wnt proteins have role in different fibrotic and inflammatory processes, like COPD and IPF. But we still don't know, are there any connections between these two processes?

In our studies 1 month and 24 months Balb/C mice lungs were compared first with computed tomographic technique. On the recordings is clearly seen the enlargement of the alveoli, and it was also proven with microscopic sections with Hematoxylin-Eosin staining.

To investigate the molecular pattern of lung, first epithelial and non-epithelial cells were separated; by EpCAM1 positivity and gene expression analysis were performed. Because for the normal lung regeneration PPARγ and ADRP are essential, they expression were measured with quantitative real time PCR, beside Wnt molecules.

Our studies have shown that Wnt4 are increased in epithelial and non-epithelial cells, which based on literature, can decrease the PPARγ expression, which will lead to the loss of normal lung function and cause COPD in elderly.

TRANSIENT RECEPTOR POTENTIAL ANKYRIN 1 (TRPA1) RECEPTOR HAS A PROTECTIVE ROLE IN DEXTRANE-SULFATE INDUCED MOUSE COLITIS

József Kun[1, 3], Zsuzsanna Helyes[1, 3], István Szitter[1, 3], Ágnes Kemény[1], Dávid Ernszt[3], Éva Szőke[1, 3], Éva Sághy[1], Tamás Kovács[2], András Bognár[2], Krisztina Pohóczky[1], Teréz Bagoly[1], Adrienn Markovics[1], Anikó Perkecz[1], Zoltán Sándor[1], Erika Pintér[1, 3]
[1]Department of Pharmacology;
[2]Department of Immunology and Biotechnology, Faculty of Medicine, University of Pecs, 12 Szigeti street, H-7624 Pecs, Hungary;
[3]Molecular Pharmacology Research Group, Szentagothai Research Center, University of Pecs, 20 Ifjusag street, H-7624 Pecs, Hungary

Transient Receptor Potential Ankyrin 1 (TRPA1) cation channel activation on sensory nerves leads to the release of pro- and anti-inflammatory neuropeptides. Its expression was also described on non-neural (e.g. epithelial, immune) cells, but data on functional significance are contradictory. Therefore, we investigated its role in a colitis mouse model.

Dextrane-sulfate (DSS) was added to the drinking water of male TRPA1 knockout (KO, n=11) and wildtype (WT, n=9) mice for 10 days. Disease activity index (DAI) was calculated from weight loss, stool consistency and blood content. Expression of TRPA1, the pro-inflammatory tachykinins substance P, neurokinin A (NKA), neurokinin B (NKB) and NK1 tachykinin receptor was measured by qPCR, radioimmunassay (RIA) and immunohistochemistry. The levels of interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNFα), B-lymphocyte chemoattractant (BLC) mRNA and protein were measured by qPCR and Luminex bead-based assay, respectively. TRPA1 activation by the agonist mustard oil (200 µmol) was determined on isolated peritoneal macrophages by fluorescence calcium imaging and on lymph node-derived T cells by flow cytometry.

TRPA1 is present on enteric ganglion cells and macrophages, DSS upregulated its expression 4-fold on day 7 compared to the untreated control. In TRPA1 KO mice, DAI was significantly higher compared to WTs which is supported by the significantly elevated levels of substance P, NKA, NKB, NK1 receptor, IL-1beta, TNFα mRNA and BLC peptide levels in the distal colon. The functionality of TRPA1 ion channel was evidenced by Ca^{2+} influx as a response to mustard oil in WT macrophages and T cells but not in TRPA1 KO cells.

TRPA1 is upregulated in the inflamed colon, macrophages and T cells express functional channels. It exerts a clear protective role in DSS-induced colitis by decreasing tachykinin, NK1 receptor and cytokine /chemokine expressions.

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T CELL RECONSTITUTION STUDIES IN ZAP-70 DEFICIENT MICE

Réka Kugyelka, Katalin Olasz, Lilla Prenek, Péter Németh, Tímea Berki, Ferenc Boldizsár
Department of Immunology and Biotechnology, Faculty of Medicine, University of Pécs

The ZAP-70 kinase (70kDa Zeta-Chain Associated Protein) plays a central role in signal transduction through the antigen receptor during T cell activation. The importance of the molecule is clearly demostrated when it is absent: several signaling pathways are inhibited, and severe T-cell immunodeficiency appears both in humans and mice. The reason of the latter is that ZAP-70 is indispensable in T cell differentiation: in its absence the maturation of T cells in the thymus is blocked in the double positive ($CD4^+CD8^+$) stage, and, as a consequence no mature T-cells can be found in the peripheral lymphoid organs.

In our work we studied the possibilities of T cell reconstitution in ZAP-70 deficient mice. We performed adoptive transfer experiments, where ZAP-70–/– mice were reconstituted with bone marrow or thymus cells from their wild type (ZAP-70 expressing) siblings intrahepatically or intraperitoneally.

According to our results both transfer techniques were effective in restoring T cells. After the cell transfers, blood was taken every 2 weeks to detect the presence of T cells in the blood. The survival of those animals which had T cells reconstituted exceeded significantly those which were immunodeficient. Both flow cytometric measurements and immunohistochemistrical staining performed after the experiments (following the transfers) proved that T cells appeared in the spleen, lymph nodes and gut associated lymphoid tissues of the animals. Furthermore, during the investigation of cell constitution of the thymus, we have found that the ratio of $CD4^+$ or $CD8^+$ single positive cells increased significantly, which indicated the normalisation of T-cell maturation.

Thus, we managed to establish stable chimerism in ZAP-70 deficient mice with two methods. We proved that both thymus and bone marrow originated cells were able to restore the development of T-cells.

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MOLECULES INVOLVED IN THYMIC EPITHELIAL SENESCENCE *IN VITRO* AND *IN VIVO* RESULTS

Krisztian Kvell[1], Aniko Fejes[1], David Ernszt[1], Jan-Erik Werry[1], Petra Gal[1], Laszlo Nagy[2], Roland Foisner[3], Judit Pongracz[1]

[1]Department of Pharmacological Biotechnology, University of Pecs, Hungary,
[2]Department of Biochemistry and Molecular Biology, University of Debrecen, Hungary,
[3]Max F. Perutz Laboratories, Medical University, Vienna, Austria

Introduction: The thymus undergoes rapid involution compared to other organs. The thymic epithelium shrinks and gives place to adipose tissue. This is followed by functional decline in naive T-cell production and subsequently increased incidence of infections, cancers and autoimmune diseases. It is of high importance to identify molecules responsible for thymic adipose involution or central immune senescence to prolong imune fitness.

Methods: For our experiments we have useda mode cell line (primary derived mouse thymic epithelial cell line or TEP1), enriched mouse thymic epithelial cells from control and also knock-out mice for PPARgamma and LAP2alpha. The applied methods include qRT-PCR for gene expression, immune-fluorescent staining for histology and mTREC qPCR to assess naive T-cell production.

Results – conclusion: Our data indicate that with ageing the thymic epithelium undergoes indirect trans-differentiation towards adipocyte lineage. First there is an initial EMT (epithelial-to-mesenchymal) transition stage into fibroblast-like cells that subsequently differentiate towards adipocyte lineage. The process may be promoted by LAP2alpha or PPARgamma, and may be slowed down by Wnt4 based on in vitro data. However, in vivo data show a more complex constellation therefore further studies are required.

ANALYSIS OF THE ION AND MEMBRANE HOMEOSTASIS OF MICROVESICLES

Márk Langer, Andrea Németh, Edit Buzás, Bence György

Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest

Background: Microvesicles (MV-s) are cell-derived structures, which are bordered by a phospholipid bilayer, and have multiple functions including immune modulation, haemostasis and intercellular communication. Production of MV-s is an evolutionary conserved cell-physiological phenomenon. Extracellular vesicles can be found in all biological fluids. Their biomedical significance include their potential exploitation as biomarkers for many diseases, and/or as possible carriers of nucleic acids, or pharmaceutics.

Goal: We aimed at examining the ion homeostasis and membrane integrity of U937 monocyte cell line derived MV-s with calcium dyes, using flow cytometry.

Methods: We used CCRF and U937 cell line derived, and human and mouse originated MV-s for our measurements. We sedimentated the MV-s by differential centrifugation, and painted them, with cell permeable ion paints (Fluo4 and Sodium Green), and common vesicle marker Annexin V. We monitored the calcium and sodium concentration using flow cytometry. To prove membrane integrity we stimulated the MV-s with A23187, and monitored calcium concentration. We also used LPS, concanavalin A, and PMA as stimulants.

Results: We proved that MV-s can be labeled using ion dyes. This however requires intravesicular esterase activity, which in turn, is an indirect proof of intravesicular enzyme function. The Fluo4 positive events were also Annexin V. positive, providing evidence for the vesicular nature of the labeled structures. Further proof for this was provided by the detergent sensitivity of the fluorescent events: after the use of 0.1% Triton X-100, we detected vesicle lysis. In time we saw two phases of the vesicular calcium signal. A slow calcium uptake was followed by a sudden calcium and dye depletion 40-50 minutes later. The A23187 stimulation (in 25 independent experiments) raised the calcium signal unambiguously in all specimens (approx. with 40%, $p<0.01$ paired t-probe). Twenty minutes after stimulation, the calcium levels returned to normal. PMA, LPS, and concanavalin A had no significant influence on vesicle calcium levels whatsoever.

Conclusions: Our results show that MV-s have their own ion and membrane homeostasis similarly to cells. The physiological significance of this fact is unknown, and needs to be further investigated.

THE ROLE OF MULTINUCLEATED GIANT HEMOCYTES IN THE CELL-MEDIATED IMMUNE RESPONSE

Zita Lerner[1], Róbert Márkus[1], Viktor Honti[1], János Zsámboki[1], Szilvia Tápai, Tamás Lukacsovich[2], Árpád Párducz[3], Gábor Csordás[1], Éva Kurucz[1], István Andó[1]

[1]Immunology Unit, Institute of Genetics, BRC HAS, Szeged,
[2]Developmental Biology Center, University of California, Irvine, USA
[3]Institute of Biophysics, BRC HAS, Szeged

Insects developed a powerful immune system – the prototype of the innate immune system of vertebrates – to invading microorganisms and parasites by the help of humoral and cellular elements. In Drosophila melanogaster, the commonly used model organism to study innate immunity, the cellular elments, the phagocytic plasmatocytes, the melanizing crystal cells and the encapsulating lamellocytes are organized in three hematopoietic compartments, the lymph gland,

the circulation and the sessile hematopoietic tissue. While the immunity of D. melanogaster is extensively studied, our knowledge on the immune response of other Drosophila species is still fragmentary. The aim of our research is to understand the adaptation of the cell-mediated immunity to different parasites within Drosophilidae. In the study, we analyzed the cell mediated immunity of Drosophila ananassae, a representative of the ananassae subgroup.

To define the hemocyte subsets, functions and origin in this species, we developed a toolkit based on immunological markers and a transgenic reporter system, which allows the in vitro analysis and in vivo observation as well as manipulation of hemocytes and hematopoietic compartments.

In D. ananassae, plasmatocyte and crystal cell morphology and functions are similar to those in D. melanogaster. However, instead of lamellocytes – the encapsulating cell type of D. melanogaster –, we observed special giant cells with filopodia, which we named as Multinuclear Giant Hemocytes (MGHs). These MGHs take part in the capsule formation, and they eliminate the parasitic wasp eggs effectively without melanization, a reaction characteristic for the encapsulation in D. melanogaster. The MGHs are derived from the circulation and the sessile tissue, without the involvement of the lymph gland; the latter compartment being a source of the encapsulating lamellocytes in D. melanogaster. This, and the in vivo observation that the lymph gland primarily consists of differentiated plasmatocytes suggest that the major role of the lymph gland in this species is to provide plasmatocytes for the metamorphosis and for the adult stage.

The analysis of the immune response in D. ananassae reveals diversity in the development of the innate immune system in insects suggesting a selecting pressure of the host-parasite interactions. In addition, as MGHs are similar to vertebrate multinuclear giant cells in granulomas, therefore we believe that the D. ananassae immune system is a powerful model to understand basic mechanisms of granuloma formation in vertebrates.

OPTIMIZATION OF AUTOMATED CD8⁺ REGIONAL MEMORY T CELL ISOLATION

Nikolett Lupsa[1], Barbara Molnár-Érsek[1], Tünde Simon[1], Anett Tóth[1], Bence Bagita[1], András Falus[2], Edit Buzás[2], Zoltán Pós[1]
[1]„Lendület"Experimental and Translational Research Group, Hungarian Academy of Sciences – Semmelweis University, Budapest,
[2]Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest

Tissueresidentcytotoxic memory T cells (CD8⁺ Trm)represent a unique group of the CD8⁺ T cellmemoryprotectingperipheral tissues and providing early alarm signals upon recall antigen challenge.Depending on tissue localizationTrm cells express different homing markers; on the other hand, increased integrin αE (CD103) and CD69 expression is a common feature of all known CD8⁺ Trm subsets, regardless of their organ of residence.This project aimsata better understandingof the organ-specific biomarkers, functional features,establishment, maintenance and recall response of the CD8⁺ Trm cells.

We believe that the most adequateway to analyze this complex and largely unexplored field is,to begin witha hypothesis-free, comprehensive,genomic scalegene expression analysis of regional memory T cells of various organs. Pursuing this goal, the firstand probablymost challenging issue to be solved is the isolation of theserare and rather vulnerable cells,that requires a methodology ensuring highly pure, effective, reproducible, and gentle cell retrieval that does not decrease viability or compromises functionality of these cells. To this end we setupseveral novel automated isolation protocols, individually tailored to the needs of distinct murine CD8⁺ organ-resident Trm cells,acting by means of mechanical and enzymatic tissue processing. The process isconducted with help of ahighly sensitive cell separator platform supported by appropriateRNA isolation and amplification systems to allow subsequent microarray analysis.

We successfully isolated tissue resident CD8⁺ Trm cells from select organ samples (lung, small intestine, liver,) of mice on the C57Bl/6 background by using automated tissue processing with help of a GentleMACSOctoDissociator.Subsequent cell sorting depending on Trm markers (CD8b, CD103) was carried out on an autoMACS Pro Separator system. Pure fractions (93–98%) of Trm cells were processed by an RNeasy Micro Kit for ultra-low inputRNA amplification with an ArcturusRiboAmp HS PLUS Amplification Kit and subjected tomicroarray gene expression analysis.

This isolation strategy successfully retrievedthousandsof CD8⁺ Trm cells from various organs,is highly effective, and ensured both high purity, and reproducibility. The isolated cells were used for microarray analysis and their gene expressing profilealso confirmed their identity as CD8+ Trm cells.To our best knowledge, this is the first attempt to conduct a comparative, hypothesis-free, in-depth genomic scale analysis on CD8⁺ Trm cells to gain further insight into this unique branch of CD8⁺ T cell memory.

COMPLEMENT FRAGMENT C3d INHIBITS TLR9 AND BCR⁺TLR9 INDUCED ACTIVATION OF HUMAN B CELLS

Bernadett Mácsik-Valent[1], Mariann Kremlitzka[1], Anna Erdei[1, 2]
[1]Department of Immunology, Eötvös Loránd University,
[2]Research Group of the Hungarian Academy of Sciences, Budapest, Hungary

Introduction/Background: Complement receptors type 1

(CR1, CD35) and 2 (CR2, CD21) expressed by B lymphocytes play a crucial role in bridging innate and adaptive immunity. By binding different degradation products of the third complement component, C3 – such as C3b and C3d – these two receptors mediate opposing effects in human B lymphocytes. Namely, CR2 enhances the BCR mediated functions while CR1 inhibits those (Int. Immunol. 2013, J. Immunol. 2002).

Several microbial pathogens not only engage BCR but can trigger B cell functions by binding to Toll-like receptor 9 (TLR9), the microbial DNA sensor of the innate immune system. Besides the BCR dependent signalling, TLR9 also significantly determines the activation state of a B lymphocyte. Moreover, it is also known that, theTLR9 and BCR initiated signalling pathways synergise at the level of MAPKs.

Methods: Our aim is to get insight into how complement receptors – especially CR2 – might influence the TLR9 induced activation of human B cells. For the experiments we used C3d, the natural ligand of CR2 immobilized on the surface of the culture plates, and measured the proliferation and phosphorylation of human resting tonsillar B cells. Cells were stimulated via BCR using suboptimal dose of F(ab')2 anti-human IgG/M/A and via TLR9 using CpG ODN 2006 either separately or simultaneously.

Results and Conclusions: We show that clustering of CR2 by its natural ligand significantly reduces both the TLR9 and BCR^{+}TLR9 induced proliferation of resting human tonsillar B cells. These results demonstrate that CR2 exerts a strong inhibitory effect on TLR9 dependent signalling, which can not be prevailed by BCR triggering. To see whether additional B cell functions are also affected by the engagement of CR2, the assessment of antibody production and cytokine release is in progress in our laboratories.

Our results reveal a so far undescribed level of B cell regulation where complement might be strongly involved.

NOVEL FACTORS IN PSORIASIS PATHOGENESIS AND POTENTIAL DRUG CANDIDATES ARE FOUND WITH SYSTEMS BIOLOGY APPROACH

Máté Manczinger[1], Lajos Kemény[1, 2]
[1]Department of Dermatology and Allergology, University of Szeged, Hungary,
[2]Dermatological ResearchGroup of the Hungarian Academy of Sciences, Szeged, Hungary

Introduction: Psoriasis is a multifactorial inflammatory skin disease characterized by increased proliferation of keratinocytes, activation of immune cells and susceptibility to metabolic syndrome. Systems biology approach makes it possible to reveal novel important factors in the pathogenesis of the disease.

Methods: Protein-protein, protein-DNA, merged (containing both protein-protein and protein-DNA) interactions and chemical-protein interaction networks were constructed consisting of differentially expressed genes (DEG) between lesional and non-lesional skin samples of psoriatic patientsand/or the encoded proteins. DEGs were determined by microarray meta-analysis using MetaOMICS package. We used STRING for protein-protein, CisRED for protein-DNA and STITCH for chemical-protein interaction network construction. General network-, cluster- and motif-analysis were carried out in each network.

Results and discussion: Many proteins (BUB1B, CCNA2, FYN and PIK3R1, SGK1) and transcription factors (AR, TFDP1) were identified as hubs, suggesting that these factors might be important in psoriasis pathogenesis. BUB1B, CCNA2 and TFDP1 might play a role in the hyperproliferation of keratinocytes, whereas FYN and SGK1 may be involved in the disturbed immunity in psoriasis. AR can be an important link between inflammation and insulin resistance in psoriasis. A controller sub-network was constructed from interlinked positive feedback loops that with the capability to maintain psoriatic lesional phenotype. Analysis of chemical-protein interaction networks detected 37 drugs with previously confirmed antipsoriatic effects, 29 drugs with some experimental evidences, and 25 drugs with case reports suggesting their disease modifying effects. In addition, 108 unpublished drug candidates were also found, that might serve future treatments for psoriasis.

PROINFLAMMATORY AND ANTI-INFLAMMATORY CYTOKINES REGULATE THE EXPRESSION OF CD3ζ-CHAIN IN HUMAN T LYMPHOCYTES

Nikolett Marton[1], Barbara Molnár-Érsek[1], Edit Buzás[1], György Nagy[1, 2]
[1]Department of Genetics, Cell- and Immunbiology, Semmelweis University, Budapest,
[2]Department of Rheumatology, Semmelweis University, Budapest

Background: The CD3 ζ-chain is critically important in T lymphocyte activation, decreased expression of the ζ-chain has been reported in inflammatory, autoimmune and malignant diseases. ζ-chain downregulation is common at sites of chronic inflammation. According to our earlier data TNF treatment of human T lymphocytes selectively downregulates CD3 ζ-chain expression in a dose dependent manner, and decreases the activation induced IL-2 synthesis. In the present study we investigated the effect of proinflammatory and anti-inflammatory cytokines on the regulation of CD3 ζ expression.

Materials and methods: Jurkat cells and peripheral blood mononuclear cells (PBMCs) were treated with 5, 15 or 40

ng/ml of TNF, 20 or 80 ng/ml IL-1, IL-4, IL-6, IL-8, IL-10, IL-13, IL-17 for 24 and 48 hours or left untreated. ζ-chain expression was measured by Western blot and by flow cytometry.

Results: according to our present data IL-6, IL-8, IL-10, IL-13, IL-17 decrease, while IL- 1 and IL- 4 do not alter the expression of the CD3ζ-chain.

Conclusion: Our present data indicate that both proinflammatory and anti-inflammatory cytokines may regulate T cell activation through regulating CD3 ζ–chain expression.

INVESTIGATING THE PERIPHERAL BLOOD TIM-3 POSITIVE NK AND CD8$^+$ T CELLS DURING PREGNANCY

Matyas Meggyes[1, 4], Eva Miko[1, 4], Beata Polgar[1, 4], Balint Farkas[2], Zsolt Illes[3], Laszlo Szereday[1, 4]

[1]University of Pecs, Clinical Centre, Department of Medical Microbiology and Immunology, Pecs, Hungary,
[2]University of Pecs, Clinical Centre, Department of Obstetrics and Gynaecology, Pecs, Hungary,
[3]Department of Neurology, Odense University Hospital, Odense, Denmark,
[4]Janos Szentagothai Research Centre, Pecs, Hungary

Problem: TIM-3 (T-cell immunoglobulin and mucin domain-3) is a relatively newly described molecule with a conserved structure and important immunological functions. Multiple immune cells expressing TIM-3 therefore it has been implicated both in activation and inhibition of immune responses. Identification of Galectin-9 as a ligand for TIM-3 has established the Galectin-9/TIM-3 pathway as an important negative regulator of Th1 immunity and tolerance induction. Data about the role of TIM-3/Gal-9 pathway in the pathogenesis of human diseases is emerging, but data about their role during human pregnancy is still not clear. We have therefore investigated the number, phenotype and functional activity of TIM-3$^+$ peripheral blood mononuclear cells during healthy human pregnancy.

Methods of study: 30 healthy pregnant women [first trimester (n=10); second trimester (n=10); third trimester (n=10)] and 15 non-pregnant controls were included in this study. We measured the surface expression of TIM-3 by cytotoxic T cells, NK cells and NK cell subsets and Galectin-9 expression by regulatory T cells by flow cytometry. We analyzed the cytokine production and cytotoxicity of TIM3+ and TIM3- CD8 T and NK cells of non-pregnant and healthy pregnant women at different stages of pregnancy by flow cytometry. Serum Galectin-9 levels were measured by ELISA.

Results: Our results show that the numbers of NK and cytotoxic T cells and their TIM-3 expression do not change between the first, second and third trimesters of pregnancy. Compared to non pregnant individuals, regulatory T cells show higher level of Galectin-9 expression as pregnancy proceeds, which is in line with the data obtained analyzing sera for soluble Galectin-9. Cytotoxic T cells, NK cells and NK cell subsets expressing TIM-3 molecule show altered cytokine production and cytotoxicity during pregnancy compared to non pregnant state.

Conclusion: Our results indicate that Galectin-9 expressing regulatory T cells, TIM-3$^+$ cytotoxic T cells and NK cells could play an important role in the maintenance of healthy pregnancy.

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NOVEL EVIDENCES ON THE IMPORTANT CONNECTION BETWEEN ALLERGIC SENSITIZATION AND SKIN BARRIER ALTERATIONS

Gábor Mócsai[1, 2], Beáta Tóth[3], László Maródi[3], Krisztián Gáspár[1, 2], Anikó Kapitány[1, 2], Zsolt Dajnoki[1, 2], Andrea Szegedi[1, 2]

[1]Department of Dermatology,
[2]Department of Dermatological Allergology,
[3]Department of Infectious and Pediatric Immunology, University of Debrecen, Medical and Health Science Center, Debrecen, Hungary

Introduction: Recently, in the development of allergic diseases the role of sensitization through damaged barrier surfaces had become more appreciated. Genetic mutations or acquired alterations in the expression of different skin barrier molecules (filaggrin, tight junction proteins and serine proteases) are often connected to an increased risk of IgE-mediated allergic sensitization.

In the present study our aim was to investigate whether a well-functioning skin barrier could inhibit the development of allergic sensitization in spite of extremely increased total IgE levels. Therefore we compared skin barrier parameters and allergic sensitization in patients with atopic dermatitis (AD) and Hyper-IgE syndrome (HIES), which disease is often accompanied by AD-like skin lesions.

Methods: In our experiments, STAT3 and FLG mutation analyses were performed in HIES (n=7) and AD (n=30) patients. Laboratory parameters (serum LDH and peripheral blood eosinophil counts), immunologic alterations (intracellular cytokine staining in blood T cells), data on allergic sensitization (total and specific IgE levels, medical history), and skin barrier changes [transepidermal water loss (TEWL), skin pH, serum and stratum corneum thymic stromal lymphopoietin (TSLP) levels] were examined.

Results: Mutation analysis of STAT3 showed 100% positi-

vity in HIES patients, although all of them had FLG wild-type genotype concerning the two most common mutations (R501X and 2282del4), which were found in 31% of our AD patients in heterozygous form. Impaired Th17 cell numbers, but normal barrier functions were found in HIES patients, based on TEWL, skin pH, serum and stratum corneum TSLP levels. Skin barrier parameters were significantly altered in AD patients. Allergic sensitization was detected in nearly all AD patients, while no signs of sensitization occurred in HIES.

Discussion: Our workgroup investigated skin barrier functions of HIES patients first in the literature. The well-functioning skin barrier in these patients may explain the contradiction between the extremely high total IgE levels and the lack of allergic sensitization. Our study underlines the importance of skin barrier in the development of allergic sensitization.

AZ IL-17 SZÉRUMSZINTEK EMELKEDÉSÉHEZ A sRANK LIGAND FOKOZOTT KÉPZŐDÉSE TÁRSUL POSZTMENOPAUZÁLIS OSTEOPOROSISBAN

Ildikó Molnár[1], Ilona Bohaty[2], Vári Éva Somogyiné[1]

[1]EndoMed, Immunendokrinológia és Osteoporosis Centrum, Debrecen,
[2]Regionális Vérellátó Központ – Országos Vérellátó Szolgálat, Debrecen

Bevezetés: Az IL-17A proinflammatorikus citokin jelentős csontvesztést okozhat. A posztmenopauzális osteoporosist a csontreszorpció túlsúlya jellemzi, amiben kimutatható az ösztrogénhiány és a fokozott immunreakciók egymásra hatása. Korábbi vizsgálataink emelkedett IL-17A szérumszinteket igazoltak posztmenopauzális ösztrogénhiányban. Az IL-17-szintek emelkedése szignifikáns összefüggést mutatott a csontvesztés mértékével. Újabb vizsgálatainkkal választ kerestünk, hogy az emelkedett IL-17 szérumszintek milyen módon okozzák az osteoporosis kialakulását.

Módszerek: A szérum IL-17A, sRANK (szolubilis receptor aktivátor NF-κB) ligand, OPG (osteoprotegerin) szinteket enzyme-linked immunosorbent assay (ELISA) módszerrel mértük 94 (22 prae- és 72 posztmenopauzában lévő) nőnél. A csontvesztés mértékét a lumbalis gerinc (L1-L4) DEXA vizsgálatával és a bone mineral density (BMD) ill. a T-score értékek megadásával értékeltük. A betegek nem részesültek csontritkulás elleni kezelésben, és kizártuk az onkológiai, endokrin és autoimmun betegségben szenvedőket.

Eredmények: Posztmenopauzális osteoporosisban (n=41) jelentősen emelkedett IL-17A és sRANK ligand szinteket, illetve mérsékelten emelkedett OPG -szinteket kaptunk az osteopeniás (n=31) nőkhöz képest (3,65±0,61 ng/ml vs 3,31±0,43 ng/ml, P<0,007 IL-17A esetében; 2,88±0,84 ng/ml vs 2,49±0,61 ng/ml, P<0,027 sRANK ligand estében; 1,43±0,07 ng/ml vs 1,39±0,07 ng/ml, P<0,038 OPG esetében). Az IL-17A szintek negativan korreláltak a BMD (P<0,022) és T-score (P<0,023) értékekkel. Ugyanakkor pozitivan korreláltak a sRANK ligand (P<0,009) és sRANK/OPG hányados (P<0,023) értékekkel, de az OPG szintekkel nem mutattak összefüggést.

Következtetések: A posztmeno-pauzális IL-17A szintek emelkedése a fokozott csontvesztést a csontreszorpciót fokozó sRANK ligand jelentős emelkedésével okozza.

IL28B CC GENOTYPE: A PREDICTOR OF RESPONSE TO INTERFERON IN CHRONIC HEPATITIS C VÍRUS (hcv) INFECTION, AND IT IS ASSOCIATED WITH INCREASED TH1 CYTOKINE PRODUCTION OF ACTIVATED PERIPHERAL BLOOD MONOCYTES AND LYMPHOCYTES

Alajos Pár[1], Gabriella Pár[1], Timea Berki[2], László Pálinkás[2], Péter Kisfali[3], Béla Melegh[3] and the Hungarian Viral Hepatitis Study Group*.

[1]First Department of Medicine,
[2]Department of Immunology and Biotechnology,
[3]Department of Medical genetics, University of Pécs, Pécs
* I. Tornai, E. Fráter, M. Papp (Debrecen), D. Várszegi, A.Vincze, B. Hunyady, Zs. Nemes, Z. Péterfi (Pécs), F. Szalay, G. Lengyel, J. Fehér, J. Schuller (Budapest), M. Varga (Békéscsaba), J. Gervain (Székesfehérvár), A.Tusnádi (Szolnok), A. Haragh, Zs. Szinku (Kaposvár)

Aim: The effect of IL28B polymorphisms on the outcome of chronic HCV genotype 1 infection, as well as the association between IL28B genotypes and the Th1/Th2 cytokine production of activated peripheral blood monocytes and lymphocytes were investigated.

Materials and methods: Total of 748 chronic HCV genotype 1 positive (HCV1) patients (365 male, 383 female; 18–82 years) have been enrolled; 420 of them were treated with PEG-IFN pus ribavirin (P/R) for 24–72 weeks, and 195 patients (46.4%) achieved sustained virological response (SVR). For genotyping studies, DNA was isolated from peripheral blood by standard desalting method. IL28B rs12979860 SNP was determined using Custom Taqman SNP Genotyping Assays. For cytokine studies, in 40 HCV patients TNF-α, IFN-γ, IL-2, IL-4 and IL-6 production by LPS-stimulated monocytes and PMA-ionomycine activated lymphocytes was measured from the supernatant of the cells, using FACS-CBA Becton Dickinson test. The cytokine levels were compared in the patients with different (CC, CT, TT) IL28B genotypes.

Results: IL28B rs12979860 CC genotype occurred with lower frequency in HCV patients than in healthy controls (26.1% vs 51.4%, p<0.001). The patients carried T allele with higher frequency than controls (73.9%, vs 48.6%, p<0.001). P/R treated patients with the IL28B CC genotype achieved higher SVR rate, than those with CT (58.6% vs 40.8%, p=0.002), or who

carried T allele (41.8%, p=0.002). LPS-induced TLR-4 activation of monocytes resulted in higher TNF-a production in patients with IL28B CC genotype compared to non-CC individuals (p<0.01). Similarly, increased TNF-a, IL-2 and IFN-g production by lymphocytes was found in IL28B CC carriers (p<0.01)

Conclusion: IL28B CC genotype exerts protective effect against chronic HCV infection and may be a pretreatment predictor of SVR during P/R therapy. It is associated with increased Th1 cytokine production of activated peripheral blood monocytes and lymphocytes, which may play a role in IFN-induced rapid immune control and sustained virological response of P/R treated HCV1 patients.

INCREASED BASELINE PROINFLAMMATORY CYTOKINE PRODUCTION IN CHRONIC HEPATITIS C PATIENTS WITH RAPID VIROLOGICAL RESPONSE TO PEGINTERFERON PLUS RIBAVIRIN

Gabriella Pár[1], Laszlo Szereday[2, 6], Timea Berki[3], Laszlo Palinkas[3], Melinda Halasz[2, 6] Attila Miseta[14], Geza Hegedus[15], Julia Szekeres-Bartho[2], Aron Vincze[1], Bela Hunyady[1], Alajos Par[1]

[1]University of Pecs, First Department of Medicine, Clinical Centre,
[2]Department of Medical Microbiology and Immunology,
[3] of Immunology and Biotechnology,
[4]Department of Laboratory Medicine,
[5]Department of Pathology,
[6] Szentagothai Research Centre, Pecs, Hungary

Background: Chronic hepatitis C (CHC) patients achieving rapid virological response (RVR) on PEG-IFN/ribavirin (P/R) therapy have high chance of sustained virological response (SVR). To analyze host immunological factors associated with RVR, viral kinetics, phenotype distribution and Th1/Th2 cytokine production by peripheral blood mononuclear cells (PBMC) were studied prior to and during P/R therapy.

Methods: TNF-α, IFN-γ, IL-2, IL-6, IL-4 and IL-10 production by PBMC were measured after Toll-like receptor 4 (TLR-4) or phorbol myristate acetate /Ionomycin stimulation in 20 healthy controls and in 50 CHC patients before receiving and during P/R therapy. RVR was achieved by 14, complete early virological response (cEVR) by 19 patients and 17 patients were null-responders (NR).

Results: Patients with RVR showed an increased baseline TNF-α and IL-6 production by TLR-4 activated monocytes and increased IFN-γ decreased IL-4 and IL-10 production by lymphocytes compared to non-RVR patients. SVR was also associated with increased baseline TNF-α production and decreased IL-10 levels compared to patients who did not achieve SVR. Baseline IL-2 production was higher in cEVR compared to NR patients. Antiviral treatment increased TNF-α, IL-6 production by monocytes and IFN-γ secretion by lymphocytes and decreased IL-4 and IL-10 production by lymphocytes in cEVR compared to NR patients.

Conclusion: RVR was associated with increased baseline proinflammatory cytokine production by TLR-4 stimulated monocytes and by activated lymphocytes. In null-responders and in patients who did not achieve SVR both TLR-4 sensing function and proinflammatory cytokine production were impaired, suggesting that modulation of TLR activity and controlled induction of inflammatory cytokine production may provide further therapeutic strategy for CHC patients non-responding to P/R treatment.

EFFECT OF NATIVE AND OXIDATIVELY MODIFIED EXOGENOUS MITOCHONDRIAL DNA ON THE FUNCTIONS OF HUMAN PLASMACYTOID DENDRITIC CELLS

Kitti Linda Pázmándi[1], Viktória Sógor[1], István Boldogh[2], Éva Rajnavölgyi[1], Attila Bácsi[1]

[1]Department of Immunology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary;
[2]Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX, USA

Introduction: Plasmacytoid dendritic cells (pDCs) are a unique and rare cell population of the immune system. They are specialized for the recognition of nucleic acids of invading microbes by their selectively expressed endosomal nucleic acid-sensing Toll-like receptors (TLRs) such as TLR7 and TLR9. It has been recently demonstrated that extracellular mitochondrial DNA (mtDNA) released from injured or even living cells during inflammation can act as endogenous damage-associated molecular pattern (DAMP) molecule. Mitochondria are evolutionary endosymbionts derived from bacteria and so might carry bacterium-associated molecular motifs so we suppose that extracellular mtDNA is able to induce activation of pDCs.

Methods: mtDNA was extracted from non-treated and oxidative stress-exposed human cells. The levels of the 7,8-dihydro-8-oxoguanine (8-oxoG) in the purified mtDNA, which correlate with the oxidized state of the DNA, were measured by dot blot method. Phenotypic changes of pDCs after mtDNA treatments were monitored by flow cytometry and the cytokine and chemokine secretion of the cells was detected by ELISA.

Results: We found that treatment with mtDNA up-regulated the expression of several cell surface proteins (CD40, CD80, CD83, CD86, HLA-DQ) on pDCs and increased the type I interferon, TNF-α, and IL-8 secretion by the cells. These effects were more apparent when pDCs were treated with high 8-oxoG-containing mtDNA purified from oxidative-stress exposed cells, indicating that 8-oxoG enriched mtDNA sequences arisen under oxidative stress conditions can be more potent activators of the human pDCs than the native ones. In addition, pre-treatment of the cells with TLR9 antagonist

(ODN TTAGGG sequence), strongly diminished the ability of mtDNA to induce phenotypic and functional changes in pDCs, indicating that these activation processes were manly mediated through TLR9.

Conclusions: Collectively, our data suggest that the cell-free mtDNA enriched in the extracellular matrix or circulated in the blood-stream after cell injury or inflammation is fully capable of activating human pDCs via TLR9. Furthermore, the oxidatively modified mtDNA generated during the inflammatory reactions may have a greater potencial to initiate and maintain of the immune responses.

INVESTIGATION OF GLUCOCORTICOID INDUCED MITOCHONDRIAL APOPTOTIC PATHWAY IN MOUSE THYMOCYTES

Lilla Prenek, Ferenc Boldizsar, Tímea Berki

University of Pécs, Medical Center,
Department of Immunology and Biotechnology

Introduction: Glucocorticoids are essential in the development of T-cell in the thymus. Glucocorticoids play important roles in the selection and apoptosis of double-positive (DP) thymocytes. Classically, the ligand-bound glucocorticoid receptor (GR) regulating the gene expression after translocating into the nucleus. But next to these genomic effects, rapid non-genomic effects have emerged, for example the interaction of GR with cytoplasmatic proteins and the translocation of GR to the mitochondrium, which was described especially in apoptosis-sensitive cells. Mitochondrial translocation of activated GR in DP thymocytes has been demonstrated in our department. Based on these previous results we have researched the activation of the mitochondrial apoptotic pathway caused by the translocation of GR to the mitochondrium, and the association of GR with Bcl-2 family pro-apoptotic members.

Methods: Thymocytes were isolated from thymi of four-week-old mice, which were treated in vitro with 10-6M dexamethason (DX) for an hour. DX is a synthetic steroid compound which has only glucocorticoid-like effects. After treatment the cells were lysed, cytoplasmic and mitochondrial fractions were separated. The apoptotic proteins: cytochrome c, activated caspase 3,8,9,12 and Bax were analysed by Western-blot. The association of GR with Bim, and Bcl-x were investigated by coprecipitation experiments.

Results: Elevated cytochrome c and activated caspase 3,8,9 were detected in thymocytes after DX treatment. The level of activated caspase 12 did not change after treatment. Accumulation of Bax in the mitochondrium was found and association of GR with Bim and Bcl-x were also observed.

Conclusion: Our results have supported our hypothesis that in the glucocorticoid-induced apoptosis of thymocytes the mitochondrial pathway plays an important role, which was proved by activation of caspase 9. Probably the interaction between GR and Bim and the acculmulation of Bax in the mitochondrium play also part in it. Interestingly the activation of caspase 8 was also detected, probably caused by the different glucocorticoid-induced apoptotic mechanism of non-DP cells, which are presented in lower portion in the thymus.

GENERATION OF VASCULARIZED IN VITRO LUNG CONSTRUCT – A NOVEL THERAPEUTIC IMPLICATION OF ENGINEERED TISSUE CONSTRUCTS

Judit Rapp, Veronika Csöngei, Tamás Kovács, Mónika Avdicevic, Edit Kiss, Krisztian Kvell, Judit. E. Pongrácz

Department of Pharmaceutical Biotechnology,
Medical School, University of Pécs, Hungary

Introduction: Most of our knowledge about cell-cell interactions, signalling pathways or molecular biology is based on experiments by using monolayer cell cultures or animal models. To understand the more complex human body or set up a realistic model for pharmaceutical testing; 3D tissue cultures represent a promising technology. Tissue engineering has been a quickly developing field of biotechnological research, but the efficient induction of capillary vessel network in in vitro created tissue types remained a mystery. Irrespectively of the tissue type, the efficient blood supply is an essential criterion for making viable tissues as nutrients; oxygen, etc. reach the tissue through the blood vessel system. Without sufficient capillary network the implantation and survival of an engineered tissue is limited.

Aims: Our primary aim was to set up complex human three dimensional lung tissues that following implantation into a host body would be quickly and sufficiently vascularized for increased viability.

Materials and methods: Three dimensional (3D) lung tissue model was set up using human non-cancerous small airway epithelial cells (SAEC) and normal lung fibroblasts (NHLF). To investigate the process of vascularization of the implanted engineered tissue, the aggregates were implanted subcutaneously into the back or into ears of immunodeficient mice. Vascularization was monitored using non-invasive SPECT/CT, histology using hematoxylin-eosin (HE) and immunofluorescent staining to differentiate mouse and human-derived tissues in the implants.

Results: Three dimensional lung tissues were created using SAEC and NHLF cells. The implantations were performed successfully and increased perfusion was observed at the implantation site. HE staining confirmed the presence of the implanted tissue in the ears of test animals. Immunohystochemistry using specific antibodies identified mouse derived endothelial tissues in the 3D human tissue complexes.

Conclusion: In the presented work three dimensional tissue complexes were set up from cell types which are representing the two major cell types of the lung. We theorized that the third and extremely important cell type the endothelial cells are not needed during in vitro tissue engineering, as endothelial cells would grow into the engineered tissue once implanted into the host. Using mice as test animals, we succeeded showing that vascularization is possible in such circumstances. These findings suggest that the 3D complex can connect to the host vascular system after implantation. The successful vascularization leads us one step closer to creating an applicable tissue construct beyond a minimum size.

CR3 AND CR4 DIFFERENTLY MEDIATE THE ADHERENCE TO FIBRINOGEN OF HUMAN DENDRITIC CELLS

Noémi Sándor[1], Csilla Kriston[2], Anna Erdei[1, 2], Zsuzsa Bajtay[2]
[1]MTA-ELTE Research Group of Immunology,
[2]ELTE TTK, Institute of Biology, Department of Immunology

CR3 and CR4 are members of the family of β2 integrins, both expressed by human monocytes, neutrophil granulocytes (PMN), macrophages and dendritic cells. They consist of a common β-chain (CD18) and a unique α chain (CD11b in CR3 and CD11c in CR4). Their similarity is very high regarding their extracellular domain; 87% respectively. This is why ligand specificity of CR3 and CR4 is very much overlapping, and their main ligands – such as iC3b, fibrinogen, ICAM-1 – are the same. They differ however, in their intracellular domain, which suggests fundamental differences between the two receptors. So far however, very little is known about the function of CD11c.

We recently demonstrated that CD11b dominates iC3b mediated phagocytosis over CD11c, the latter having only a supportive role in this process. In our present work we analyzed the role of CD11b/CD18 and CD11c/CD18 in another important cellular function of β2 integrins, namely adherence to fibrinogen, a common ligand of both receptors. Fibrinogen is an acute phase protein present on inflamed endothelium as well as a component of the extracellular matrix. We studied the adhesion of primary human monocytes and monocyte-derived dendritic cells and macrophages to this protein in the presence or absence of blocking antibodies binding to CD11b or CD11c. Interestingly we found that in contrast to phagocytosis, adherence is mainly mediated through CD11c ligation. These results provide further evidence that CD11b/CD18 and CD11c/CD18 have different roles despite their structural similarites.

CANDIDA ALBICANS SECRETED ASPARTIC PROTEASE 2 CLEAVES HUMAN FACTOR H AND THE MACROPHAGE FACTOR H-RECEPTORS

Andrea E. Schneider[4], Mihály Józsi[1, 4], Eliška Svobodová[1], Peter Staib[2], Mariann Kremlitzka[3], Dagmar Barz[5]
[1]Junior Research Group for Cellular Immunobiology and
[2]Junior Research Group for Fundamental Molecular Biology of Pathogenic Fungi, Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany;
[3] of Immunology, Eötvös Loránd University, Budapest, Hungary;
[4]MTA-ELTE „Lendület" Complement Research Group at the Department of Immunology, Eötvös Loránd University, Budapest, Hungary;
[5]Institute for Transfusion Medicine, University Hospital of Jena, Germany

Introduction: The human-pathogenic yeast Candida albicans employs several mechanisms to escape from the human complement system. This protective armory includes the acquisition of host complement regulators, the release of molecules that scavenge complement proteins or block cellular receptors, and the secretion of proteases that inactivate complement components. Secreted aspartic protease 2 (Sap2) was previously shown to cleave C3b, C4b and C5. On the other hand, while C. albicans recruits the complement inhibitor factor H (FH), we previously showed that yeast-bound FH, by binding to complement receptor type 3 (CR3), can enhance the antifungal activity of human neutrophil granulocytes. The aim of this work was to assess the ability of C. albicans to inhibit this kind of host protection mechanism.

Materials and methods: Human monocyte-derived macrophages were assessed for their ability to bind FH using flow cytometry and Western blot. Cytokine release from macrophages upon coincubation with C. albicans was measured from culture supernatants using commercial ELISA kits. Sap2 expression was induced in yeasts and the supernatant was incubated with purified FH and with macrophages. Factor H cleavage was visualized by Western blot. Receptor expression was measured by flow cytometry.

Results: FH bound dose-dependently to human monocyte-derived macrophages. The binding was inhibited by antibodies against CD11b, CD11c and CD18, indicating that both CR3 (CD11b/CD18) and CR4 (CD11c/CD18) function as FH receptors on human macrophages. C. albicans yeasts preincubated with FH induced increased production of IL-1β and IL-6 in macrophages, compared to yeasts without FH. Similarly, FH enhanced zymosan-induced production of these cytokines. C. albicans Sap2 cleaved FH, which then lost its complement regulatory activity. Furthermore, Sap2 cleaved CR3 and CR4 on the surface of macrophages.

Conclusion: These data show that FH, when bound to C.

albicans, enhances the activation of human macrophages. However, the fungus can proteolytically degrade both FH and its receptors on macrophages by secreting Sap2. This mechanism represents an additional means to evade the host innate immune system.

ANALYSIS OF THE SPLEEN OF BFCRN TG MICE WITH AUGMENTED IMMUNE RESPONSE

Zita Schneider[1], Péter Balogh[2], Imre Kacskovics[1, 3]

[1]Department of Immunology, Eötvös Loránd University, Budapest, Hungary;
[2]Department of Immunology & Biotechnology, University of Pécs, Faculty of Medicine, Hungary;
[3]ImmunoGenes Kft, Budakeszi, Hungary

Introduction: The neonatal Fc receptor (FcRn) plays a key role in IgG and albumin homeostasis, and is involved in antigen presentation in case of antigen-IgG immune complexes. We have previously demonstrated that bovine FcRn (bFcRn) overexpression in transgenic (Tg) mice significantly augments the humoral immune response producing higher titer of antigen specific antibodies and increased number of antigen-specific B cells and hybridomas, offering a great advantage in polyclonal and monoclonal antibody production.

Methods: To gain further insight into the mechanisms of this enhanced humoral immune response we examined the spleen structure of untreated and immunized bFcRn Tg and wild type (wt) mice, and localized bFcRn positive cells with a newly developed bFcRn-specific monoclonal antibody.

Results: Thy-1, B220 and CR1/2 staining demonstrated normal localization of T and B-cell zones and follicular dendritic cells in the white pulp, both in untreated and immunized bFcRn Tg mice. Furthermore, MARCO and CD169 staining indicated a preserved distribution of marginal zone macrophages and marginal metallophilic macrophages in Tg mice. Germinal centers (GCs) formed in bFcRn Tg mice upon booster immunization with ovalbumin were twice as large as compared to wt animals, indicating improved recall response.

To determine the topographic relationship between bFcRn-expressing cells and GC formation, spleen sections were stained with our recently developed bFcRn-specific monoclonal antibody that does not cross-react with mouse FcRn. We detected strong bFcRn expression in the marginal zone macrophages and marginal metallophilic macrophages. In addition, other bFcRn-positive cells in the T-cell zone and red pulp were found, possibly corresponding to dendritic cells and red pulp macrophages.

Conclusions: The general lymphoid architecture of the spleen was unchanged in bFcRn Tg mice. The strong bFcRn expression of splenic macrophages that are essential for the formation of germinal centers and dendritic cells with highly effective immune complex presentation capacity probably contributes to the GC enlargement and augmented humoral immune response in bFcRn Tg mice.

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COMPLEMENT MASP-1 INDUCES ENDOTHELIAL CELLS TO ATTRACT AND BIND NEUTROPHIL GRANULOCYTES

Endre Schwaner[1], Péter K. Jani[1], Erika Kajdácsi[1], Márta L. Debreczeni[1], Márton Megyeri[2], József Dobó[2], Zoltán Doleschall[3], Krisztina Futosi[4], Csaba I. Timár[4], Attila Mócsai[4], Péter Gál[2], László Cervenak[1]

[1]Research Lab, 3rd Department of Medicine, Semmelweis University, Budapest, Hungary,
[2]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary,
[3]Department of Pathogenetics, National Institute of Oncology, Budapest, Hungary,
[4]Department of Physiology, Semmelweis University School of Medicine, Budapest, Hungary

Background and objectives: The complement system and neutrophil granulocytes (PMNs) are substantially important in immune response against bacteria and fungi. Endothelial cells, besides many other functions, can also participate in antimicrobial immunity through their cytokine production and homing regulation by adhesion molecules. We previously demonstrated that complement mannan-binding lectin associated serine protease 1 (MASP-1) is able to activate Ca-, NFκB and p38 MAPK signaling pathways in endothelial cells by cleaving protease activated receptors (PARs). Moreover, MASP-1 stimulated endothelial cells produce IL-6 and IL-8. However, the results of the downstream events have not been studied so far. Therefore, we aimed to assess if endothelial cells induced by MASP-1 have the capability to attract and bind PMNs.

Methods: We used human umbilical vein endothelial cells, freshly prepared PMNs and PLB-985 cell line (as a model for PMNs) for our experiments. ELISA, immunofluorescence and quantitative PCR were used to determine the level of cytokines and adhesion molecules, transwell and plate-based adhesion test were utilized to assess chemotaxis and adhesion.

Results: The unique cytokine pattern induced by MASP-1 may have an important role in the activation of PMNs, since we demonstrated that supernatant of MASP-1 treated endothelial cells triggered PMN chemotaxis. MASP-1 did not influence the expression of ICAM-1 and VCAM-1, whereas ICAM-2 was moderately down-regulated and E-Selectin expression was significantly increased. Furthermore, PLB-985 cells differentiated towards PMNs were able to adhere better to MASP-1 treated endothelial cells than to non-treated ones.

Conclusions: The expression of VCAM-1 is required for the transmigration of T cells and monocytes, while for PMNs E-selectin may be sufficient (in the presence of basal levels of ICAMs). MCP-1 and IL-8 are very potent chemotactic factors for monocytes and PMNs, respectively. The expression of E-selectin together with increased production of IL-6 and IL-8 suggests that MASP-1 stimulation of endothelial cells selectively favors the activation of neutrophils. Our findings suggest a novel connection between the two antibacterial/antifungal immune mediators – the complement system and neutrophil granulocytes.

IS THERE A RELATION BETWEEN THE NATURAL ANTIBODY NETWORK AND THE INFECTION RELATED ANTIBODY FORMATON IN CARDIAC SURGERY PATIENTS?

Diána Simon[1], Orsolya Gilicze[1, 2], László Lénárd[2], Sándor Szabados[2], Nelli Farkas[3], Zoltán Prohászka[4], Péter Németh[1], Tímea Berki[1]

[1]Department of Immunology and Biotechnology,
[2]Heart Institute,
[3]Institute of Bioanalysis, University of Pécs, Pécs,
[4]Research Laboratory, 3rd Department of Medicine, Semmelweis University, Budapest

Introduction: The number of certain infectious pathogens to which an individual has been exposed to (pathogen burden) has been linked to the development and prognosis of coronary artery disease. Natural antibody network is considered to have a role in pathogen specific antibody formation. High levels of autoantibodies against 60-kDa members of the heat shock protein family (HSP60) have been associated with atherosclerotic vascular diseases. Anti-citrate synthase antibodies, previously proven by our research group to belong to the pool of natural antibodies may also play a part.

Methods: Pericardial fluidand plasma samples of 36 cardiac surgery patients (12 AVR, 12 CABG with AMI and 12 CABG with no AMI in anamnesis) were tested for anti-CS and anti-HSP60 antibodies with previously developed in-house ELISA techniques, while antibodies against Chlamydia pneumoniae, Mycoplasma pneumoniae, Helicobacter pylori, Yersinia enterocolitica and Borreliaburgdorferi were measured with commercially available serological tests.

Results: Anti-HSP60 and anti-CS antibodies were present in pericardial fluid, at significantly lower amounts than in plasma with strong correlation between quantities. Anti-CS IgG antibodies were at highest amounts both in plasma and pericardial fluid. No significant differences were found in the levels of natural antibodies in the given disease groups. All patients' sera contained antibodies against at least one pathogen which couldn't be observed in every respective pericardial fluid sample. No significant associations between defined disease groups and pathogen specific antibodies were found. Pathogen burden significantly increased the amounts of anti-CS and anti-HSP60 antibodies.

Conclusions: According to our results, the impact of pathogen burden on amounts of natural antibodies cannot be explained by molecular mimicry, because the bacterial and human HSP60 proteinsequences show high similarities and Chlamydia and Mycoplasma pneumoniae have no citrate synthase. Since no significant differences were found in the given disease groups, it can be hypothesized that infection triggered inflammation and tissue damage can rather be the causative factors of the observed differences in the level of natural autoantibodies, than the extent of atherosclerosis or myocardial infarction.

THE USE OF A NEW IN VITRO LABORATORY TEST FOR THE DIAGNOSIS OF LATENT TUBERCULOSIS

Sándor Sipka[1], Zsuzsa Papp[2], Erika Lantos[3], Szilvia Bősze[4, 5], Kata Horvát[4, 5], Ildikó Kovács[1], Sándor Baráth[1], György Lustyik[3], Mária Szilasi[2], Ferenc Hudecz[4, 5]

[1]Regional Immunological Laboratory, Division of Clinical Immunology, Debrecen,
[2]Department of Pulmonology,
[3]Soft Flow Ltd, Pécs,
[4] Research Group, HAS, Budapest,
[5]Department of Organic Chemistry, Budapest

Indroduction: The need for laboratory tests to diagnose latent tucerculosis (tbc) has increased in the present age of biological therapies. The serological QuantiFERON-TB Gold (USA) and the traditional Mantoux dermal tests are used now for this purpose. Both tests are based upon the release of interferon gamma induced by tbc peptides.

Aim: To study the diagnostic value of a new in vitro test for latent tbc based upon the release of tumor necrosis α (TNFα) induced by a suspension of 10 peptides specific for pathogenic tbc bacteria In parallel, this test was compared to the values of QuantiFERON (QF) and Mantoux (M) tests.

Patients: 15 health care workers dealing with patients with tbc; 7 pateints with active treated tbc; 13 healthy controls.

Methods: a) in the culture supernatants IL-1β, IL-6, IL-10, ***TNF***α ELISA. b) cell culturing: 1 ml heparinized blood, activators: LPS, PPD, tbc peptides, 37 ºC, 5% CO_2 20 hours. Positive result: tbc peptid induced TNFα pg/ml > 1.5x PPD TNFα pg/ml.

Results: The values of coincidence were found as follows:

„TNFα – QuantiFeron": health care workers: 76.88; active patients: 85.7%,

„TNFα – Mantoux": health care workers: 66.66; active patients: 71.4%

QuantiFERON – Mantoux: health care workers: 88.46; active patients: 85.7%

Conclusions: (1) 6 of the 15 heath care workers could be

regarded to have latent tbc. (2) 4 of the 7 active treated tbc patients were positive by the TNFα test. (3) The TNFα measurements show a greater sensitivity to demonstrate a increased risk of active disease both in patients with latent and active tbc than the QuantiFERON and Mantoux tests.

APPLICATION OF MONOCYTES ON ANTIGEN MICROARRAYS - DETECTING INFLAMMATORY ACTIVATION

Zoltán Szittner[1], Krisztián Papp[2, 3], Noémi Sándor[2], Zsuzsa Bajtay[1] and József Prechl[2, 3]

[1]Department of Immunology, Eötvös Loránd University;
[2] Research Group of the Hungarian Academy of Sciences at Eötvös Loránd University, H-1117, Pázmány Péter s. 1/C, Budapest, Hungary;
[3]Diagnosticum Ltd., H-1047, Attila u. 126, Budapest, Hungary

Background: In our earlier studies we demonstrated the applicability of monocytoid cell line U937 to detect antigen bound IgG. We found that this adhesion of the cells is mainly determined by the Fcγ receptor – IgG interactions. This interaction depends on the affinity of Fcγ receptors towards the IgG subclasses that are sensitve to glycolysation as well. Therefore detection of antibodies with monocytes is possibly a useful tool to simply determine effector functions of an antibody from the cells' point of view. Activating Fcγ receptors after binding their ligands are known to activate the Nf-κB pathway, an inflammatory pathway. In our present work we investigated how this inflammatory activation of the cells could be detected.

Methods: To investigate the inflammatory activation of the cells we transfected U937 monocytoid cells with plasmids coding EGFP under the regulation of Nf-κB responsive elements and an other plasmid coding iRFP, a fluorescent protein. Following the cloning of the cells we characterized the cells Nf-κB response to various stimuli.

Results: First we investigated the activating properties of LPS towards these cells and determined the kinetics and the dose dependence of the activation and found the mean fluorescence intensity of these cells to be close to the peak after 8 hours of incubation in the 0,1 -– 10 μg/ml LPS range. We compared the activating properties of IgG subclasses in solution and in coat as well. In summary we found that while IgG 1,3 and 4 in coat activates the Nf-κB pathway, in solution none of them did just like IgG2 which we found to be less significant in activation of the cells both in solution and in coat. We also found that this activation is blockable by masking Fc parts of IgG molecules.

Conclusion: In agreement with our previous results we demonstrated how U937 cells through their Fcγ receptors differentiate between IgG subclasses bound to a solid surface and that this activation does not only result in the adhesion of the cells but in the activation of their Nf-κB pathway as well.

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REZISZTENS JIMT-1 EMLŐTUMOR XENOGRAFTOK CÉLZOTT TÁMADÁSA ERBB2 ANTIGÉNT FELISMERNI KÉPES KIMÉRA RECEPTORRAL ÚJRAPROGRAMOZOTT CITOLITIKUS T-LIMFOCITÁKKAL SCID EGEREKBEN

Szöőr Árpád[1], Simon László[1], Liebrecht Valerie[2], Holzinger[2], Szöllősi János[1], Abken Hinrich[2], Vereb György[1]

[1]Debreceni Egyetem OEC Biofizikai és Sejtbiológiai Intézet,
[2]Uniklinik Köln, Labor für Tumorgenetik und Immunologie

Célkitűzések: Munkacsoportunk korábbi eredményeiben bemutatta, hogy a JIMT-1 ErbB2 pozitív emlőtumor sejtvonal trastuzumab és egyéb ErbB2 antigént célzó humanizált antitest terápiákkal szemben fennálló rezisztenciája a tumorok méretén túl azok jól szervezett extracelluláris mátrixának sztérikus ellenállásán is alapul. Jelen pilot studynak tekinthető kísérletünkben arra kerestük a választ, hogy olyan nagy kiterjedésű daganatok esetén, ahol az antitestek passzív diffúzióját az extracelluláris mátrix korlátozza, vajon az aktív mozgásra képes és az ErbB2 antigén ugyanazon epitópját felismerő kiméra antigén receptorral (CAR) transzdukált citolitikus T-limfociták hatékony tumorellenes hatást érnek-e el.

Módszerek: Kísérleteinkben JIMT-1 xenograft modellt használtunk SCID egerekben (állatonként négy oltás). Amikor az átlagos tumorméret meghaladta a kritikus 250 mm^3-t, az egerek egy csoportja heti egyszeri intraperitoneális (IP) trastuzumab kezelést, a másik pedig anti-ErbB2 CAR transzdukált egér T-sejt készítményt kapott egy oltásban (3-3 egér csoportonként). Mivel az IP adott trastuzumab hatása jól ismert ezen a modellrendszeren, ezért jelen munkánkban a terápiás különbözőségeket csak a trastuzumab és az anti-ErbB2 CAR T-sejtes kezelés között vizsgáltuk a felhasznált kísérleti állatok számának ésszerű csökkentésével. Az átprogramozott T-limfociták specifikus aktivációját (IFN-gamma termelés) és citotoxikus hatását (XTT esszé) a sejtkészítmény beadását megelőzően in vitro teszteltük. A tumorok növekedését heti kétszer mértük. Az állatokat a kísérlet 66. napján CO2- belélegeztetéssel elaltattuk, majd felboncoltuk. A vérmintákat a szív közvetlen megszúrásával nyertük.

Eredmények: Korábbi feltételezéseinket megerősítve 250 mm^3-es tumorméret felett a trastuzumab kezelésnek nem volt hatása a JIMT-1 xenograftok növekedésére, ugyanakkor a kiméra antigen receptorral transzdukált T-sejtek mar-

káns antitumor hatást mutattak. Mind a xenograftok száma, mind azok mérete jelentősen csökkent. A három, CAR T-limfocita kezelést kapó egér tizenegy darab tumora közül hét teljesen eltűnt, négynek pedig folyamatosan csökkent a mérete a kísérlet befejezéséig. Ebben a csoportban a véráramban sem lehetett keringő tumorsejteket kimutatni. A kezelés mellékhatása vészes súlyvesztés volt.

Konklúzió: Az aktívan mozgó, specifikus tumorantigént felismerni képes T-limfociták képesek elpusztítani azokat a daganatsejteket, amelyeket a jól szervezett ectracelluláris mátrix véd a passzívan diffundáló antitestektől.

STUDIES ON THE EFFECT OF VARIOUS *PROPIONIBACTERIUM ACNES* STRAINS ON THE CELLULAR FUNCTIONS OF HPV-KER CELLS

Gábor Tax[1], Lilla Erdei[1], Beáta Szilvia Bolla[1], Edit Urbán[3], Lajos Kemény[1, 2], Kornélia Szabó[2]
[1]Department of Dermatology and Allergology, University of Szeged, Hungary,
[2]MTA-SZTE Dermatological Research Group, Szeged, Hungary,
[3]Institute of Clinical Microbiology, University of Szeged, Hungary

Introduction: Acne vulgaris is the most common multifactorial inflammatory skin disease of the pilosebaceous unit. The Propionibacterium acnes (P. acnes) bacterium has been shown to affect the cellular properties of skin cells, and plays a role in acne lesion formation, even though it is also a common member of the skin's commensal bacterial flora.

Methods: In order to investigate this phenomenon in more detail, we monitored the effect of three P. acnes strains (889, 6609, ATCC 11828) belonging to various phylogenetic groups within the species applied in different doses (multiplicity of infection, MOI= 25, 50, 100, 200, 300) on the proliferation and viability of HPV-KER cells using cell biological and molecular methods.

Results: First we monitored the cellular changes using a real-time impedance measurement-based technology. We found that only the P. acnes 889 strain applied in high doses induced increased cell index (CI) values compared to the untreated control cells at 24–36 hours post-treatment. At later time-points (36-72 h post-treatment), however, the CIs showed a rapid decrease in the P. acnes 889 and ATCC 11828 treated cells, when applied in high doses (MOI=200, 300). The P. acnes 6609 strain had no measurable effect in any applied conditions during the time course of the experiment. The observed changes were the result of the differential effect of various P. acnes strains on the proliferation and viability of HPV-KER cells, proved the cell number and morphological changes using Bürker-chamber and fluorescent microscopic analysis.

We also started to analyze the strain and dose specific signaling differences induced in HPV-KER cells using a real-time RT-PCR method. We found that the mRNA expression of key pro-inflammatory cytokines (TNFα, IL-1α) increased parallel to the elevating P. acnes doses at 6 hours after the bacterial treatment. This appeared to be the result of the dose dependent increase we detected in the nuclear translocation and the parallel activation of the NF-κB transcription factor, shown by Western blotting.

Conclusions: These results suggest that assorted P. acnes strains have different effects on the proliferation and viability of keratinocytes. These strain specific differences can be important in the determination of the severity of individual acne symptoms.

EFFECT OF RAGWEED POLLEN EXTRACT ON THE IL-1β EXPRESSION OF MACROPHAGES AND DENDRITIC CELLS

Aliz Varga[1], Marietta Margit Budai[1], László Csernoch[1], József Tőzsér[2], Szilvia Benkő[1]
University of Debrecen, Medical and Health Science Center,
[1]Department of Physiology,
[2]Department of Biochemistry and Molecular Biology

Introduction: Innate immunity has important role in the recognition of pathogen-derived molecular patterns and altered self-motifs. These patterns can be recognized by pattern recognition receptors, like intracellular Nod-like receptors.

Some members of NLR family can form protein complexes, which are called inflammasomes. NLRP3 inflammasome contains NLRP3 sensor, ASC adaptor and effector caspase-1 enzyme. Upon activation of inflammasome, caspase-1 cleaves inactive pro-IL-1β into active, pro-inflammatory IL-1β. NLRP3 inflammasome activation requires two signals. The first signal is necessary for the expression of inflammasome components, the second is important for the protein complex assembly.

Main sources of IL-1β are macrophages and dendritic cells. It is known, that macrophage and dendritic cell-produced IL-1β has important role in the development of pollen-induced allergic rhinitis symptoms. Enhanced presence of IL-1β has been demonstrated in patients suffering from allergic rhinitis, but it is unclear whether NLRP3 inflammasome is involved in this process in macrophages and dendritic cells. Therefore we aimed to study the effect of ragweed pollen extract on IL-1β expression in human monocyte-derived macrophages, dendritic cells, and in THP-1 macrophage cell line.

Pollens are often contaminated with bacterial motifs, like lypopolysaccharide (LPS). It is known, that LPS can trigger the first signal of NLRP3 inflammasome activation, therefore we studied the effect of ragweed pollen in combination with LPS as well.

Methods: Monocytes were separated from human „buffy

coat", then differentiated into macrophages and dendritic cells. Cells were treated with ragweed pollen extract and LPS, and the IL-1β secretion was determined by ELISA, pro-IL-1β and NLRP3 gene expression changes were studied by quantitative RT-PCR.

Results: Our results show that ragweed pollen extract alone is not able to induce IL-1β and NLRP3 expression in THP-1 macrophages and dendritic cells, but in GM-CSF-macrophages we detected moderate enhances in the IL-1β mRNA and cytokine expression. We found that ragweed pollen extract enhanced LPS-induced IL-1β secretion in human macrophages and dendritic cells. We also demonstrated, that LPS-induced mRNA expression of pro-IL-1β and NLRP3 can be further enhanced with ragweed pollen extract in macrophages and dendritic cells as well.

Summary: Ragweed pollen extract enhances LPS-induced IL-1β secretion and NLRP3 expression in human macrophages and dendritic cells.

CELL-DIFFERENTIATION IN THE HEMATOPOIETIC COMPARTMENTS OF *DROSOPHILA MELANOGASTER*

B. I. Gergely Varga[1], Viktor Honti[1], Gábor Csordás[1], Ferenc Jankovics[2], Róbert Márkus[1], Éva Kurucz[1] and István Andó[1]

[1]Hungarian Academy of Sciences, Biological Research Centre Institute of Genetics, Immunulogy Unit, Szeged,
[2]Hungarian Academy of Sciences, Biological Research Centre Institute of Genetics, Developmental Genetics Unit, Szeged

Similarly to mammalian blood cells, the hemocytes of the fruit fly (Drosophila melanogaster) differentiate in several waves and are located in separate compartments. These compartments – the sessile tissue, the circulation and the lymph gland – give rise to three classes of effector cell types, the phagocytic plasmatocytes, the melanizing crystal cells and the encapsulating lamellocytes. The differentiation of these cells is under the control of phylogenetically conserved epigenetic and transcription factors. In our previous cell lineage tracing studies, we observed that although the three larval hematopoietic compartments arise from different embryonic mesodermal anlagen, they all take part in the formation of the effector cells. In addition, the phagocytic plasmatocytes show a high level of plasticity. Upon immune induction, plasmatocytes leave the sessile tissue, lose phagocytic capacity and transform into lamellocytes. Since our knowledge on the molecular mechanisms of the cellular immune reactions in Drosophila is fragmentary, we set out to study the possible involvement of the genes which could be instrumental in the control of hemocyte development as well as in the formation and regulation of the integrity of the sessile tissue. The selected genes were overexpressed and silenced in transgenic strains and a combination of immunological and in vivo genetic markers were used to follow blood cell differentiation and the organization of the sessile tissue. We found that two factors – Headcase and Eater – are involved in the regulation, the development and maintenance of the structure of the sessile tissue, without affecting lamellocyte differentiation. To study the expression pattern of hdc, we generated hdc-Gal4 driver lines with gene conversion. We found that the headcase gene is expressed in the lymph gland, but the hemocytes leaving the organ and transforming into effector cells lose hdc expression. The silencing of hdc by RNA interference resulted in strong reduction of the number of mature plasmatocytes in all hematopoietic compartments, although hdc is not expressed by either the circulating, or the sessile hemocytes. Eater is the main phagocytosis receptor of Drosophila. Interestingly, the silencing of eater results in the mobilization of the sessile islets without the differentiation of lamellocytes. This observation indicates that Eater is necessary for maintaining the integrity of the sessile tissue, and, contrary to previous hypotheses, the disintegration of this tissue by itself is not sufficient for lamellocyte differentiation. Our findings suggest the existence of cell autonomous and non-autonomous regulatory networks that control in concert the development of the hematopoietic compartments and the differentiation of hemocytes.

THE ROLE OF C1Q AND ANTI-C1Q AUTOANTIBODY IN HEREDITARY ANGIOEDEMA CAUSED BY C1-INHIBITOR DEFICIENCY

Nóra Veszeli, Dorottya Csuka, Zsuzsanna Zotter, Viktória Kinga Kőhalmi, Henriette Farkas, Lilian Varga

Semmelweis University, 3rd Department of Internal Medicine, Budapest, Hungary

Introduction: Hereditary angioedema resulting from the deficiency of the C1-inhibitor (HAE-C1-INH) is an autosomal dominant disorder. It is characterized by episodic recurrences of bradykinin-mediated edema formation in the subcutis and/or the submucosa. C1q determination has differential diagnostic value in distinguishing between the hereditary and the acquired forms of C1-INH deficiency. Although the C1q levels of the HAE-C1-INH patients are usually within the normal range, and the presence of anti-C1q autoantibodies is not characteristic either, both were abnormal in 13% of our cases. Our objective was to investigate C1q and anti-C1q levels in HAE-C1-INH.

Methods: The study population comprised 141 patients with HAE-C1-INH (80 females and 61 males, mean age: 37 years (min: 3, max: 82), 130 with HAE type I, and 11 with HAE type II. We measured C1q, anti-C1q, C4, functional and antigenic C1-INH levels and then, recorded the number and location of edematous episodes, as well as monitored drug therapy over the subsequent year.

Results: C1q correlated with C4 and functional C1-INH levels, as well as – in patients with HAE type 1 – with antigenic C1-INH level (r=0.2154, p=0.0106; r=0.1966, p=0.0195; and r=0.2413, p=0.0079). Anti-C1q unrelated to all the other complement parameters in all patients. Although C1q was not related to the number of edematous episodes either, anti-C1q level showed a positive relationship with the number of laryngeal edema attacks (r=0.2463, p=0.0040). Analyzing female and male patients separately, we found that anti-C1q correlated with total attack number (r=0.2783, p=0.0143), as well as with the number of submucosal (laryngeal and abdominal) episodes (r=0.2834, p=0.0125, and r=0.2317 p=0.0426) in women only. The comparison of patients treated/not treated with danazol revealed a relationship between anti-C1q level and facial edema in danazol-treated patients, as well as a correlation between the former and laryngeal edema patients not receiving danazol (r=-0.3708, p=0.0132; r=0.2232, p=0.0377).

Discussion: As indicated, in particular, by the clinical relationships found in females and in patients with more severe disease (requiring treatment with danazol), the presence of anti-C1q autoantibodies might influence the clinical situation or might be a clinical marker even if their level is normal. The lack of a similar correlation with C1q suggests immunoregulatory relationships in the pathomechanism of HAE-C-INH, rather than activation triggering the classical pathway.

IN VITRO DOWNREGULATION OF THE INCREASED TRAF6 EXPRESSION IN THE MONONUCLEAR CELLS OF PATIENTS WITH SJÖGREN'S SYNDROME BY AN EBV-EBER-SPECIFIC SYNTHETIC SINGLE STRANDED COMPLEMENTER DNA MOLECULE

Erika Zilahi[1], Sándor Sipka[1], Gábor Papp[1], Gábor Nagy[1], József Kónya[2], Katalin Hegyi[3], Margit Zeher[1]
[1]Division of Clinical Immunology, Department of Internal Medicine, University of Debrecen, Debrecen, Hungary 4032,
[2]Department of Microbiology, University of Debrecen, Debrecen, Hungary 4032,
[3]Department of Pathology, University of Debrecen, Debrecen, Hungary 4032

Introduction: We described earlier that the increased expression of miRNA-146 a/b is surprisingly accompanied by an increase in TRAF6 and a decrease in IRAK 1 gene expressions in the mononuclear cells of patients with Sjögren's syndrome.

Methods: Peripheral mononuclear cells extracted from Sjögren's syndrome patients and healthy controls were treated using poly I:C, EBV-EBER specific DNA, and poly adenylic acid for 24 hours in vitro. Then we measured the expression of TRAF6 gene in the peripheral mononuclear cells of patients with Sjögren's syndrome and healthy controls by quantitative reverse transcription polymerase chain reaction.

Results: In the current study, we present that a.) the increased TRAF6 expression remains almost unchanged tested after two years, b.) the expression levels miR146a and TRAF6 represent a significant negative correlation to each other, c.) however, neither of them shows any association with the values of immunological laboratory parameters, d.) the in vitro use of an EBV-EBER specific synthetic single stranded complementer DNA molecule can result in significant reductions in the expression of TRAF6 in the cells of patients, but not in the healthy controls, whereas the treatments with poly I:C and poly adenylic acid are not able to reduce the TRAF6 overexpression. e.) EBV-EBER specific DNA slightly stimulates the release of interferon α (IFNα) in the cells of Sjögren's syndrome patients..

Conclusion: These data support the conclusion that the decreasing effect of EBV-EBER specific DNA on the TRAF6 expression may be mediated by a pathway different from the Toll-like receptors in the mononuclear cells of Sjögren's syndrome. However, the possibility of the involvement of some Sjögren's specific EBV-EBER related effects in the increased TRAF6 expression what miR146a is unable to suppress totally, cannot be excluded.

ROLE OF DROSOPHILA NIMROD CLUSTER IN THE INNATE IMMUNITY

János Zsámboki, Gyöngyi Cinege, Gábor Csordás, Viktor Honti, István Andó, Éva Kurucz
Institute of Genetics, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

The Nimrod gene cluster is located on the second chromosome of Drosophila melanogaster and it is composed of the nimrod A, B, C, the vajk, the ance (angiotensin converting enzyme) and cenG (centaurin gamma) gene families. Similar gene composition across distantly related arthropod species indicates that the Nimrod gene cluster is a fundamental component of the innate immune response, since it remained intact since 300–350 million years.

Previously we identified NimC1 as a Drosophila blood cell specific transmembrane protein, which is involved in the phagocytosis of microorganisms. Nimrod proteins encoded by the nimA, nimB1-5 and nimC1-4 genes contain a signal peptide, characteristic NIM domains and a short conserved CCxGY motif, immediately preceding the first NIM domain. In order to study the bacterium binding properties of Nimrod proteins we have developed an immunofluorescence and flow cytometry based analysis and found that native NimC1 expressed by Drosophila phagocytic cells binds Escherichia coli, but does not bind Staphylococcus epidermidis. We produced several FLAG-tagged recombinant Nimrod proteins and analyzed them using this assay. We found, that NimA,

NimB1, NimB2 and NimC1 bind E. coli bacteria, but only NimB1 binds S. epidermidis. We also observed that neither lipopolysaccahide, nor peptidoglycan, but a protein molecule serves as ligand for the NimC1 receptor.

We further analyzed the Nimrod gene cluster by studying the vajk genes and noticed that they encode proteins having similar sequence properties; they contain N-terminal signal peptide, low complexity regions and at least 20% valine amino acids. To analyze the expression of the vajk1–4 genes in different developmental stages of the fruit fly we used RT-PCR. We found that all vajk transcripts are present in the embryo, only vajk-2 and vajk-3 are expressed during larval stages, and none of the vajk genes were transcribed in adults, therefore we also examined the vajk-1 and vajk-4 gene expression upon immune induction provoked by parasitoid wasp infection, wounding and/or bacterial infection. To look into the expression of the Vajk proteins in the body, we developed antibodies against recombinant Vajk proteins. We test the function of vajk genes after silencing them with RNA interference constructs.