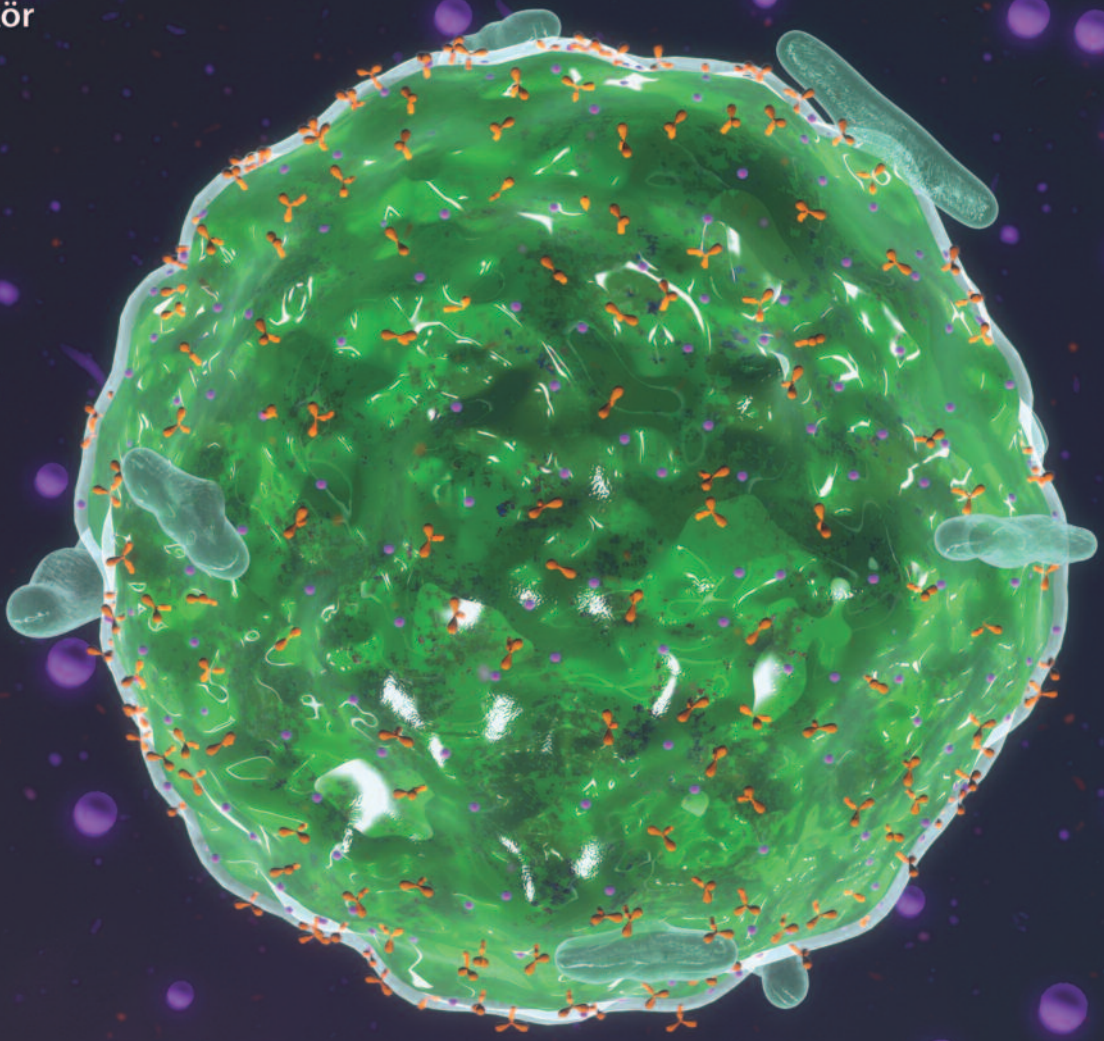
X. ÉVFOLYAM 3. SZÁM 2018. OKTÓBER

IMMUNOLÓGIAI SZEMLE

NEGYEDÉVENTE MEGJELENŐ ORVOSTUDOMÁNYI FOLYÓIRAT

IMMUNOLOGY QUARTERLY

ALAPÍTVA 2009-BEN | MEDICINA KÖNYVKIADÓ | WWW.MEDICINA-KIADO.HU



ISSN 2061–0203

Egy más jövő lehetősége SPA-ban, HUMIRA-val

HS = Hidradenitis Suppurativa, Ps = Psoriasis, pPs =Gyermekkori Psoriasis, JIA = Juvenilis Idiopathias Arthritis, ERA = Enthesitis Asszociált Arthritis, nr-AxSpA = Non röntgen Axialis Spondyloarthritis, SPA = Spondylitis Ankylopoetica, RA = Rheumatoid Arthritis, AP = Arthritis Psoriatica, UV = nem infekciózus non-anterior uveitis, pUV = Gyermekkori non-infekciózus anterior uveitis, pCD = Gyermekkori Crohn-betegség, CD = Crohn-betegség, UC = Colitis Ulcerosa

Humira 40 mg oldatos injekció előretöltött fecskendőben (adalimumab), Humira 40 mg oldatos injekció előretöltött injekciós tollban (adalimumab), Humira 40 mg/0,8 ml oldatos injekció gyermekgyógyászati alkalmazásra (adalimumab), Humira 80 mg oldatos injekció előretöltött fecskendőben (adalimumab), Humira 80 mg oldatos injekció előretöltött injekciós tollban (adalimumab), Humira 20 mg oldatos injekció előretöltött fecskendőben (adalimumab)

http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000481/human_med_000822.jsp&mid=WC0b01ac058001d124

Bővebb információért olvassa el a gyógyszer alkalmazási előírását! Forgalomba hozatali engedély jogosultjának helyi képviselete: AbbVie Kft. 1095 Budapest, Lechner Ödön fasor 7. Telefonszám: +36 1 455 8600. **A közfinanszírozás alapjául elfogadott ár:** 275 090 Ft. Támogatás összege: 0 Ft. Térítési díj: 275 090 Ft. Forrás: www.neak. gov.hu. Letöltés: 2017.03.20. Az aktuális árak megtalálhatók a www.neak.gov.hu oldalon. A Humira 2012. február 1-től tételes finanszírozás keretében érhető el (OENO kód: 06052) Finanszírozott indikációs kör: rheumatoid arthritis, spondylitis ankylopoetica, arthritis psoriatica, juvenilis idiopathiás arthritis; Crohn-betegség felnőtt- és gyermekkori, colitis ulcerosa, psoriasis. A 9/1993. (IV. 2.) NM rendelet alapján.

Referencia:

1: Van der Heijde és mtsai, Arthritis Rheum 2006; 54(7):2136-2146.
2. Sieper J és mtsai, Ann Rheum Dis 2012; 71(5): 700-706
3: Humira alkalmazási előírás http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000481/human_med_000822.jsp&mid=WC0b01ac058001d124

Kód: HUSPA180064 • Lezárás dátuma: 2018.04.26.

HUMIRA® adalimumab
destination you.™

abbvie

AbbVie Kft. · 1095 Budapest Lechner Ödön fasor 7. · Tel: +36 1 455 8600 · Fax: +36 1 455 8699

X. ÉVFOLYAM • 3. SZÁM • 2018. OKTÓBER

IMMUNOLÓGIAI SZEMLE

NEGYEDÉVENTE MEGJELENŐ ORVOSTUDOMÁNYI FOLYÓIRAT

IMMUNOLOGY QUARTERLY

ALAPÍTVA 2009-BEN • KIADJA A MEDICINA KÖNYVKIADÓ ZRT. • WWW.MEDICINAKIADO.HU

A Magyar Immunológiai Társaság hivatalos lapja
Szerkesztőség/kiadó: Medicina Könyvkiadó Zrt.
1072 Budapest, Rákóczi út 16.
Postacím: 1245 Budapest 5, Pf. 1012
Telefon: (1) 331 0781, (1) 312 2650; fax: (1) 312 2450;
e-mail: medkiad@euroweb.hu

ISSN 2061-0203

Egyéni előfizetési díj egy évre bruttó 2800 Ft.
Előfizetéssel kapcsolatos információt kereskedelmi osztályunk munkatársai adnak: **Durán Ágnes, Lengyelné Barta Anetta**
e-mail: kerosztály@medicinazrt.hu

A kiadásért felel: **Farkasvölgyi Frigyesné** vezérigazgató
Borítóterv, tipográfia: **Bede Tamásné**
Nyomdai előkészítés: **Gar–Wind Bt.**

Nyomdai munkálatok: Pauker Nyomdaipari Kft., Budapest
Felelős vezető: **Vértes Gábor** ügyvezető igazgató

Tartalom

Tisztelt Kolléganők és Kollégák!

Az Immunológiai Szemle szerkesztősége nevében köszöntöm a Magyar Immunológiai Társaság 47. kongresszusának résztvevőit. Szokás szerint e lapszámot döntően a konferencia előadás- és poszterkivonatai teszik ki. Örömteli, hogy ennyire megnövekedett terjedelemmel „kényszerülünk" megjelentetni az idei 3. számot, ami a kongresszus iránti nagy, aktív érdeklődést jelzi. Emellett Szamosi Szilvia és munkatársai (Debrecen) áttekintik az interleukin-6-receptor-gátló tocilizumabbal kapcsolatos legfontosabb adatokat. Nemcsak a gyógyszermolekula lényeges, hanem az a tény, hogy a célzott terápia során mennyi új mechanizmus felderítésére nyílik mód. Ugyancsak érdekes lehet a MIT kongresszus szempontjából is Gergely Péter professzor (Budapest) kritikus közleménye az „immunerősítőkről". Számos ilyen készítményt hirdetnek, a cikk segít eligazodni és helyükre tenni ezeket.

Érezzék jól magukat a konferencián, de akik nem tudnak személyesen részt venni, azoknak is jó böngészést kívánok!

Szekanecz Zoltán
főszerkesztő

Immunológiai Szemle/Immunology Quarterly • 2018. X. évfolyam, 3. szám

Welcome to the 47th Annual Meeting of the Hungarian Society for Immunology!

The Hungarian Society of Immunology (MIT) welcomes all the participants – speakers, sponsors and researchers from all over Hungary to the 47th Annual Meeting of the Society, between October 17-19 in Bük. We are very much honored to invite you all to exchange and share your views and experience on basic and clinical immunology.

This year's meeting is arranged by the topic 'Extracellular vesicles and Immunology' and tumor immunology. Research on extracellular vesicles is one of the most dynamically developing, exciting new science areas of the last decade. The number of research teams involved in extracellular vesicle research also grows continuously in Hungary, and speakers from these groups will allow a wide-ranging insight into the results achieved also in the border area of immunology and extracellular vesicle research. The intention of the program organizers with a series of focused lectures was to draw the attention of our immunologist community to this new science field that is inherently linked to immunological research. In this regard MIT is privileged to have Professor Francisco Sánchez Madrid, this year's recipient of EFIS-IL award, as keynote speaker. Professor Sánchez Madrid's research has focused on the exosome-mediated communication between dendritic cells and T cells, allowing seminal observations to be made, thus promoting our understanding on immune synapse formation and initiation of anti-pathogen immune responses. We are delighted to look forward to his presentation entitled "Immune cell-cell communication: transfer of genetic information through the immunological synapse".

In addition to identifying novel communication mechanisms and events contributing to normal immune responses, the involvement of immune system in several metabolic conditions is also widely acknowledged. An important field is the vascular damages occurring in atherosclerosis, where both innate and adaptive immune participants, including macrophages, complement and immunoglobulins, combined with biochemical pathways leading to lipid oxidation, have important pathological roles. This topic will be addressed by our special guest for this year's MIT conference, Christoph J. Binder, MD, PhD, Professor of Atherosclerosis Research from the Department of Laboratory Medicine, at the Medical University of Vienna, in his talk entitled "Role and function of microvesicles carrying oxidation-specific epitopes".

The other topic in spotlight is the intertwining relationship between chronic inflammation, autoimmunity and malignancies. This topic will be extensively covered from various angles, including gene expression alterations of mucosal tissue regeneration in inflammatory bowel diseases, effector lymphocyte functions and the experimental as well as therapeutic modulation of their interactions, possible analytical and diagnostic expansion approaches. This theme will be concluded with two in-depth updates on the yin-yang of rheumatologic or oncological consequences of checkpoint-targeted immunotherapies.

During the conference – aside from further invited and sponsored lectures – the program is organized into oral and poster presentations, with enough time allocated for discussion of the new results and idea. The number of original abstracts submitted to the Annual Meetings of our Society is steadily at high level (87-92-80-79-85-98 between 2013 and 2018), thus marking the strength of this field in Hungary. The meeting is prepared to provide a stimulating forum for sharing experience, exchanging ideas, and establishing fruitful collaborations. We wish you an stimulating Conference experience and an unforgettable stay in Bük. Your presence and presentation will be the key to the success of our Meeting.

Zoltán Prohászka, President
Péter Balogh, Secretary General
Hungarian Society for Immunology

Edit Buzás
President of the Scientific Committee of the 47th Annual Meeting of the Hungarian Society for Immunology

A MAGYAR IMMUNOLÓGIAI TÁRSASÁG 47. VÁNDORGYŰLÉSÉNEK ÖSSZEFOGLALÓI

ORAL PRESENTATIONS

ORAL TOLERANCE AND REGULATORY T-CELL FUNCTION ARE IMPAIRED IN MADCAM-1–/– MICE BUT NOT IN NKX2.3–/– MICE

Tareq Abu-Dakah[1], Zoltán Kellermayer[1], Angela Schippers[2], Norbert Wagner[2], Péter Balogh[1]
[1]Department of Immunology and Biotechnology, University of Pécs
[2]University Hospital RWTH Aachen

Introduction: Endothelial MAdCAM-1 is the main addressin recruiting regulatory T cells (Tregs) to the mucosa. As an important transcriptional regulator, Nkx2.3 promotes MAdCAM-1 expression, its absence leads to the postnatal loss of MAdCAM-1. Tregs are important in the development of oral tolerance and their impaired function has been shown to play a role in the pathogenesis of inflammatory bowel diseases, where Nkx2-3 may also be involved. In our work we examined how the absence of MAdCAM-1 affects Treg homing and function in murine models for oral tolerance and colitis.

Materials and Methods: Adult BALB/c, Nkx2.3–/–, C57BL/6 and MAdCAM-1–/– were used. Oral tolerance was induced by giving mice 5mg/ml ovalbumin (OVA) in drinking water for 7 days. Mice were then injected intraperitoneally with OVA:complete Freund's adjuvant on day 7 and OVA: incomplete Freund's adjuvant on day 14. On day 21 Treg frequencies were measured with flow cytometry from mLN and LP samples, while serum anti-OVA IgG levels were measured with ELISA. To induce colitis mice received 2.5% DSS in drinking water for 7 days. Weights were measured daily. Colonic Tregs were measured with flow cytometry. RNA was isolated from colonic samples and qPCR was performed to measure anti-inflammatory IL-10 and TGFβ mRNA levels at various time points.

Results: Tolerization with OVA blocked anti-OVA antibody production in Nkx2.3–/– mice. In contrast, feeding MAdCAM-1–/– mice with OVA did not prevent anti-OVA antibody production. Upon DSS treatment, Nkx2.3–/– mice lost less weight and showed milder signs of colitis compared to controls, while MAdCAM-1–/– mice developed more severe colitis. Colonic Tregs were significantly increased at day 7 in the absence of Nkx2.3 compared to BALB/c mice. mRNA for IL-10 was significantly higher at D0 and D7 in Nkx2.3–/– mice, while TGFβ was lower at D0 compared to BALB/c mice. We also found higher Treg numbers in MAdCAM-1–/– mice at D7; however this was not coupled with an increase in mRNA for IL-10 or TGFβ.

Conclusions: Our results indicate that in Nkx2.3–/– mice the increased presence and function of colonic Tregs may protect mice from colitis and induce oral tolerance independent of endothelial MAdCAM-1, as MAdCAM-1–/– mice showed severe signs of colitis and did not develop oral tolerance. As Nkx2.3 is not expressed in hematopoietic cells, we hypothesize that these differences may be due to an altered stromal microenvironment in mice lacking Nkx2.3.

Supported by OTKA grant K108429

EFFECT OF HLA PROMISCUITY ON ANTITUMOR IMMUNITY

Gergő Mihály Balogh[1], Balázs Koncz[1], Máté Manczinger[1, 2]
[1]University of Szeged, Faculty of Medicine, Department of Dermatology and Allergology
[2]Hungarian Academy of Sciences, Biological Research Centre

Introduction: HLA class I molecules are encoded by the most variable regions of the human genome. They present peptide fragments of intracellular pathogens and own proteins to T cells. Missense mutations in tumor cells cause amino acid changes in peptides, which are presented to CD8+ T-cells by HLA-I molecules leading to the destruction of the cell. This process is fundamental in antitumor immunity. Different HLA variants have different size of peptide repertoire. Some alleles tend to bind only a smaller subset of peptides, while others can present a wide range of them. This feature of an allele is called binding promiscuity. According to our hypothesis, higher HLA promiscuity leads to the presentation of more mutant peptides, and this might lead to a more efficient antitumor immunity.

Methods: We examined the relationship between allele promiscuity and peptide diversity presented by HLA-I molecules on 91 tumor cell lines and samples. HLA allele promiscuity was determined by assessing general peptide-binding characteristics of each allele. For this, we predicted the binding strength of allele-associated peptides collected from the IEDB database with the NetMHCpan-4.0 software. To investigate the effect of allele promiscuity on cancer immunotherapy, we calculated HLA promiscuity levels in patients of a cancer immunotherapy cohort (N = 370). We used Kaplan-Meier analysis and Cox regression models.

Results: We found that high promiscuity of HLA-A and HLA-B leads to the presentation of a more diverse peptide set on the cell surface, while alleles of locus HLA-C seem to have

a negligible effect on tumor antigen presentation. Paradoxically, the efficiency of immunotherapy was lower in patients with highly promiscuous alleles.

Conclusions: Promiscuity of HLA-A and HLA-B, but not HLA-C alleles determine the breadth of peptides presented on cancer cells. Surprisingly, high promiscuity was associated with worse survival in cancer immunotherapy patients. Further research is needed to explain this phenomenon.

CHEMOKINE-DRIVEN COMPARTMENTALIZATION OF LYMPHOCYTES AND HIGH-GRADE B-CELL LYMPHOMA SUBTYPES IN FOLIATE LYMPHOID AGGREGATES (FLAGS) OF THE SEROSA

Péter Balogh[1], Gergely Berta[2], Bálint Botz[3], Jia Xinkai[1]
[1]Department of Immunology and Biotechnology, University of Pécs
[2]Department of Medical Biology, University of Pécs
[3]Department of Pharmacology and Pharmacotherapy, University of Pécs

Introduction: In contrast to the lymph nodes and mucosal lymphoid tissues with well-defined entry and exit routes, the movement of leukocytes in the peritoneal cavity is largely unknown. In addition to the omental milky spots (MS), we found that several organized lymphoid aggregates on the serosal surface of the adipose streaks around the mesenteric vessels can also bind high-grade B-cell lymphoma (DLBCL) including a new form of lymphoid organoids denoted as foliate lymphoid aggregate (FLAg). In this work we studied high-grade B-cell lymphoma subtypes colonize FLAgs, and correlated their distribution pattern with the regional display of homeostatic chemokine ligands CCL21 and CXCL13.

Materials and Methods: The distribution of lymphoid aggregates was monitored by whole mount immunohistology for B220, Thy-1/CD90 and LYVE-1 markers and CCL21 and CXCL13 chemokines, combined with laser scanning confocal microscopy. Tracing of A20 (germinal center DLBCL) and Bc-DLFL.1 (extrafollicular DLBCL) cells was performed after labeling with Far Red Cell Tracing and CFSE or by X-gal staining of A20 cells transduced with LacZ. The expression of CCR7 and CXCR5 was determined by flow cytometry. The role of macrophages in the early binding of ip. injected cells was investigated by clodronate liposome-mediated depletion followed by XenoLight DiR near-infrared fluorescence-based detection of lymphoma cells.

Results: We found that shortly (2-4 hours) after intraperitoneal injection both A20 and Bc-DLFL.1 cells efficiently homed to the FLAgs. Here A20 cells gathered at the edge of FLAgs, Bc-DLFL.1 cells entered the central part in a pattern corresponding to the T:B clusters within the FLAgs of untreated mice. The segregation of lymphoma cells was coupled with the upregulation of CCR7 and loss of CXCR5 on the Bc-DLFL.1 cells and with the inverse receptor expression pattern of A20 cells, respectively. The location of CXCL13 chemokine production corresponded to the site of B cells' and A20 lymphoma cells' preferential accumulation at the LYVE-1+ peripheral rim of FLAGs, whereas the CCL21 production seemed less confined. In addition, the mesenteric lymphatic vessels also contained focal CCL21+ segments. The elimination of LYVE-1 macrophages by clodronate liposome treatment significantly reduced the in vivo binding of Bc-DLFL.1 cells.

Conclusions: Partial compartmentalization of FLAgs includes segregation of chemokine-producing regions with LYVE-1+ macrophages exerting both adhesive and chemotactic roles in the peritoneal B-cell placement.

Supported by OTKA K108429, GINOP-232-15-2016-00050 and EFOP-361-16-2016-00004.

TRANSGENIC EXOSOMES FOR THYMUS REGENERATION

Krisztina Banfai[1, 2], Kitti Garai[1, 2], David Ernszt[2, 3], Judit E. Pongracz[1, 2], Krisztian Kvell[1, 2*]
[1]Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Pecs, Hungary
[2]Szentagothai Research Center, University of Pecs, Hungary
[3]Institute of Physiology, Faculty of Medicine, University of Pecs, Hungary

With senescence Wnt4 expression is down-regulated, while PPARgamma expression increases in the thymus. Together these changes allow for thymic degeneration to occur, observed as adipose involution. However, when restored, Wnt4 can efficiently counteract PPARgamma and prevent thymic senescence from developing. In addition to Wnt4, miR27b has also been reported to inhibit PPARgamma. Our goal was to evaluate the Wnt4 and miR27b levels of transgenic thymic epithelial cell (TEC) exosomes, show their regenerative potential against age-related thymic degeneration and visualize their binding and distribution both in vitro and in vivo.

First, transgenic exosomes were harvested from Wnt4 over-expressing TECs and analyzed by transmission electron microscopy showing exosomes ranging 50-100 nm in size. Exosomal Wnt4 protein content was assayed by ELISA, while miR27b levels were measured by Taqman qPCR, both showing elevated levels in transgenic exosomes of Wnt4 over-expressing TECs relative to their control counterparts. Then, for functional studies steroid (Dexamethasone or DX)-induced TECs were used as cellular aging model in which DX-triggered cellular aging was efficiently prevented by transgenic exosomes. Finally, DiI-labeled exosomes were applied on mouse thymus sections and also iv-injected into mice, for in vitro binding and in vivo tracking, respectively. We have observed distinct staining patterns using DiI-labeled transgenic exosomes on sections of young and aged murine thymus samples. Moreover, in vivo injected DiI-labeled transgenic exosomes showed detectable homing to the thymus.

In summary our findings prove that exosomal Wnt4 and

miR27b can efficiently prevent thymic adipose involution. Our results appoint transgenic TEC exosomes as promising tools of immune rejuvenation and contribute to the characterization of the immune-modulatory effects of extracellular vesicles in the context of regenerative medicine.

Keywords: aging, thymus, exosome, Wnt4, miR27b

HUMAN PLASMACYTOID AND MONOCYTE-DERIVED DENDRITIC CELLS DISPLAY DISTINCT METABOLIC PROFILE UPON RIG-I ACTIVATION

Dóra Bencze, Tünde Fekete, Kitti Pázmándi
Department of Immunology, Faculty of Medicine, University of Debrecen

Introduction: Metabolic reprogramming is required for adequate antiviral responses of dendritic cells (DCs) that possess the capacity to initiate both innate and adaptive immune responses. Several reports indicate that Toll-like receptor (TLR) stimulation of DCs is accompanied by a rapid induction of glycolysis; however, the metabolic requirements of retinoic-acid inducible gene I (RIG-I)-like receptor (RLR) activation have not defined either in conventional DCs or in plasmacytoid DCs (pDCs) showing different RLR expression profile. Therefore, in this study we aimed to compare the metabolic requirements of RIG-I stimulated human pDCs and monocyte-derived DCs (moDCs) displaying distinct viral sensing machinery.

Methods: Metabolic changes in human pDCs and moDCs were monitored by extracellular flux analysis and the activation profile of TLR- or RIG-I-stimulated cells was analyzed by Q-PCR, ELISA or western blot methods in response to metabolic inhibitors.

Results: We found that TLR9-driven activation of human pDCs leads to a metabolic transition to glycolysis supporting the production of type I IFNs, whereas RIG-I-mediated responses of pDCs do not require glycolysis and rather rely on oxidative phosphorylation activity. In particular, TLR9-activated pDCs show increased extracellular acidification rate (ECAR), lactate production and upregulation of key glycolytic genes indicating an elevation in glycolytic flux. Furthermore, administration of 2-deoxy-D-glucose (2-DG), an inhibitor of glycolysis, significantly impairs the TLR9-induced secretion of type I IFNs by human pDCs. In contrast, RIG-I stimulation of pDCs does not result in any alterations of ECAR, and type I IFN production is not inhibited but rather promoted by 2-DG treatment. Moreover, pDCs activated via TLR9 but not RIG-I in the presence of 2-DG are impaired in their capacity to prime naïve CD8+ T cell proliferation. Interestingly, human moDCs triggered via RIG-I show a commitment to glycolysis to promote type I IFN production and T cell priming in contrast to pDCs.

Conclusion: Our findings reveal for the first time, that pDCs display a unique metabolic profile; TLR9-driven but not RIG-I-mediated activation of pDCs requires glycolytic reprogramming. Nevertheless, the metabolic signature of RIG-I-stimulated moDCs is characterized by glycolysis suggesting that RIG-I-induced metabolic alterations are rather cell type-specific and not receptor-specific.

Support: This work was supported by NKFIH-PD 115776 and PD_16 120887, EFOP-3.6.3-VEKOP-16-2017-00009 to KP and GINOP-2.3.2-15-2016-00050 projects. The project is co-financed by the European Union and the European Regional Development Fund. KP was also supported by the János Bolyai Research Scholarship from the Hungarian Academy of Sciences.

ROLE AND FUNCTION OF MICROVESICLES CARRYING OXIDATION-SPECIFIC EPITOPES

Christoph J. Binder
Medical University of Vienna, Vienna, Austria & CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

A major consequence of increased oxidative stress is lipid peroxidation, which generates a number of breakdown products of membrane phospholipids that form proinflammatory oxidation-specific epitopes (OSE) such as malondialdehyde (MDA), phosphocholine of oxidized phospholipids, and 4-hydroxynonenal. We previously found that a subset of circulating microvesicles (MVs) of different cellular origin is characterized by the presence of OSE and MDA in particular. We further showed that MDA+ MVs are induced during inflammation and increased in blood obtained from patients with atherosclerotic cardiovascular disease. We could further demonstrate that this subset of MVs is enriched in mitochondrial content and that mitochondrial activity of the parental cells shedding MDA+ MVs is essential for their pro-inflammatory activities. Moreover, targeting MDA with specific antibodies provides novel approaches to interfere with both the pro-inflammatory as well as the pro-coagulatory properties of MVs. Recent insights on these unique properties of OSE+ MVs and their role in cardiovascular pathologies will be discussed.

RELATIONSHIP OF CONGENITAL HEART DISEASE WITH THE STRUCTURE OF HUMAN THYMUS

Ildiko Bodi[1], Krisztina H.-Minko[1], Botond Ferenczy[1], Ivan Varga[2], Zsolt Prodan[3], Nandor Nagy[1], Imre Olah[1]
[1]Semmelweis University, Faculty of Medicine, Department of Anatomy, Histology and Embryology, Budapest, Hungary
[2]Comenius University of Bratislava, Institute of Histology and Emriology, Bratislava, Slovakia
[3]Gottsegen György Hungarian Institute of Cardiology, Budapest, Hungary

The epithelial network of thymus, responsible for the development of T cells has endodermal origin which requires the presence of mesenchyme developing from the neural crest

as it is demonstrated on animal models. Hematoxylin-eosin staining shows the histological units of the human thymic tissue as cortex and medulla but the anti-pancytokeratin immunostaining reveals two sub-compartments in the organ described as keratin negative area (KNA) and keratin positive network (KPN) of epithelial cells (Bódi et al., 2018). According to these results we conclude that the structure of the human thymus is much more complex than previously described. The Foxn1 transcription factor known to be specific to the thymic epithelial cells is expressed during embryogenesis by several other cell types in the liver, lung, kidney and urinary tract [Itoi et al., 2007], and during the migration and maturation of neural crest cells participating in thymus development [Nowell et al., 2007]. According to our present knowledge, Foxn-1 also plays a role in the development of thymus vascularization [Mori et al., 2010].

Human thymuses were obtained from children under 6 years of age from cardiac surgical intervention and from children diagnosed with sudden infant disease syndrome (SIDS) as a control group. The thymic lobes were analysed on frozen sections by numerous monoclonal antibodies and histological methods (light, fluorescence, confocal and transmission electron microscopy).

Based on our observations, we found evidence that Foxn1 is expressed in the nucleus of cytokeratin positive cells and in the cytokeratin negative cells of the KNA. In children where congenital heart disease was classified as septal defect we could not observe cytokeratin and Foxn1 expression in the cortex of the thymuses. Additionally, our electron microscopical studies show that the blood vessels are obliterated in the cortical area of these thymuses.

It is assumed that the abnormally developed thymus is a parallel symptom to the septal defect and the real cause of this phenomenon could be the defective regulation of signaling processes in the neural crest cells.

UNRAVELLING THE HAZARDS OF METAL NANOMATERIALS: COMPARATIVE OBSERVATIONS ON INVERTEBRATE PHAGOCYTES

Kornélia Bodó[1], Gellért Gerencsér[2], Albert Kéri[3], Gábor Galbács[3], Elek Telek[4], Mária Mészáros[5], Gréta Tolnai[1], Bohdana Kokhanyuk[1], Péter Németh[1], Péter Engelmann[1]
[1]Dept. of Immunology and Biotechnology, University of Pécs
[2]Dept.of Public Health Medicine, University of Pécs
[3]Dept. of Inorganic and Analytical Chemistry, University of Szeged
[4]Dept.of Biophysics, University of Pécs
[5]Inst. of Biophysics, Biological Research Centre of the Hungarian Academy of Sciences, Szeged

Little is known about the immunological consequences of different metal nanoparticle (NP) exposure. We presumed that earthworm's immune cells as macrophage-like cells are highly susceptible to the toxicity of NPs.

We exposed Eisenia andrei and E. fetida coelomocytes to 10 nm silver (Ag) NP and gold (Au) NP with different concentrations (1.25 µg – 40 µg) and incubation intervals (4 and 24 hours). Our aim was to assess the effects of NPs at molecular and cellular levels in earthworm coelomocytes.

After the psychical characterization of NPs, cellular effects were ascertained by flow cytometry and TUNEL assay, while DNA damage was determined with Comet assay. Q-PCR analyses were performed to measure the expression of pattern recognition receptor (TLR), stress molecules (Cu/Zn SOD, metallothionein) and antimicrobial factors (lysenin, lumbricin, lumbricin-related peptide) at different time periods.

We observed concentration-dependent cell death in both species (E. andrei LC10: 1.64 µg, LC50: 16.7 µg, and LC90: 27.53 µg; E. fetida LC10: 1.18 µg, LC50: 5.24 µg, and LC90: 23.25 µg) exposed to AgNP. AgNP raised the level of ROS, therefore elevated the number of apoptotic cells as well as induced mitochondrial and DNA damage. AuNP yielded no notable alterations in the viability of coelomocytes in either species. At the cellular level, E. fetida coelomocytes showed higher sensitivity compared to E. andrei coelomocytes. Q-PCR measurements revealed that both NPs treatment resulted in changed mRNA expression patterns in studied genes, which were rather similar in both species. AgNP exposure for 24 hours evoked significantly up-regulated mRNA expression level of TLR, Cu/Zn-SOD, lumbricin, and lumbricin-related peptide genes and reduced the lysenin mRNA expression. AuNP treatment had no major influence on the investigated genes.

Comparison of different metal NPs unfolded some striking contrasts in their mechanisms of action. Based on our results AgNP is more toxic to coelomocytes, although E. fetida coelomocytes are more sensitive to NPs treatments compared to E. andrei coelomocytes.

This work was aided by the Bolyai János Research Scholarship of the Hungarian Academy of Sciences, Medical School Foundation, University of Pécs (PTE-ÁOK-KA 2017/4) GINOP-232-15-2016-00050 and EFOP-361-16-2016-00004.

CUTIBACTERIUM ACNES CONTRIBUTES TO THE MAINTENANCE OF SKIN BARRIER AND HOMEOSTASIS

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

The skin provides a physical barrier to separate our body from the external environment and provides protection from harmful invaders. Little is known how the cutaneous microbiota may affect the barrier functions. Our aim was to investigate the role of Cutinebacterium acnes (C. acnes), a member of the

skin microflora in these events using an in vitro cultured human keratinocyte cell line (HPV-KER) and 3D organotypic skin (OS) models.

We used confluent HPV-KER cultures kept in high (HPV-KER-high) or low (HPV-KER-low) Ca^{2+}ion-containing media and treated them with C. acnes bacterium. Barrier changes were followed by transepithelial electrical resistance (TEER) measurement and xCELLIgence analysis. Lucifer yellow (LY) penetration assays were used to monitor the integrity of the used models and expression changes of tight junction (TJ) proteins (CLDN1, 4, OCLN and ZO-1) were also analysed by western blotting and immunohistochemical staining.

Following a high dose C. acnes treatment, the barrier properties of HPV-KER monolayer cultures changed. In the HPV-KER-low cultures the measured TEER and cell index (Ci) values transiently increased, followed by a rapid decrease. In the HPV-KER-high cultures only decreasing TEER and Ci values were detected, and parallel with these changes LY dye penetration increased 72 hours after bacterial treatment.

In the OS models, similar to the HPV-KER-high cultures, increased LY penetration was noted 72 hours after C. acnes treatment.

Western blot analysis of selected TJ proteins revealed changes in their level and distribution: changes in the HPV-KER-low cultures were comparable to what we observed in the less differentiated layers of the OS models (increased CLDN4 levels). In contrast to that, HPV-KER-high cultures exhibited similarities to the granular layer of the OS cultures (increased OCLN and ZO-1 and decreased CLDN1 levels) after C. acnes treatment.

These suggests that HPV-KER monolayer cultures may serve as a good model to study the properties of different epidermal layers of the human skin. We also hypothesize that C. acnes may actively modify the barrier properties of our epidermis. It can change the expression and localization of certain TJ proteins which may contribute to the alteration of the tightness of cell-to-cell contacts. Overall the skin microbiome may play a role in the maintenance of cutaneous homeostasis.

INTERDEPENDENT GENE- AND MICRORNA-EXPRESSION PATTERN CONTRIBUTES TO THE EPITHELIAL TO MESENCHYMAL TRANSITION IN INFLAMMATORY BOWEL DISEASE

Éva Boros[1], Zoltán Kellermayer[2], Péter Balogh[2], Patrícia Sarlós[3], Áron Vincze[3], Csaba Varga[4] and István Nagy[1]
[1]Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary
[2]Department of Immunology and Biotechnology, University of Pécs, Pécs, Hungary
[3]1st Department of Internal Medicine, University of Pécs, Pécs, Hungary
[4]Deptartment of Physiology, Anatomy and Neuroscience, University of Szeged, Szeged, Hungary

Chronic inflammation along the gastrointestinal tract is the main symptom of Inflammatory Bowel Diseases (IBD) that highly reduces the quality of life and significantly increases the risk of colorectal cancer. Since the incidence of IBD shows a worldwide increase, it is of global importance to understand the molecular mechanisms underlying the pathogenesis of the disease.

We sought to investigate the global transcriptional as well as miRNA expression profile in a rat model of experimental colitis. For this, inflammation in male Wistar rats was induced with 2,4,6-trinitrobenzene sulphonic acid (TNBS) and subsequent RNA-Seq and miRNA-Seq was performed from the same input material. Validation was performed on both rat as well as human samples.

The global expression analysis identified numerous genes and microRNAs with altered expression in experimental colitis. Among several inflammation-related signaling pathways, we observed the activation of epithelial to mesenchymal transition (EMT) that may be a possible molecular link between inflammation and tumorigenesis in IBD patients. Upregulation in the gene expression profile of EMT inducers was associated with the synchronised reduction of their inhibitory miRNAs (both precursor as well as mature molecules). All members of the MIR-8 (miR-200a, -200b, -200c, -141 and -429) and MIR-192 (miR-192 and -215) families were downregulated; in contrast, their common mRNA target ZEB2 showed upregulation in the inflamed colon regions. Moreover, we detected the same reciprocal correlation in the expression profile of inflammatory genes and their miRNA regulators, such as MMP13 and miR-27b.

Our findings suggest that downregulation of inhibitory miRNAs may promote the enhanced expression of inflammation and EMT inducer genes in IBD and their further examination may have the potential to develop novel therapeutic strategies.

FHR1 AND FHR5 COMPETE WITH FACTOR H FOR BINDING TO BACTERIAL PROTEINS AND ENHANCE COMPLEMENT ACTIVATION

Bianca Brandus[1], Alexandra Papp[1], Marcell Cserhalmi[1], Barbara Uzonyi[1], David Ermert[2], Angela Silva Barbosa[3], Anna M. Blom[2], Lourdes Isaac[4], Seppo Meri[5], Mihály Józsi[1]
[1]Department of Immunology, Eötvös Loránd University, Budapest, Hungary
[2]Division of Medical Protein Chemistry, Department of Translational Medicine, Lund University, Malmö, Sweden
[3]Laboratory of Bacteriology, Butantan Institute, São Paulo, Brazil
[4]Department of Immunology, University of São Paulo, Brazil
[5]Department of Bacteriology and Immunology, Helsinki University, Finland

The complement system is a major humoral arm of the innate immune response and serves as a first line of defense against pathogens. However, many pathogens have developed different strategies to avoid the destructive effect of host complement. A commonly used microbial strategy is the

binding of complement inhibitors, such as factor H (FH), from body fluids to escape from complement-mediated destruction. Several microbes have been shown to bind not only FH but also FH-related proteins (FHRs). FHR1 and FHR5 belong to the human FH protein family and are structurally related to FH. The C-terminal complement control protein (CCP) domains of FH (CCPs 19-20), which are responsible for binding various ligands and surfaces, show strong sequence similarity to homologous domains in FHR1/FHR5. Recent data suggest that these FHRs, contrary to FH, are positive regulators of complement activation. Our aim was to study the interaction between FHR1/FHR5 and selected bacterial proteins and to determine the influence of FHR1/FHR5 on the activity of FH and on complement activation.

The microbial proteins leptospiral LcpA, Lig-A and Lig-B, borrelial OspE and streptococcal GBS-enolase were recombinantly expressed. Binding studies, competition assays, cofactor- and complement activation assays were performed by ELISA and Western blotting.

FHR1 and FHR5 bound to the studied bacterial ligands both from serum and as recombinant proteins. FHR1 and FHR5, bound via their C-termini, dose-dependently competed with FH on OspE, caused reduced FH cofactor activity, and also enhanced alternative pathway activation and C3 fragment deposition on OspE when exposed to human serum. Similarly, FHR5 bound via CCPs 3-7 to GBS-enolase and the tested leptospiral proteins, competed with FH and increased alternative pathway activation in human serum, measured by enhanced deposition of C3-fragments and Bb in wells coated with the bacterial proteins.

Our results indicate that by competing with FH on microbial proteins, FHR1 and FHR5 reduce the complement inhibitory activity of FH and may also directly promote alternative pathway activation and enhance the opsonization of microbes.

Supported by the National Research, Development and Innovation Fund (grant K109055) and the Institutional Excellence Program of the Ministry of Human Capacities of Hungary.

INTRODUCTION TO EXTRACELLULAR VESICLES

Edit Buzas[1], Anita Varga[2], Eszter Tóth[1], András Gézsi[2], Nikolett Lupsa[2], Dóra Melicher[2], Krisztina Pálóczi[1], Ádám Oszvald[1], András Försönits[1], Árpád Kovács[1], Tamás Visnovitz[1], Nóra Fekete[1], Emilia Simó[2], Anna Balogh[1], Alicia Galinsoga[1], Delaram Khamari[1], Eszter Baricza[1], György Nagy[1, 3], Éva Pállinger[1], András Falus[1]
[1]Semmelweis University, Department of Genetics, Cell- and Immunobiology
[2]MTA-se Immune-Proteogenomics Extracellular Vesicle Research Group
[3]Semmelweis University, Department of Rheumatology

Extracellular vesicles are recently identified membrane-enclosed structures secreted by all cellular life forms. Their discovery has led to paradigm shifts in all fields of biomedicine. Recent evidences show significant roles of extracellular vesicles in immunity as well. The thematic session on extracellular vesicles on the first day of the 47th Annual Meeting of the Hungarian Society for Immunology was inspired by the recent developments at the intersection of extracellular vesicle biology and immunology. This brief introductory talk will give an insight into some key aspects of extracellular vesicles as complex conveyors of intercellular communication in the immune system.

Supported by STIA-M17 and NKFIH-OTKA 111958 and 120237 and H2020 MSCA ITN TRAIN-EV

CHARACTERIZATION OF THE HUMAN FACTOR H-RELATED PROTEIN FHR2 FOR LIGAND INTERACTIONS AND ROLE IN COMPLEMENT ACTIVATION

Marcell Cserhalmi, Domonkos Czárán, Barbara Uzonyi, Mihály Józsi
Complement Research Group, Department of Immunology, Eötvös Loránd University, Budapest, Hungary

Background: Complement is a major humoral effector system of innate immunity. Its alternative activation pathway also amplifies complement activation initiated by any pathway. Therefore, the proper regulation of the alternative pathway is particularly important to avoid complement-mediated damage to the host. Factor H (FH) is the main alternative pathway inhibitor in body fluids and also when bound to cell surfaces; however, the physiological roles of the five human FH-related (FHR) proteins are still poorly understood. Recent studies on some of the FHRs strongly suggest a role for them in enhancing complement activation via competing with FH for binding to certain ligands and surfaces. The FHR proteins consist of varying number of complement control protein (CCP) domains that display various degrees of sequence identity to the respective domains of FH. The plasma glycoprotein FHR2 consists of 4 CCP domains, and was described to bind C3b. The aim of our current study was to further characterize FHR2 for its ligand binding capacities and role in complement activation/regulation.

Methods: To this end, recombinant FHR2 was expressed in insect cells. Binding of C3 fragments, monomeric C-reactive protein (mCRP) and pentraxin 3 (PTX3), as well as assembly of the C3bBb convertase and complement activation were measured by ELISA. Hemolysis assay was performed using sheep erythrocytes.

Results: FHR2 bound C3b, iC3b and C3d, but not C3c. FHR2 supported the assembly of a functionally active C3bBb alternative pathway C3 convertase via its interaction with C3b. The FHR2 bound convertase was active as shown by the conversion of C3 to C3a. This was confirmed by demonstrating C3 deposition and factor B binding to immobilized FHR2 from human serum. FHR2 also bound to the pentraxins PTX3 and mCRP. FHR2 when bound to mCRP supported complement

activation. FHR2 bound PTX3 could bind C1q, the initiator of classical pathway activation. However, FHR2 inhibited the terminal pathway in a zymosan-induced complement activation assay and in hemolysis assay.

Conclusions: These data show that on the one hand, FHR2 promotes complement activation at the C3 level by supporting the assembly of an active C3bBb alternative pathway C3 convertase and by interactions with the pentraxins PTX3 and mCRP. On the other hand, FHR2 reduces activation of the terminal pathway. Altogether, our results describe ligand interactions and identify novel, dual roles of FHR2 in complement activation and regulation.

Support: These studies were supported by the National Research, Development and Innovation Office (grant nr. K 125219) and the Institutional Excellence Program of the Ministry of Human Capacities of Hungary.

CONNECTION BETWEEN BARRIER ALTERATIONS AND ADAPTIVE IMMUNE ACTIVITIES IN TOPOGRAPHICALLY DIFFERENT SKIN AREAS

Zsolt Dajnoki[1, 2], Barbara Medgyesi[1, 2], Gabriella Béke[1, 2], Krisztián Gáspár[1, 2], Imre Lőrinc Szabó[1, 2], Eszter Anna Janka[2], Szilárd Póliska[3], Zoltán Hendrik[4], Dániel Törőcsik[2], Tamás Bíró[5], Anikó Kapitány[1, 2] Andrea Szegedi[1, 2]
[1]Division of Dermatological Allergology
[2]Department of Dermatology
[3]Genomic Medicine and Bioinformatic Core Facility, Department of Biochemistry and Molecular Biology
[4]Department of Pathology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[5]Department of Immunology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Introduction: Investigations revealed that disturbances of barriers (gut, skin) play an essential role in the initiation and orientation of adaptive immune activities on these surfaces resulting in well-known immune-mediated diseases (e.g. atopic dermatitis [AD], Crohn's disease) and the developed inflammation has a backward influence on barrier functions. In this study, we asked the question whether a sebaceous gland rich (SGR) skin region specific Th1/Th17 mediated disease (rosacea) is accompanied by barrier alterations similar to or distinct from the barrier damage reported in AD.

Methods: RNASeq has been performed on 8 SGR and 8 rosacea skin samples. Pathway analyses were performed by Cytoscape software ClueGo application. Validations were performed by RT-PCR and immunohistochemistry.

Results: By using RNASequencing technique, 5136 gene showed significantly different expression; out of these, 3133 genes showed higher, whereas 2003 genes exhibited lower expressions in rosacea samples (fold change ≥ 1,5). Pathway analysis revealed multiple genes exhibiting roles in the formation of skin barrier and cell junctions in rosacea samples. In our further investigations, we focused on validating the expression of these genes. In rosacea, markers having role in proliferation (KRT6, 16, 17) showed significantly higher expression while molecules taking part in differentiation (FLG, LCE1, LOR, KRT1, 10) and cell junction formation (CLDN1, 16, 23, CDH1, CDSN, DSC1, DSG1, PKP1) were significantly downregulated compared to SGR. The expression of antimicrobial peptides (S100A7, 8, 9, hBD2, LCN2, LL37) was also significantly higher in rosacea.

Conclusions: Our results indicate that barrier alterations of different healthy skin regions may be adjuvants for the development of distinct T helper type immune-mediated skin diseases. Since we could detect unexpectedly similar barrier alterations in rosacea to that found in AD, we hypothesize that barrier damage has an initiating role in the manifestation of both diseases. In AD, it is well-known that barrier damage of sebaceous gland poor skin leads to the appearance of inflammatory TSLP resulting in a Th2/Th22 type inflammation. In contrast, our results suggest that a very similar barrier damage of SGR skin may decrease the level of homeostatic TSLP leading to the Th1/Th17 type inflammation of rosacea.

PRINS LONG NON-CODING RNA REGULATES INTERLEUKIN EXPRESSION IN KERATINOCYTES

Judit Danis[1], Anikó Göblös[1, 2], Zsuzsanna Bata-Csörgő[1, 2], Lajos Kemény[1, 2], Márta Széll[2, 3]
[1]MTA-SZTE Dermatological Research Group, Szeged, Hungary
[2]Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary
[3]Department of Medical Genetics, University of Szeged, Szeged, Hungary

Background: We have previously identified a non-coding RNA, PRINS, as a differentially expressed transcript in psoriatic uninvolved and healthy epidermis. Further studies highlighted its possible contribution to keratinocyte stress responses, as the expression of PRINS is altered after exposure to various stressors and its forced downregulation decreased cell viability during stress. Recently, we have demonstrated that PRINS regulates the expression of interleukin (IL)-6 and chemokine (C-C motif) ligand (CCL)-5 through sequence specific binding to the mRNA of these inflammatory molecules. The aim of our recent study was to identify additional targets for PRINS-mediated regulation in psoriasis-associated inflammatory reactions.

Methods: Cytosolic DNA exposure was used to model psoriasis associated inflammatory reactions in normal human epidermal keratinocytes (NHEKs) with or without plasmid-based overexpression of PRINS lncRNA. The expression of inflammatory cytokines was studied by real-time RT-PCR.

Results: While cytosolic DNA is a potent inducer of the mRNA expression of several cytokines (IL-1α, IL-1β, IL-8,

IL23, TNF-α) it results in decreased PRINS gene expression in NHEKs. Overexpression of PRINS during the inflammation induction resulted in decreased IL-8 and IL-23 expression, similarly to IL-6 and CCL-5 expression, while the expression of IL-1α, IL-1β and TNF-α was not affected. In silico analysis was performed to reveal whether the mRNAs of IL-8 and IL-23 harbor similar interaction sites to PRINS, as previously found on the mRNAs of IL-6 and CCL-5. While no interaction site could be found on the IL-8 mRNA, two potential interaction sites were identified on the mRNA of IL-23. Previously we demonstrated that destruction of the interaction site to IL-6 mRNA on the PRINS construct (ΔPRINS) inhibited the ability of PRINS to decrease IL-6 expression. One of the IL-23 interacting sites overlaps this region, thus we analyzed how ΔPRINS affects IL-23 expression. While in certain donors PRINS overexpression decreased IL-23 expression, ΔPRINS overexpression did not affect IL-23 expression. These observed high intra–individual differences in IL-23 expression might be the consequence of the genetic variant on the polymorphic IL-23 gene.

Conclusions: We propose that PRINS not only acts as a regulator of IL-6 and CCL-5 but also IL-23 expression, through different lncRNA–mRNA interaction sites. IL-23 polymorphisms are risk–factors for psoriasis, and might also modify the binding of IL-23 mRNA to PRINS lncRNA, thus inhibiting the anti-inflammatory functions of PRINS in psoriatic keratinocytes.

A NEW LABEL-FREE METHOD REVEALS NOVEL MECHANISMS OF INNATE IMMUNITY

Márta Lídia Debreczeni[1], Inna Székács[2], Boglárka Kovács[2], András Saftics[2], Beatrix Péter[2], Sándor Kurunczi[2], József Dobó[3], Róbert Horváth[2], László Cervenak[1]
[1]3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary
[2]Nanobiosensorics Momentum Group, Institute of Technical Physics and Materials Science, Centre for Energy Research, Hungarian Academy of Sciences, Budapest, Hungary
[3]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary

Introduction: As constituents of the inner lining of vessel walls, endothelial cells (ECs) are among the first to come into contact with blood components, e.g. plasma proteases important in innate immunity, most of which have not yet been tested on ECs. Although there are several methods suitable for studying EC functions, these usually rely on the detection of labeled molecules, which can per se affect cellular functions. Therefore, we aimed to develop a high-throughput, label-free optical biosensor assay suitable for the investigation of EC responses to plasma enzymes.

Methods: We used the Epic BenchTop (Corning) platform, which can monitor the changes in the refractive index in a ~200 nm thick layer over the sensor surface by the illumination of a 384 well plate. The response curve is an integrated signal of dynamic mass redistribution coming from intracellular rearrangements and changes in cell-cell, or cell-substrate adhesion. The plate was coated with gelatin and a confluent monolayer of ECs was created on the surface. Changes in the refractive index were monitored real-time.

Results: From the plasma proteases tested to date – beside thrombin used as positive control – kallikrein and mannan-binding lectin-associated serine protease-1 (MASP-1) dose-dependently changed EC functions causing characteristic response curves. Effects of these proteases on ECs were verified in further functional assays; both proteases increased EC permeability by the disruption of VE-cadherin junctions, and triggered Ca^{2+} mobilization. These effects were completely blocked by C1-inhibitor.

Conclusions: We have successfully adapted a new, high-throughput, label-free optical system for the real-time investigation of complex EC behavior. Our results indicate that the new platform is suitable for the simple and sensitive screening of drug candidates and other agents, as both intracellular and extracellular rearrangements can affect the refractive index. We are also the first to characterize the cellular effects of kallikrein and MASP 1 in real time. The finding that plasma serine proteases can affect EC behavior proposes yet unexplored mechanisms of innate immunity, as some of these enzymes may have an additional function to induce ECs actively facilitate the leakage of complement into the interstitial space at the site of infection.

Supported by: NKFI K115623, EFOP 3.6.3-VEKOP-16-2017-00009

THE ROLE OF TNFAIP3 IN THE REGULATION OF TLR SIGNALING EVENTS IN HUMAN EPIDERMAL KERATINOCYTES

Lilla Erdei[1], Beáta Szilvia Bolla[1], Renáta Bozó[1], Gábor Tax[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

The human skin microbiome plays an important role in the maintenance of epidermal homeostasis but also has a role in the pathogenesis of skin diseases due to exaggerated induction of innate immune and inflammatory events mostly through TLR activation, likewise Cutibacterium acnes (C. acnes) in the pathogenesis of acne vulgaris. Little is known about the negative regulation of these processes which can protect the host from prolonged inflammation.

Our aim was to study the role of TNFAIP3, which is a negative regulator of NF-κB, in the regulation of TLR signaling

and C. acnes-induced innate immune and inflammatory events in human epidermal keratinocytes. For that, we used a human immortalized keratinocyte cell line (HPV-KER) and analyzed the expression of TNFAIP3 by real-time RT-PCR and western blot analyzes in response to different TLR ligands and C. acnes treatment. Next to this, we monitored the expression changes of pro-inflammatory mediators upon TNFAIP3-silencing by RT-PCR and ELISA after bacterial treat-ment.

Our results show that TNFAIP3 was expressed in keratinocytes and its mRNA and protein expression rapidly increased in response to different TLR ligands and C. acnes treatment. We found that basal and C. acnes-induced expression changes of TNFAIP3 were dose-dependent and regulated by NF-κB and JNK mediated pathways. TNFAIP3 attenuation by siRNA mediated silencing increased the basal promoter activity of NF-κB transcription factor. Subsequently, the basal and bacterium-induced mRNA expression of TNFα, IL-1α, IL-6, CXCL8 and CCL5 also increased in TNFAIP3-silenced cells. Parallel to that secreted IL-6, CXCL8 and CCL5 levels also increased.

Based on our results, TNFAIP3 is one of the negative regulators in keratinocytes, which through the modulation of TLR signaling pathway, may regulates C. acnes-induced signaling events and plays a role in the maintenance of epidermal homeostasis.

MELANOMA ASSOCIATED FIBROBLAST DERIVED ARGINASE REGULATES CD8+ T CELL IMMUNE CHECKPOINT RECEPTOR EXPRESSION

Barbara Érsek[1, 2], Pálma Silló[3], András Bencsik[1], Eva Mezey[4], Sarolta Kárpáti[3], Krisztián Németh[3], Zoltán Pós[1]
[1]Experimental and Translational Immunomics Research Group, Dept. of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest
[2]Office for Research Groups Attached to Universities and Other Institutions of the Hungarian Academy of Sciences, Budapest
[3]Semmelweis University, Dept. of Dermatology, Venereology and Dermatooncology, Budapest
[4]NIAID, NIH, Bethesda, MD, USA

Introduction: Melanoma associated fibroblasts (MAFs) represent a highly heterogeneous population of fibroblast-like cells residing in the tumor stroma, the exact origin of which is a matter of ongoing debate. MAFs have well-known immunosuppressive properties, display high proliferation rate, release a skewed spectrum of extracellular matrix components and actively secrete both inflammatory and immunosuppressive cytokines, such as TGFβ, VEGF, and IL-6. Immune checkpoint receptors are known regulators of the T cell activation and involved in the antitumor immune response. Nowadays, several therapeutic approaches are based on the modification of these molecules, e.g. in malignant melanoma.

Methods: MAFs and normal fibroblasts (NFs) were isolated from enzymatically disintegrated melanomas and healthy skin, respectively. MAFs were identified as Melan-A-/gp100-/α-SMA+ cells by flow cytometry. Untouched CD8+ cytotoxic T cells were isolated from healthy donors by magnetic sorting, and activated by anti-CD3/CD28 in the presence of MAF- and NF-conditioned media (CM). In order to define the impact of MAF-released soluble mediators on CD8+ T cells, markers of activation, cytotoxic degranulation, cytokine production, immune checkpoint regulators and killing activity were analyzed, the latter by redirected killing assay.

Results: We found that upon cognate activation of MAF-CM-treated CD8+ T cells, expression of the early activation molecule CD69 and granzyme B production were suppressed as compared to NF-CM-treated cells. Killing capacity of CD8+ T cells was markedly reduced by MAF-CM too. Besides, the expression of the immune checkpoint inhibitors TIGIT and BTLA expression was enhanced by the treatment with MAF-CM. To reveal key mechanisms responsible for this phenomenon, several soluble factors and immunomodulatory pathways were measured in MAFs and their CMs by ELISA and flow cytometry. According to our findings MAF cells produce much less nitric oxide, but on the contrary, their arginase enzyme activity is significantly elevated. Selective blockade of arginase enzymes could partly restore the phenotype of the CD8+ T cells, especially in the immune checkpoint receptor expression.

Conclusion: Taken together, these data indicate that MAF cells are involved in the regulation of the antitumor response within the tumor microenvironment.

REGULATORY NLRS CONTROL THE RLR-MEDIATED TYPE I INTERFERON AND INFLAMMATORY RESPONSES IN HUMAN DENDRITIC CELLS

Tünde Fekete, Dóra Bencze, Kitti Pázmándi
Department of Immunology, Faculty of Medicine, University of Debrecen

Introduction: Unique members of the nucleotide-binding domain leucine-rich repeat (NLR) family have been found to regulate intracellular signaling pathways initiated by retinoic-acid inducible gene I (RIG-I)-like receptors (RLRs). Plasmacytoid dendritic cells (pDCs), the most powerful type I interferon (IFN) producing cells, preferentially employ endosomal Toll-like receptors (TLRs) to elicit antiviral IFN responses. By contrast, conventional DCs predominantly use cytosolic RLRs, which are constitutively expressed in them. Previously we have reported that, though RIG-I is absent from resting pDCs, it is inducible upon TLR stimulation. In the recent study we investigated the regulatory ability of NLRs, namely NLRC5 and NLRX1 directly associated with the RLR-mediated signaling pathway in DC subtypes showing different RLR expression, particularly in pDCs and monocyte-derived DCs (moDCs).

Methods: The changes in RLR-mediated responses of NLRC5- or NLRX1-depleted cells were monitored at the mRNA level by Q-PCR and at the protein level by ELISA or western blotting.

Results: We found that similarly to RLRs, NLRC5 is also inducible upon TLR9 stimulation, whereas NLRX1 is constitutively expressed in pDCs. Depletion of NLRC5 and NLRX1 in pDCs augmented the RLR-stimulated expression of type I IFNs but did not affect the pro-inflammatory cytokine production. Further we showed that moDCs constantly express RLRs, NLRX1 and NLRC5 that are gradually upregulated during their differentiation. Similarly to pDCs, NLRX1 suppression increased the RLR-induced production of type I IFNs in moDCs. Interestingly, RLR stimulation of NLRX1-silenced moDCs also led to a significant increase in inflammatory cytokine production and IκBα degradation, suggesting increased NF-κB activity. On the contrary, NLRC5 does not seem to have any effect on the RLR-mediated responses in moDCs.

Conclusion: In summary, our results indicate that NLRX1 negatively regulates the RLR-mediated type I IFN production both in pDCs and moDCs. Furthermore, NLRX1 inhibits pro-inflammatory cytokine secretion in moDCs but not in pDCs following RLR stimulation. Interestingly, NLRC5 suppresses the RLR-induced type I IFN secretion in pDCs but does not have any regulatory function on the RLR pathway in moDCs. Collectively, our work demonstrates that RLR-mediated innate immune responses are primarily regulated by NLRX1 and partly controlled by NLRC5 in human DCs.

Support: This work was supported by NKFIH-PD 115776 and PD_16 120887, EFOP-3.6.3-VEKOP-16-2017-00009 to KP and GINOP-2.3.2-15-2016-00050 projects. The project is co-financed by the European Union and the European Regional Development Fund. KP was also supported by the János Bolyai Research Scholarship from the Hungarian Academy of Sciences.

CARD14 GENE VARIANTS AND NUCLEAR FACTOR κB (NFκB) ACTIVATION IN PITYRIASIS RUBRA PILARIS

Anikó Göblös[1, 2], Judit Danis[1], Brigitta Gál[2], Katalin Farkas[3], Nikoletta Nagy[3], Lajos Kemény[1, 2], Zsuzsanna Bata-Csörgő[1, 2], Márta Széll[1, 3]

[1]MTA-SZTE Dermatological Research Group, University of Szeged

[2]Department of Dermatology and Allergology, University of Szeged

[3]Department of Medical Genetics, University of Szeged

Background: Pityriasis rubra pilaris (PRP) is a rare, chronic inflammatory papulosquamous skin disease with unknown etiology. PRP is phenotypically related to psoriasis and other skin diseases making the diagnosis often difficult. Based on phenotypical features we distinguish certain subtypes (I-V) of the disease. The rare familial cases show associations with particular CARD14 gene mutations, while in the genetic background of the sporadic form of the disease CARD14 polymorphisms have been implicated. Data in the literature suggest that the CARD14 variants alter the activation of NFκB signal transduction thus contributes to disease pathogenesis.

Methods: The genetic screening of CARD14 gene was performed in 19 PRP patients. Further in vitro and in situ functional analyses were carried out on samples (skin biopsies, PBMC, keratinocytes) derived from type V PRP patient.

Results: Ten of 19 PRP patients carried different CARD14 variants alone or in combination. The genetic screening revealed two mutations (rs114688446, rs117918077) and six polymorphisms (rs28674001, rs2066964, rs34367357, rs11653893, rs11652075, rs2289541).

The atypical juvenile (type V) PRP patient carries four CARD14 variants, thus to examine the consequences of the identified variants further in vitro and in situ functional studies were carried out. Immunofluorescent staining revealed nuclear localization of the NFκB p65 subunit in PRP skin specimen, indicating the activation of NFκB, in contrast in healthy skin sections only cytoplasmic staining of inactivated NFκB was detected. Moreover, p65 staining showed higher p65 positivity in PBMC derived from PRP patient compared to healthy samples. NFκB–luciferase reporter assay demonstrated significantly increased NFκB activity in keratinocytes from the PRP patient compared to healthy keratinocytes. Investigation of the functional consequences of higher NFκB activation in PRP cells revealed significantly higher inflammatory cytokine expression and secretion in both keratinocytes and PBMCs; moreover PRP samples demonstrated higher cytokine responses to inflammatory stimuli compared to healthy cells.

Conclusions: Half of the investigated PRP patients carried rare or common CARD14 variants alone or in combination. The higher inflammatory state established in the biological samples of a type V PRP patient cannot be explained solely by any of the carried variants but the cumulative effects of the four variants may contribute to the increased NFκB activity. The multifactorial nature of the disease indicates the involvement of additional genetic factors; therefore we propose further large scale genetic investigations for the better understanding of the molecular mechanisms of the disease.

MELANOMA EXOSOMES INDUCE PD-1 OVEREXPRESSION AND TUMOR PROGRESSION VIA MESENCHYMAL STEM CELL ONCOGENIC REPROGRAMMING

Edina Gyukity-Sebestyén[1], Mária Harmati[1], Gabriella Dobra[1], István Balázs Németh[2], Johanna Mihály[3], Ágnes Zvara[1], Éva Hunyadi-Gulyás[1], Róbert Katona[1], István Nagy[1], P. Horváth[1], Árpád Bálind[1], Ábel Szkalisity[1], M. Kovács[1], Tibor Pankotai[2]2, Balázs Borsos[2], Miklós Erdélyi[2], Zsolt Szegletes[1], Attila Farkas[1], Zoltán János Veréb[2], Edit Irén Buzás[4], Lajos Kemény[2], Tamás Bíró[3], Krisztina Buzás[1, 2*]

[1]Biological Research Centre of Hungarian Academy of Sciences
[2]University of Szeged
[3]University of Debrecen
[4]Semmelweis University

Introduction: Metastatic complications are responsible for 90% of cancer-related mortality and one of the most effective metastasis-immunotherapies today is based on the blockade of PD-1:PD-1L interaction. Recently, it has been described that PD-1 overexpressing melanoma cells are highly aggressive. However, until now it has not been defined which factors lead to the generation of PD-1 overexpressing subpopulations.

In the current study, we aimed at specifically addressing the following questions:

1. Can we define the cellular and molecular signs of melanoma derived exosome-induced, intercellular communication-mediated malignant transformation of MSC cultures?
2. Can we detect the melanoma derived exosome-induced tumor progression in vivo?

Methods: We investigated the effect of exosomes on biological processes (e.g. proliferation, survival, malignant transformation, etc.) of MSCs, which are generally considered as proper in vitro models of tumor stroma. For these experiments, MSC cultures were initiated from mouse abdominal adipose tissue and were subjected to melanoma-derived exosome treatment.

Results: We presented that melanoma-derived exosomes, conveying oncogenic molecular reprogramming, induce the formation of a melanoma-like, PD-1 overexpressing cell population (mMSC^{PD-1+}) from naïve mesenchymal stem cells. Exosomes and mMSC^{PD-1+} cells induced tumor progression and expression of oncogenic factors in vivo. Finally, we revealed a characteristic, tumorigenic signaling network combining the upregulated molecules (e.g., PD-1, MET, RAF1, BCL2, MTOR) and their upstream exosomal regulating proteins and miRNAs. Our study highlights the complexity of exosomal communication during tumor progression and contributes to the detailed understanding of metastatic processes.

Discussion: We found that melanoma exosomes reeducate naïve mesenchymal stem cells and generate a PD-1 overexpressing, melanoma-like subpopulation and induce tumor progression. The identified associative network of upregulated tumorigenic molecules and their upstream exosomal regulating proteins and miRNAs may help to identify novel therapeutic targets. Moreover, our data establish a novel concept; i.e. besides the usual "one-molecule and one-pathway-focused research", the assessment of "molecular-signaling-patterns" as complex information packages is justified and requires extensive, system-level, multidisciplinary approaches.

Grant support: GINOP-2.3.2-15-2016-00015 and 2.3.2-15-2016-00036, KTIA_13_NAP-A-V/3 and and János Bolyai Research Scholarship

PROTECTION FROM COLITIS IN MICE IN THE ABSENCE OF NKX2.3 TRANSCRIPTION FACTOR IS INDEPENDENT OF IL-22

Zoltán Kellermayer[1], Dóra Vojkovics[1], Béla Kajtár[2], Péter Balogh[1]
[1]Department of Immunology and Biotechnology, University of Pécs
[2]Department of Pathology, University of Pécs

Introduction: Single nucleotide polymorphisms (SNPs) in the coding region of the Nkx2.3 homeodomain transcription factor have been reported to act as susceptibility factors for Crohn's disease (CD) and ulcerative colitis (UC). We have previously shown that mice lacking the transcription factor Nkx2.3 are protected from dextran sodium sulfate (DSS)-induced colitis. Protection was coupled with enhanced production of the protective cytokine IL-22. In this work, we examined whether the increased IL-22 is responsible for the absence of colitis in Nkx2.3-/- mice.

Materials and Methods: Nkx2-3-/- mice received 2.5% DSS in drinking water for 7 days. Mice were treated with 150 μg of anti-IL-22 monoclonal antibody (clone 8E11, Genentech) or isotype control (clone GP120:9709, Genentech) intraperitoneally on days 2, 3, 4, 5, and 6. Weights were measured daily. Mice were sacrificed on day 7 and colons were isolated for histology, flow cytometry, and mRNA. 6 mice were used per group and treatments were performed twice. Prior to termination, 6-6 mice/group received 5'-ethynyl-2'-deoxyuridin (EdU) ip. to determine epithelial cell proliferation in combination with EpCAM-PE antibody. We examined the stromal expression of Nkx2.3 using LacZ-Nkx2.3+/- reporter mice.

Results: Survival, weights and other macroscopic characteristics did not differ between Nkx2.3-/- mice treated with anti-IL-22 antibody or control antibody. Histological scoring of colons and analysis of solitary intestinal lymphoid tissues (SILT) revealed no difference between the two groups. mRNA for RegIIIβ and RegIIIγ, genes downstream of IL-22 signaling, were decreased in mice treated with anti-IL-22, indicating an effective blockade of IL-22. Proliferation of epithelial cells was also significantly lower in anti-IL-22 treated mice compared

to mice receiving control antibody. In LacZ-Nkx2.3+/- reporter mice we observed Nkx2.3 expression restricted to cells with a myofibroblast-like morphology. These cells were had a pericryptal localization and expressed VAP-1.

Conclusions: Our results indicate that although the protective cytokine IL-22 is increased in the absence of Nkx2.3, it is not the (sole) reason for protection from colitis in these mice. Instead, the presence of Nkx2.3 in pericryptal cells in the vicinity of epithelial stem cells indicate that Nkx2.3 might have a role in the homeostasis of the epithelium, leading to resistance to colitis via modulation of epithelial cell survival and turnover.

Supported by OTKA grant K108429

THE NUMBER OF CIRCULATING IMMATURE TRANSITIONAL B CELLS CORRELATES WITH THE TYPE 3 INNATE LYMPHOID CELLS IN HEMATOPOIETIC STEM CELL-TRANSPLANTED PATIENTS WITH ACUTE GRAFT-VERSUS-HOST DISEASE

Zsolt I. Komlósi[1, 2], Nóra Kovács[3], Zoltán Pós[1], Nikolett Lupsa[1], Gábor Barna[4], Péter P. Reményi[5], Gábor Tatai[5], Gergely Kriván[5], Tamás Masszi[5], György Losonczy[6], Cezmi A. Akdis[2]
[1]Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary
[2]Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich; CK-CARE: Christine Kühne Center for Allergy Research and Education, Davos, Switzerland
[3]Lung Health Hospital, Törökbálint, Hungary
[4]1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary
[5]Department of Hematology and Stem Cell Transplantation, South Pest Centrum Hospital Budapest, Hungary
[6]Department of Pulmonology, Semmelweis University, Budapest, Hungary

Activated type 3 innate lymphoid cell (ILC3) population is expanded after hematopoietic stem cell-transplantation (HSCT), and have a protective effect against graft-versus-host disease (GVHD). It has been demonstrated both in mouse models and in human patients that GVHD is accompanied by IL-10-producing B cell deficiency that probably contribute to the development of the disease. We have recently shown that activated ILC3s and B cells are in a mutually beneficial relationship. ILC3s strongly support survival and proliferation of B cells and differentiation of IL-10-producing, PD-L1-expressing immature transitional B cells with immune regulatory properties (itB_{reg} cells) in a CD40L- and BAFF-dependent manner. The impact of the activated ILC3 populations on B cells after HSCT, and their role in the prevention of GVHD have not been investigated thus far. Therefore, we aimed to examine the interrelation of ILC3s and itB cells in acute GVHD. Sixteen patients of less than 60 days post-HSCT were included in the study, out of which eight were suffered from acute cutaneous GVHD. Their peripheral blood samples were analyzed before initiation of the treatment. Peripheral blood Lineage–$CD127^+CD161^+c\text{-}Kit^+$ ILC3s, as well as $CD19^+IgD^+CD24^{high}CD38^{high}$ itB cells were measured by flow cytometry. The prevalence of ILC3s was lower in acute GVHD patients compared to HSCT patients without GVHD. Both the absolute numbers and the percentages of ILC3s and itB cells correlated with each other, and the correlation was particularly strong in acute GVHD patients. Our results suggest that activated ILC3s may have a favorable influence on the early post-HSCT period via its itB cell-inducing capacity; and itB cells with regulatory properties may have a protective role against acute GVHD. Considering the relative T helper cell–deficiency in early post–HSCT period, the ILC3s may play a critical role in the regulation of B cells reconstitution.

Funded by Hungarian Paediatric Oncology Network.

IMMUNOMODULATORY EFFECTS OF BEWO TROPHOBLASTIC CELL-DERIVED EXTRACELLULAR VESICLES ON HUMAN LYMPHOCYTES

Árpád Ferenc Kovács[1], Nóra Fekete[1], Bálint Alasztics[2], János Rigó Jr[2], Edit Irén Buzás[1], Éva Pállinger[1]
[1]Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary
[2]1st Department of Obstetrics and Gynaecology, Semmelweis University, Budapest, Hungary

Introduction: The success of pregnancy relays on a tightly balanced immune milieu at the feto-maternal interface. One of the key regulators of local immune homeostasis is the placental trophoblast cell population. These cells release an increased amount of extracellular vesicles (EVs) which can be detected also in the maternal circulation. These EVs operate as an intercellular signalling pathway between the trophoblasts and the maternal immune cells. Our aim was to investigate the immunomodulatory proteins found in trophoblast-derived EVs.

Methods: BeWo trophoblastic cell line and human lymphocyte co-culture in vitro system was used. Interaction of Calcein-AM labelled BeWo-derived intermedier sized EVs (iEVs) with lymphocytes was detected via fluorescent microscopy and flow cytometry. EV treated lymphocyte immunophenotyping, as well as iEVs and lymphocyte interactions were analysed by flow cytometry. We carried out mass spectrometry-based protein analysis on BeWo-derived iEVs and performed screening for immunomodulatory proteins. In silico analysis of immunomodulatory proteins and IL-6 signalling pathway interaction was evaluated.

Results: iEVs were taken up by lymphocytes and induced a significant decrease of CD126 (interleukin-6 receptor) on lymphocytes. Furthermore, mass spectrometry analysis yielded the HSPE1 protein as a potential immunomodulatory protein found in BeWo-derived iEVs. In silico analysis of the HSPE1 and IL-6 signalling pathways was also analysed. HSPE1

showed an interaction with the following proteins of the IL-6 pathway: AKT1, IL-6, IFNG, MYC, NFKB1, NFKBIA, TLR4, MAPK1, MAPK8 and MAPK14.

Conclusion: Our preliminary data suggest that BeWo-derived iEVs have an immunomodulatory protein cargo which may have an impact on the success of pregnancy.

DISTINCT MOLECULAR PATHWAYS IN BIOGENESIS OF DIFFERENT TYPES OF EXTRACELLULAR VESICLES FROM NEUTROPHILIC GRANULOCYTES

Erzsébet Ligeti[1], Ákos Lőrincz[1], Balázs Bartos[1], Dávid Szombath[1], Attila Mócsai[1], Dániel Veres[1], Lilla Turiák[2], László Drahos[2], Ágnes Kittel[3]
[1]Semmelweis University
[2]MS Proteomic Research Group, HAS
[3]Experimental Medical Research Institute, HAS

Introduction: In previous works we characterized three physiologically occurring types of EVs released from granulocytes spontaneously (sEV), during apoptosis (apoEV) or upon activation with opsonized particles (aEV). The latter EVs are specifically enriched in granule proteins and possess antibacterial effect. Our goal was to identify receptor(s) and signaling pathway(s) responsible for specific aEV formation.

Methods: Medium-size EVs were obtained from isolated neutrophils (PMN) by two-step centrifugation and characterized by dynamic light scattering and electron microscopy. EV generation was assessed on the basis of protein content and of EV count determined by flow cytometry. Protein identification was carried out by mass spectrometry and proteomic analysis.

Results: Potential involvement of Ig-binding Fc receptors (FcR), complement-binding CR3 (Mac-1 integrin) and pattern recognition receptors (PRR) was investigated. Stimulation of PRR affected minor increase in EV release whereas activation of CR3/Mac-1 resulted in significant aEV generation. FcRs did not seem to be involved in aEV production. Genetic deletion of either chain of Mac-1 prevented aEV formation in murine neutrophils whereas deletion of the common gamma chain of FcRs had no effect. Activation of Mac-1/CR3 also resulted in alteration of cargo in the resulting EVs. Specifically, proteins originating from azurophilic and specific granules were significantly enriched in aEVs. Both aEV formation and granule protein enrichment depended on tyrosine kinase activity, as indicated by sensitivity to dasatinib. Key role of Mac-1/CR3 in aEV formation was substantiated also in an in vivo inflammation model. In contrast, spontaneous formation of EVs was fully independent both from Mac-1/CR3 and tyrosine kinase inhibition.

Conclusion: At least two different molecular pathways are involved in EV biogenesis in neutrophils resulting in vesicles of different composition and different functional properties.

Funding: NKFIH K119236, Hungary

CALCIUM SIGNALING IN THE BIOGENESIS OF NEUTROPHIL DERIVED EV

Lőrincz M. Ákos,[1] Balázs Bartos[1], Dávid Szombat[1], Ágnes Kittel[2], Dániel S. Veres[1], Attila Mócsai[1], Erzsébet Ligeti[1]
[1]Semmelweis University
[2]Medical Research Institute, HAS

Introduction: It is known in case of several cell types that initiation of a calcium signal by application of artificial means such as calcium ionophores induces secretion of extracellular vesicles (EVs). However, the role and requirement of calcium signals triggered by natural stimuli in production of different types of EVs released from the same cell is largely unknown. We used our previous described neutrophil EV secretion system for examination of the role of biological calcium signal on EV biogenesis.

Methods: Medium-sized EVs were obtained in two centrifugation and filtration steps from neutrophils (PMN) isolated from human peripheral blood or murine (WT and phospholipaseC γ2 KO) bone marrow. Murine PMN-EVs were characterized in detail using dynamic light scattering and electron microscopy. EVs were enumerated by flow cytometry and protein measurements. Parallel to EV production phagocytosis was measured to compare signaling pathways of the two processes.

Results: EV production from human neutrophilic granulocytes occurring spontaneously (sEV) and upon stimulation with opsonized particles (aEV) was compared in the absence and presence of extracellular calcium. Generation of aEV was seriously impaired by calcium deficiency whereas release of sEV was not affected. These results were supported in similar experiments carried out on neutrophils isolated from murine bone marrow. Murine neutrophils deficient in phospholipaseC γ2, the key enzyme for intracellular calcium signaling, were also impeded in release of aEVs whereas sEV production proceeded undisturbed. Similar to sEV production phagocytosis was also unaffected by disturbed calcium signaling.

Conclusion: Requirement for extracellular calcium supply and intracellular calcium signaling strongly diverges in generation of different types of EVs or in phagocytosis. These findings supply molecular data on the existence of distinguishable cellular pathways of EV production.

Funding: NKFIH K119236, Hungary

PATHOGEN DIVERSITY DRIVES THE EVOLUTION OF GENERALIST ANTIGEN PRESENTATION IN HUMAN POPULATIONS

Máté Manczinger[1, 2, 3], Gábor Boross[1], Lajos Kemény[2, 3], Viktor Müller[4], Tobias L. Lenz[5], Balázs Papp[1*] & Csaba Pál[1*]
[1]Synthetic and Systems Biology Unit, Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary
[2]Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary
[3]MTA-SZTE Dermatological Research Group, University of Szeged, Szeged, Hungary
[4]Institute of Biology, Eötvös Loránd University, Budapest, Hungary
[5]Research Group for Evolutionary Immunogenomics, Max Planck Institute for Evolutionary Biology, Plön, Germany

Introduction: Central players of the adaptive immune system are the groups of proteins encoded in the major histocompatibility complex (MHC), which shape the immune response against pathogens and tolerance to self-peptides. The corresponding genomic region is of particular interest, as it harbours more disease associations than any other region in the human genome, including associations with infectious diseases, autoimmune disorders, cancers and neuropsychiatric diseases. Certain MHC alleles are generalists: they present an exceptionally large variety of antigenic peptides, while specialists initiate immune response against only a relatively few antigens. However, the functional implications of such elevated molecular promiscuity in the MHC molecules are largely unknown. According to what we term the pathogen-driven promiscuity hypothesis, generalists provide protection against a broad range of pathogens, and therefore they are favored in pathogen diverse areas.

Methods: We determined molecular promiscuity of prevalent HLA-DRB1 alleles using in vitro data and computational prediction. We calculated mean allele promiscuity in indigenous populations and the number of intra- and extracellular pathogens in corresponding geographical regions using public databases.

Results: We found that in pathogen-rich geographical regions, humans are more likely to carry generalist MHC alleles.

Discussion: Taken together, our work indicates that pathogen load maintains generalist adaptive immune recognition. Our study also offers a conceptually novel mechanism to explain the global distribution of allelic variants of key human immune genes. Finally, our work highlights the hitherto neglected role of epitope binding repertoire in immune defense, with implications for medical genetics and epidemiology.

A NEW ROLE FOR EXOSOMES IN CANCER: A RADICAL HYPOTHESIS

Viktor Müller[1, 2], Péter Apari[2, 1]
[1]Institute of Biology, Eötvös Loránd University, Budapest, Hungary
[2]Evolutionary Systems Research Group, MTA Centre for Ecological Research, Tihany, Hungary

Background and Methods: The etiology of cancer involves intricate cellular and molecular mechanisms that apparently emerge on the short time scale of a single lifetime. Using evolutionary reasoning, we demonstrate that some of these traits are remarkable not only for their complexity, but also because it is hard to conceive selection pressures that would favor their evolution within the local competitive microenvironment of the tumor. Building on a broad survey of recent empirical data we put forward alternative hypotheses that might explain the origin of these 'paradoxical tumor traits'.

Results and Conclusions: We discuss three possible scenarios for the origin of these characters: somatic selection driven by specific selection regimes; non-adaptive emergence due to inherent vulnerabilities in the organism; and manipulation by putative transmissible agents that contribute to and benefit from these traits. As most tumor types that display 'paradoxical tumor traits' are not associated with known oncogenic pathogens, the recurrent emergence of these traits in cancer might hint at the existence of unknown transmissible agents that either encode these traits or facilitate their emergence. We hypothesize that a radically novel class of agents exhibiting prion-like spread via exosomes might be responsible for some of these cases, and offer testable predictions that might guide further research to identify the agents (or refute the hypothesis).

EXTRACELLULAR VESICLES IN RHEUMATOID ARTHRITIS

György Nagy[1, 2], Nikolett Marton[1], Orsolya Kovács[1], Edit Buzás[1]
[1]Department of Genetics, Cell and Immunobiology, Semmelweis University
[2]Department of Rheumatology, 3rd Department of Internal Medicine, Semmelweis University

Rheumatoid arthritis (RA) is a common chronic autoimmune disease that is associated with systemic complications and progressive disability. EVs are emerging biomarkers of RA and have been implicated in several pathogenic events, such as antigen presentation, fibroblast activation or induction of proinflammatory cytokine production. Recent data support the role of EVs in the development of cardiovascular comorbidities as well. On the other hand EVs may have anti-inflammatory properties; our data suggest that blood-derived EVs regulate the human osteoclastogenesis. In our presentation we aim to provide an overview of the various aspects of EVs in RA.

EXTRACELLULAR VESICLES IN PREGNANCY

Éva Pállinger[1], Árpád Kovács[1], Nóra Fekete[1], Bálint Alasztics[2], János Rigó Jr[2], Edit Irén Buzás[1]
[1]Dept. of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary
[2]1st Department of Obstetrics and Gynaecology, Semmelweis University, Budapest, Hungary

The immunological balance between the feto–placental immune system and the maternal immunity is manifested in local and systemic mechanisms. It is mediated by direct cell-cell interactions, soluble mediators and extracellular vesicles (EVs) as well. Placenta-derived EVs may have crucial roles in regulating the maternal immune response for successful preg-nancy outcome.

Objective: The aim of our study was to characterize the cellular origin and the functional activity of circulating EVs of healthy and preeclamptic pregnants. We also followed up the immune regulatory effects of trophoblast-derived EVs.

Methods: Circulating EVs were assessed by multicolor immunophenotyping. BeWo choriocarcinoma cell line was used as a model system for the investigations of the effects of trophoblast-derived EVs including NK cell function and the cytokine production of PBMC.

Results: Distinct EV patterns were detected in healthy and preeclamptic pregnants. We have shown that platelet- and trophoblast-derived EVs bind to peripheral T lymphocytes, and have an effect on T cell function. EV-binding inhibited the IL-2-induced STAT3 and STAT6 phosphorylation in T lymphocytes and the ConA-induced cell proliferation. We assumed that EV-lymphocyte interactions regulate T cell differentiation through modulation of the local cytokine production. A characteristic shift in the cytokine production of CD4+ lymphocytes was observed: binding of BeWo-derived EVs induced IL-10 production, but neither IL-17 nor IFNg could be detected. EV-lymphocyte interaction induced the decrease of IL6Ra ex-pression of Th cells. Increased level of apoptotic bodies in preeclamptic patients was associated with an altered exo-facial "eat me" signal expression.

Conclusion: Our results underlie the complex immunomodulatory role of circulating EVs during pregnancy and verify their associations with pregnancy complications.

HLA-II HOMOZYGOZITY PROTECTS FROM BRONCHIAL ASTHMA

Benjamin Tamás Papp[1, 3], Lajos Kemény[1, 2], Máté Manczinger[1, 2, 3, 4]
[1]Department of Dermatology and Allergology, University of Szeged, Hungary
[2]MTA–SZTE Dermatological Research Group, University of Szeged, Hungary
[3]Szeged Scientists Academy, Hungary
[4]Biological Research Center of the Hungarian Academy of Sciences

Introduction: Bronchial asthma is an excessively common obstructive pulmonary disease. In the majority of asthmatic patients, the presence of certain antigens in the airways results in a bronchial hypersensitivity reaction. The antigen is presented by the HLA-II molecules, which are the most variable proteins in our body. Several HLA-II alleles have been associated with bronchial asthma. The connection between HLA-II homozygozity and asthma have not been studied yet. In our study we assume that HLA-II homozygozity protects from asthma, as homozygotes carry the same HLA-II alleles resulting in a small epitope binding repertoire.

Methods: We examined the number of HLA-II homo- and heterozygotes in asthmatic and control groups. We carried out HLA-DR and HLA-DQ genotyping of 190 severe asthmatic patients of the NHLBI GO-ESP: Lung Cohorts Exome Sequencing Project using HLA HD software. The results were compared with a healthy control group. We also predicted the number of allergy-associated epitopes bound by the individuals' HLA-II molecules using NetMHCIIpan-3.2 software. Epitope sequences were downloaded from IEDB database

Results: The number of HLA-II homozygotes was significantly higher in the control group. Homozygotes recognized a significantly lower number of epitopes. We found many asthma associated epitopes which were more likely to be recognized by asthmatic patients.

Discussion: Our results suggest that HLA-II homozygozity protects from the development of bronchial asthma, because homozygous individuals recognise a significantly lower number of asthma-associated epitopes.

MICROSATELLITE INSTABILITY (MSI) – BIOMARKER FOR IMMUNOTHERAPY

Shaun Peterson
Clinical Diagnostics StrategicBusinessUnit, Promega Corporation, Madison, Wisconsin, USA

Microsatellite instability has become an increasingly relevant tool in genetic and immuno-oncology research. Deficiencies in DNA mismatch-repair (dMMR) can be caused by hereditary, germline mutations or hypermethylation. Either mechanism disrupts expression of functional MMR proteins, allowing replication errors to accumulate across the genome. Global genomic mutations disrupt normal cellular function and can lead to unchecked growth and cancers, but also produces novel proteins. These "foreign" proteins can be immunogenic, recruiting immune effector cells to that tissue. Mononucleotide repeat microsatellite sequences are particularly sensitive to replication errors (mutation) and can be the first evidence of an MMR deficiency.

This talk focuses on microsatellite instability, and its functional capability for detecting DNA mismatch-repair deficiency which has been described as a predictive biomarker in an immuno-oncology research setting. The MSI biomarker has risen in importance following the recent FDA drug approval decision based in part on the use of laboratory developed

MSI tests as an indicator of response to immunotherapeutic drug treatment. The MSI-H/dMMR indication for an immune checkpoint blockade drug marked the first time the FDA approved a drug based on a molecular signature in cancers found in different parts of the body, rather than in the organ where the cancer originated.

www.promega.com

IMMUNE CELL-CELL COMMUNICATION: TRANSFER OF GENETIC INFORMATION THROUGH THE IMMUNOLOGICAL SYNAPSE

Francisco Sánchez-Madrid
Centro Nacional Investigaciones Cardiovasculares (CNIC), Madrid, Servicio de Inmunología, Hospital Universitario de la Princesa, Instituto de Investigación Sanitaria La Princesa (IIS-IP), Universidad Autónoma de Madrid, CIBER de Enfermedades Cardiovasculares, ISCIII (CIBERCV), Madrid, Spain

Activation of the adaptive immune response requires the formation of intimate contacts between antigen-presenting cells (e.g. dendritic cells, DC) and T lymphocytes. These contacts ("immune synapses") act as hubs to transmit activating information from the DC to the T cell, to drive differentiation and proliferation. However, information also travels in reverse, from the T cell to the DC. Reverse transmission depends on receptor-dependent adhesive contacts, soluble factors (e.g. cytokines/chemokines) and vesicles (e.g. exosomes). Exosomes are small extracellular nanovesicles formed at multivesicular bodies (MVB) and released towards the DC through MVB fusion with the plasma membrane. Exosomal content is sorted through precise mechanisms and differs from the global cell cytosolic content. I will discuss how the information transferred from T cells towards DCs through synaptic contacts induces an Alert State of Immune Cells (ASIC): getting innate immune cells READY to fight future infections. The regulation of anti-viral and anti-bacterial genes determining the ability of DCs to respond to these challenges will define their Alert State. I will also dissect the role of exosomes during cognate immune interactions, and the function of the transfer of exosomal miRNAS and mitochondrial DNA and as immune regulators and ASIC generators.

COMPLEX IMMUNOPHENOTYPING BY SINGLE CELL MASS CYTOMETRY

Gábor J. Szebeni[1], József Ágoston Balog[1], Ágnes Zvara[1], László Kovács[2], Klára Szalontai[3], István Andó[1], Attila Balog[2], László G. Puskás[1]
[1]Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary
[2]Department of Rheumatology and Immunology, Faculty of Medicine, Albert Szent-Gyorgyi Health Centre, University of Szeged, Szeged, Hungary
[3]Csongrád County Hospital of Chest Diseases, Deszk, Hungary

High resolution measurement characterizing the large number of cellular features is in the focus of recent cell biological research. To achieve these goals single cell mass cytometry combines advantages of the single cell resolution of traditional fluorescence-based flow cytometry with the multiplexicity of inductively coupled plasma-mass spectrometry. Instead of fluorophores detection for mass cytometry is based on stable heavy-metal isotope labeled antibodies. Thus, the autofluorescence and spectral overlapping are eliminated. This unique feature enables researchers to multiplex up to 45 different antibodies in one single tube.

Extensive mapping of signaling networks in single cells, surface receptor quantification has been also achieved by single cell mass cytometry. Sample multiplexing is also possible by barcoding prior the antibody labeling which enables the combination of 20 different samples in one single tube. Cell types, cellular populations of interest can be visualized on dot plots and the protein expression levels are demonstrated by histograms. There are several novel algorithmic approaches to process large datasets such as: SPADE, viSNE, Citrus.

The monitoring of the complex immunophenotype is highly relevant in several human diseases which have been previously restricted to limited number of markers with flow cytometry compared to single cell mass cytometry. PBMCs of human systemic autoimmune diseases and non-small cell lung cancer patients are under investigation. The cellular complexity and functional heterogeneity of solid tumors, inflammatory diseases and animal models will be also analyzed in our laboratory by single cell mass cytometry.

Funding: GINOP-2.3.2-15-2016-00030; János Bolyai Research Scholarship (GJSz, BO/00139/17/8) Bolyai+ Research Scholarship (GJSz, UNKP-18-4-SZTE-73)

AUTOIMMUNE PHENOMENA DUE TO CHECKPOINT INHIBITION IN ONCOLOGY

Éva Szekanecz, Zoltán Szekanecz
University of Debrecen, Faculty of Oncology, Division of Reumatology, Debrecen

Costimulation and coinhibition are associated with the development of immune responses, antitumor immunity and autoimmunity. B7-CTLA4-mediated coinhibition is involved in the priming phase of immune responses in the lymph node. PD1 on T-cells and PD-L1 on tumor cells drive coinhibition in the peripheral tissues. Thus, tumor cells can escape from immune surveillance. Based on these phenomena, CTLA4, PD1 and PD-L1 inhibitors have been developed and successfully used in cancer immunotherapy. Logically, this "inhibition of coinhibition" may aggravate autoimmune responses, as well as tu-mor immunity.

In animal models, checkpoint knockout lead to the development of various autoimmune diseases, such as diabetes mellitus, autoimmune arthritis and encephalomyelitis. Administration of PD1-Fc or PD-L1-Fc constructs prevented autoimmunity.

To date, thousands of cancer patients have been treated by checkpoint inhibitors. Along with the growing number of treated patients, numerous cases developed autoimmune phenomena or even full-blown autoimmune disease. Mucositis, arthralgia, sicca syndrome or myalgia/myositis are among the most common manifestations. Here we will summarize the current understanding of checkpoint inhibition-triggered autoimmunity and present a recent case of Raynaud's phenomenon after nivolumab therapy.

DEVELOPMENT OF SECONDARY MALIGNANCIES IN AUTOIMMUNE-RHEUMATIC DISEASES AND AFTER TARGETED IMMUNOTHERAPY OF ARTHRITIS

Zoltán Szekanecz, Éva Szekanecz
University of Debrecen, Faculty of Medicine, Department of Internal Medicine
Division of Rheumatology

Sustained systemic inflammation may lead to increased risk of malignancies. Chronic B-cell stimulation may lead to the development of lymphomas in SLE, rheumatoid arthritis (RA) or systemic sclerosis (SSc). Solid tumors may occur in tissues mostly affected by the underlying disease. For example, lung cancer is more common in SSc or RA, partly due to smoking. We have been conducting numerous studies on patients with autoimmune rheumatic conditions. We assessed the prevalence of secondary malignancies, the production of soluble tumor antigens. We also reviewed current knowledge about the possible carcinogenicity of antirheumatic therapies.

We found significantly higher prevalence of malignancies among patients with RA, SSc and SLE. We will discuss the possible oncogenic effects of antirheumatic drugs. We detected increased levels of soluble tumor antigens in RA, SSc and SLE.

There are numerous relationships between rheumatic and malignant diseases. Our studies may draw attention to the importance of collaboration between medical specialties including rheumatology, immunology and oncology.

THE ROLE OF TRANSIENT RECEPTOR POTENTIAL VANILLOID 4 ON MONOCYTE-DERIVED LANGERHANS CELLS

Attila Gábor Szöllősi[1, 2], Ágnes Gyetvai[3], Shahrzad Alimohammadi[1, 2], Zsófia Pénzes[1, 2], Tamás Bíró[1]
[1]University of Debrecen, Faculty of Medicine, Department of Immunology
[2]University of Debrecen, Faculty of Medicine, Department of Physiology
[3]Hungarian Academy of Sciences, University of Debrecen, Cell Biology and Signaling Research Group, Debrecen, Hungary

Background: Emerging evidence suggests that transient receptor potential (TRP) ion channels not only act as 'polymodal cellular sensors' on sensory neurons but are also functionally expressed by a multitude of non-neuronal cell types, including immune cells. Our own workgroup has previously shown that TRP channels influence a wide range of cellular functions on dendritic cells, including maturation, phagocytosis/endocytosis, and cytokine production among others. In our current experiments we aimed at identifying and characterizing the role of TRP channels on Langerhans cells (LCs).

Methods: Since isolation of primary LCs is difficult and often results in the unwanted activation of the cells, we differentiated LCs from monocytes of healthy donors. mRNA and protein expression of cells was determined with QPCR and flow cytometry, respectively, while the function of TRP channels was assessed with fluorimetric calcium measurements.

Results: LCs were found to express TRPV1, 2 and 4. Fluorimetric measurement of intracellular calcium concentration revealed that the TRPV1 agonist capsaicin had no measurable effect up to 10 μM. In contrast the specific TRPV4 agonist GSK1016790A dose dependently increased the intracellular calcium concentration (10-100 nM). This effect was completely blocked by the addition of the specific antagonist (1 μM HC 067047). The TRPV4 agonist applied during the differentiation process doubled the ratio of CD207/CD1a positive cells, which effect was also blocked by specific antagonist. GSK also decreased the endocytotic activity of monocyte-derived LCs, while minimally increasing the maturation of the cells.

Conclusions: TRPV4, which may be activated by many epidermal compartment-specific factors such as hyperosmolarity, and UV radiation, may have an important role in both the development and function of Langerhans cells.

SURFACE CARGO OF BLOOD PLASMA EXTRACELLULAR VESICLES IN INFLAMMATORY AND NON-INFLAMMATORY MICROENVIRONMENTS

Eszter Ágnes Tóth, Anita Varga, Anna Magdolna Balogh, Katalin Éva Szabó-Taylor, Edit Buzás
Semmelweis University, Department of Genetics, Cell- and Immunobiology

Introduction: Nanoparticles, usually <100nm, utilized mostly in targeted therapeutic approaches develop diverse protein coronas once administered into biological media. This protein corona was found to fundamentally affect the biodistribution and bioavailability of nanoparticles. Extracellular vesicles (EVs) are considered as endogenous nanoparticles of a living organism. Here we set the goal to study the surface cargo of EVs. Also, we studied whether inflammation (e.g. rheumatoid arthritis) influenced the formation of the EV protein corona.

Materials and Methods: Blood plasma samples of healthy subjects (n=9) and rheumatoid arthritis patients (n=10) were depleted both in platelets and EVs. Nascent EVs released by THP1 cells were isolated with differential centrifugation and were incubated for 30 min in the platelet- and EV-free blood

plasma samples. Plasma without addition of THP1-EVs and THP1-derived EVs incubated for 30 min in buffer, were used as controls. After washing, we analysed the samples by mass spectrometry, flow cytometry and immune electron microscopy. Data were further analysed using bioinformatic approaches. For the investigation of individual proteins, fluorescent proteins were utilized for further flow cytometric studies.

Results: A significant interaction between blood plasma molecules and extracellular vesicles was suggested by the difference between the number of proteins found by mass spectrometry (MS/MS) in plasma+EV samples and the summed number of proteins found in only plasma and only vesicle samples (p<000.1). Subtracting the proteins that were found in pure extracellular vesicle samples from the list of proteins of extracellular vesicle samples incubated in plasma for 30' and washed 2x, 66 proteins were found, out of which 16 were more characteristic of rheumatoid arthritis samples and only 2 were more prevalent in healthy samples. The interaction between fibrinogen and EVs was also confirmed by flow cytometry using fluorescent fibrinogen molecules. The presence of the alpha chain of fibrinogen in the surface cargo of EVs was also demonstrated by immune electron microscopy.

Discussion: Our data suggests the existence of a protein corona of blood plasma EVs which may significantly modify the biological fate of EVs. Differences found between patients with an inflammatory disease and healthy persons may have pathophysiological role and diagnostic potential.

Support: STIA-M17, NKFIH-OTKA 111958

ELEVATED METASTATIC ACTIVITY, TAM RECEPTOR EXPRESSION AND ERK ACTIVATION OF ORAL SQUAMOUS CELL CARCINOMA CELL LINES UPON MICROBIAL EXPOSURE

Máté Vadovics[1], Nóra Igaz[2], Éva Veres[1], Dóra Adamecz[2], Renáta Gáspár[3], Csaba Vágvölgyi[1], Mónika Kiricsi[2], Attila Gácser[1, 4]
[1]Department of Microbiology, University of Szeged, Szeged, Hungary
[2]Department of Biochemistry and Molecular Biology, University of Szeged, Szeged, Hungary
[3]Department of Biochemistry, Faculty of Medicine, University of Szeged, Szeged, Hungary
[4]Hungarian Academy of Sciences, University of Szeged, Lendület „Mycobiome" Research Group

A large number of commensal microbial species reside in the human body that have co-evolved with the human genome and adopted to the host immune system. However, defects in regulatory processes or alterations in the composition of the microbiota can lead to various diseases, including cancer. A previous study has shown that the colonisation of Candida cells is significantly higher in patients with oral squamous cell carcinoma (OSCC) compared to healthy individuals (Berkovits et al. 2016). Our hypothesis is that the dysbiotic microbiota has tumor promoting effects, and the microbes are able to promote the metastatic activity of the cancerous cells.

In order to investigate the metastatic activity of the cancerous cells after microbial treatment we used a metastatic (HO-1-N-1) and a non metastatic (HSC-2) OSCC cell line. Cell migration, matrix metalloproteinase (MMP) and proliferation activity of HSC-2 and HO-1-N-1 OSCC cells were investigated after fungal and bacterial stimuli. The migration capacity of HO-1-N-1 cells was significantly higher after the treatments compared to the untreated samples. Prominent MMP activities were detected in case of HSC-2. Both cell lines showed increased proliferation activity upon treatments, which clearly indicates that fungal or bacterial infections can accelerate cancer cell proliferation. To investigate the possible molecular mechanisms underlying the increased metastatic activity of OSCC cells after fungal or bacterial exposure, we examined the expression level and activation of ERK and AKT kinases after microbial treatment because they are involved in metastatic signaling pathways, furthermore we investigated the expression level of various TAM receptors, since they play a role in microbial recognition through inhibition of TLR signaling and TLR-induced cytokine-receptor cascades. In addition to their roles in limiting TLR and cytokine signaling they advance cancer cell migration by assisting the epithelial-mesenchymal-transition (Rankin et al. 2016). Elevated expression of ERK, pERK and Axl in OSCC cells could be verified by Western blot following microbial treatment.

These findings suggest that the presence of microbes in the oral cavity can promote cell proliferation, increased motility and metastatic activity of oral squamous cell carcinoma cells at least in part by the induction of TAM receptors and activation of ERK kinase.

DIFFERENTIAL EFFECTS OF THE ABSENCE OF NKX2-3 AND MADCAM-1 ON THE DISTRIBUTION OF INTESTINAL ILC3 CELLS AND POSTNATAL SILT FORMATION IN MICE

Dóra Vojkovics[1, 2], Zoltán Kellermayer[1, 2], Angela Schippers[3], Norbert Wagner[3], Gergely Berta[4], Péter Balogh[1, 2]
[1]Department of Immunology and Biotechnology, University of Pécs, Hungary
[2]Lymphoid Organogenesis Research Group, Szentágothai Research Center, University of Pécs, Hungary
[3]University Hospital RWTH, Aachen, Germany
[4]Department of Medical Biology, University of Pécs, Hungary

Leukocyte seeding to embryonic and early postnatal lymphoid tissues and to the mucosa throughout adulthood depends on the interaction between endothelial MAdCAM-1 addressin and $\alpha 4\beta 7$ integrin. Nkx2-3 as a transcriptional regulator of MAdCAM-1 controls vascular patterning in visceral lymphoid tissues in mice. However, the role of Nkx2-3 in the organogenesis of the solitary intestinal lymphoid tissues (SILTs) involving ILC3 cells is still unknown.

In this work we studied the effect of Nkx2-3 on the postnatal distribution of gut ILC3s and the development of SILTs, comparing these events to mice lacking MAdCAM-1, but preserving Nkx2-3.

Lamina propria lymphocytes of the small intestine (SI) and colon of 1, 2 and 4 week old mice were isolated by collagenase digestion. Cells were labeled with mAbs against CD3, CD19, CD90 and CD45 in conjunction with anti-RORγt, and were analyzed with flow cytometry. For analyzing SILT ratio multiple fluorescent labeling was performed on cryostat sections of SI and colonic Swiss rolls. Sections were viewed with fluorescence microscope or laser scanning confocal microscope.

We found that at 1 week of age SI contained significantly higher number of ILC3s relative to the colon, with a significant reduction in MAdCAM-$1^{-/-}$ mice compared to wild-type animals. One week later SI ILC3 number decreased in all genotypes, whereas the number of colonic ILC3 of wild-type BALB/c and Nkx2-3-deficient mice significantly increased. On the 4^{th} postnatal week a further reduction of SI ILC3 cells was observed in wild-type BALB/c and Nkx2-3-deficient mice, whereas the colonic ILC3 number showed a significant reduction in all genotypes.

At one week of age only sporadic SILT components were present in all genotypes. By the second week in mice lacking either Nkx2-3 or MAdCAM-1 SILT maturation was absent compared to wild-type controls, causing a lack of mature isolated lymphoid follicles (ILF). By the fourth week both Nkx2-3-deficient and wild-type mice showed a similar distribution of ILFs relative to cryptopatches (CP), whereas in MAdCAM-$1^{-/-}$ mice only CPs and immature ILFs were present.

Our data demonstrate that the different forms of MAdCAM-1-deficiency variably affect the regional distribution of intestinal ILC3 cells and the development of SILT. Furthermore, the complete absence of MAdCAM-1 still permits gut seeding of ILC3 cells and partial SILT maturation.

Supported by FIKP-20765-3/2018/FEKUTSTRAT and OTKA-108429.

CHARACTERIZATION OF THE STROMAL COMPOSITION OF SEROSAL LYMPHOID ORGANOIDS IN MICE

Jia Xinkai, Oli Jacobsen, Gábor Bedics, Péter Balogh
Department of Immunology and Biotechnology,
University of Pécs

Introduction: Our previous work on the selective intraperitoneal homing of B-cell lymphoma cell has led to the identification of a cluster of mesenteric serosal lymphoid organoids, in addition to the omental milky spots (MS). Of these organoids a particular formation (foliate lymphoid aggregates – FLAgs) with partial T:B segregation appear particularly effective to collect lymphocytes after their intraperitoneal administration, and are absent in mice lacking T and B cells. The similar appearance of FLAgs at different locations suggests similar cellular composition of their mesenchymal scaffolding. In the present work we studied the stromal organization of serosal lymphoid territories using mAbs against stromal markers, and compared wild-type and Rag$^{-/-}$ mice.

Materials and Methods: Whole-mount immunohistochemistry of microdissected mesenteric and omental tissue samples using mAbs against MAdCAM-1, VCAM-1, ICAM-1 adhesion molecules, LYVE-1 lymphatic endothelial antigen and IBL-20 shared blood-and-lymphatic endothelial antigen was used. For tissue transparency we performed benzyl-alcohol: benzyl-benzoate (BABB) transparency treatment.

Results: We found that several markers displayed by the stroma of mouse peripheral lymphoid organs were also expressed in the serosal lymphoid organoids. Their precise evaluation in the deeper parts of whole-mount samples was greatly improved by BABB treatment. The omental MS structures were highly vascularized, whereas FLAgs typically contained one or two vascular branches. Dilated LYVE-1+ lymphatic vessels were regularly present in the mesentery, while such segments were much less prominent in the omentum. In addition, LYVE-1+ macrophages outlined the area of both omental and mesenteric MS, and were also detectable at the edge of the leaf part of FLAgs. We could also identify a VCAM-1+ region within the FLAgs, in addition to its expression on the supplying vessels, which were also positive for MAdCAM-1 and ICAM-1. However, these two latter adhesion proteins were also expressed outside the endothelium by FLAg-associated macrophages in wild-type mice. In samples from Rag$^{-/-}$ mice lacking FLAg structures the basic vascular pattern of omental and mesenteric MS structures was similar to those of wild-type mice, although the stromal intensity of VCAM-1 and ICAM-1 expression were weaker, and the MAdCAM-1+ regions were significantly reduced.

Conclusions: Our findings demonstrate considerable overlap between the display of several non-hematopoietic markers in the vasculature of various forms of serosal lymphoid organoids. Their shared expression with leukocytes may indicate their involvement both in the surface-mediated colonization and homing for the blood-borne lymphocytes, which substantially affects the stromal organization.

POSTERS

LYMPHATICS MODULATE THE DEVELOPMENT OF CONTACT HYPERSENSITIVITY

Petra Aradi[1, 2], Éva Kemecsei[1, 2], Zalán Horváth[1, 2], Zoltán Jakus[1, 2]
[1]Department of Physiology, Semmelweis University School of Medicine, Budapest, Hungary
[2]MTA-SE „Lendület" Lymphatic Physiology Research Group of the Hungarian Academy of Sciences and the Semmelweis University, Budapest, Hungary

Background: Contact hypersensitivity reaction (CHS), the mouse model of human allergic contact dermatitis, can be induced by repeated exposure to contact allergens. The lymphatic system is a critical player for the regulation of the immune response in infectious diseases in the skin but the possible role of lymphatics in the development of allergic contact dermatitis remains unclear.

Methods: In this study $Flt4^{kd/+}$ mice carrying a germline point mutant kinase dead Vegfr3 allele were used. Our experiments have revealed the complete lack of lymphatics in the skin including the ear of $Flt4^{kd/+}$ mice, while the lymphatic structures were present in the lung and small intestine. CHS was initiated by the exposure of the skin to TNCB (2,4,6-trinitrochlorobenzene) followed by a second treatment. The disease progression was monitored by ear thickness measurement, H&E histology and immunostaining against lymphatic and immune cell markers.

Results: Detailed characterization of CHS development in $Flt4^{+/+}$ and $Flt4^{kd/+}$ mice indicated reduced inflammation in the ear of the $Flt4^{kd/+}$ mice. CHS development induced dynamic changes in lymphatic morphology and resulted in unexpected lymphatic growth with dilated lymphatic structures in $Flt4^{kd/+}$ ears.

Conclusions: Collectively, our results reveal that dynamic changes of lymphatic morphology occur in CHS, and the inflammation is reduced in the $Flt4^{kd/+}$ mice lacking ear lymphatics. Our findings also suggest that distinct mechanisms regulate the developmental and inflammatory lymphangiogenic program. Importantly, our results define novel aspects of the interactions between the immune and lymphatic system in allergic diseases.

ROLE OF EXOSOMES IN THYMIC REGENERATION

Krisztina Banfai[1, 2], Kitti Garai[1, 2], David Ernszt[2, 3], Judit E. Pongracz[1, 2], Krisztian Kvell[1, 2]
[1]Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Pécs
[2]Szentagothai Research Center, University of Pécs
[3]Institute of Physiology, Faculty of Medicine, University of Pécs

Introduction: During the development of the thymus Wnt4 plays a crucial role by regulating thymopoiesis and maintaining the cellular composition of TECs (thymic epithelial cells). During thymic senescence Wnt4 is down-regulated, while PPARγ is up-regulated and triggers adipose involution following epithelial to mesenchymal transition. Along Wnt4 miR27b was described to suppress PPARγ. Previous results show that Wnts and several miRNA species are located in exosomes. Our aim was to confirm the presence of Wnt4 and miR27b in TEC exosomes, detect their effect and follow their way both in vitro and in vivo.

Methods: Exosomes were collected and isolated from control and Wnt4 over-expressing TECs. Transmission electron microscopy was used to visualize exosomes. Exosomal miR27b levels were measured by TaqMan qPCR, while Wnt4 protein content was determined by ELISA. DiI-labeled exosomes were injected into mice intravenously to detect homing.

Results: Using transmission electron microscopy exosomes were detected in the expected size range. TaqMan miRNA assay measured elevated miR27b levels, while ELISA showed high Wnt4-content in Wnt4- exosomes compared to control exosomes. To create a cellular aging model, steroid (Dx)- induced TECs were used. Dx accelerated aging, but Wnt4-containing exosomes could efficiently prevent Dx-induced senescence. Finally, in vivo injected DiI-labeled Wnt4- exosomes showed detectable homing to the thymus.

Summary/conclusion: According to our results Wnt4 and miR27b are both present in TEC exosomes. Our results imply that Wnt4 may act as an inhibitor of thymic involution through miR27b.

Funding: Scientific research support was provided by PTE Virology Talent Center program and EFOP-3.6.1-16-2016-00004.

EARLY RESPONSES OF LUNG-RESIDENT MEMORY T CELLS AFTER INFLUENZA REINFECTION

András Bencsik[1, 2], Barbara Érsek[1, 2], Nikolett Lupsa[1, 2], István Jankovics[3], János Matkó[4], Edit Irén Buzás[2], Zoltán Pós[1, 2]
[1]Semmelweis University, Department of Genetics, Cell- and Immunobiology, Experimental and Translational Immunomics Research Group, Budapest
[2]Semmelweis University, Department of Genetics, Cell- and Immunobiology, Budapest
[3]National Center for Epidemiology, Division of Virology, Budapest
[4]Eötvös Loránd University, Department of Immunology, Budapest

CD8+ resident memory T cells (Trm) are sentinel-like memory cells maintaining an early warning system and also constituting the first line of defense against various recurring viral infections in diverse tissues. Nonetheless, the exact contribution of CD8+ Trm cells to recall antiviral immune responses is still elusive, in part because it seems to be fine-tuned by both the viral entity in question, and local tissue environs.

This study aims at a better understanding of the earliest response mechanisms initiated by lung-resident murine CD8+ Trm cells activated by influenza reinfection in vivo.

Lung resident CD8+ Trm cells were generated in naïve C57Bl/6J mice by intranasal infection using sublethal dose (150 X TCID50) of mouse-adapted influenza A/PR/8/34 (H1N1), and subsequently activated by lethal dose viral challenge (150.000 X TCID50) six weeks later. Successful viral infection was confirmed by monitoring weight loss, performing lung histology, and influenza-specific immunohistochemistry.

Next, lungs were subjected to automated tissue dissociation using a GentleMACS Octo platform, and establishment of virus-specific CD8+ resident memory was validated by enumerating nucleoprotein-specific Trm cells with MHC tetramer staining.

Finally, live $CD8b^{+}CD69^{+}CD103^{hi}$ (Trm) cells were FACS-sorted from dissociated lungs, harvested on days 0, 1, 2, and 3 after reinfection. Isolated CD8+ Trm cells were analyzed by transcriptome profiling and flow cytometry. CD8+ Trm-specific changes were identified by using pulmonary non-resident CD8+ T cells as reference.

Preliminary findings suggest that influenza-induced expression of several transcription factors, chemokine receptors and metalloproteases is restricted to the CD8+ Trm subset during influenza re-infection.

These data suggest that a systematic comparison of the dynamics of CD8+ Trm and non-Trm responses may disclose previously hidden aspects of the activity of tissue-resident memory cells in recurrent viral infections.

SETTING UP A MURINE EXPERIMENTAL MODEL OF RIFT VALLEY FEVER VIRUS INFECTION

András Bencsik[1, 3] and Mayke Leggewie[2, 4], Zoltán Pós[3], Esther Schnettler[2, 4], César Muñoz-Fontela[1, 4]
[1]Bernhard Nocht Institute for Tropical Medicine, Virus Immunology Research Group, Hamburg
[2]Bernhnard Nocht Institute for Tropical Medicine, Molecular Entomology Research Group, Hamburg
[3]Semmelweis University, Department of Genetics, Cell- and Immunobiology, Experimental and Translational Immunomics Research Group, Budapest
[4]German Centre for Infection Research (DZIF), partner site Hamburg-Lübeck-Borstel-Riems, Hamburg

Background: Rift Valley Fever Virus (RVFV) is a mosquito-borne pathogen mostly endemic to South- and East-Africa, actively spreading to the Arabian Peninsula and the Mediterranean coast. In affected livestock, alarming rates of mass abortions in pregnant animals, and high mortality have been reported upon RVFV infection. In humans, RVFV infection has mostly an indolent disease course, but is nevertheless capable of causing blindness, liver failure, meningoencephalitis, hemorrhagic fever, and fatalities. In addition to mosquito bites, viral transfer can occur via aerosols, contact with infected blood, body fluids or tissue samples.

Aim: Here we tested several routes of RVFV transmission to study local CD8+ T cell responses and CD8+ T cell memory developed against native RVFV in vivo in a murine host.

Methods: C57Bl/6J mice were infected with RVFV (Ar20364 isolate) by intranasal inhalation, intradermal injection, and mosquito bite. Control animals received PBS instead of virus suspension or were exposed to uninfected mosquitoes. Analysis of morbidity was done following weight loss, drop in body temperature, and limb paralysis. Serum, spleen, liver and brain were harvested from moribund or from control animals and the viral load was measured in these organs via RVFV-specific qPCR. Presence of RVFV-specific IgG antibodies in sera was checked by indirect immunofluorescence assay.

Results: Intranasal infection resulted in extremely rapid disease progression and complete lethality within 4-10 days. Hence, this route was deemed inadequate to study CD8+ T cell responses and memory formation. Intradermal injection induced mortality in one out of four mice. In line with this, virus-specific qPCR disclosed the presence of the RVFV in the brain and spleen of the deceased animal, and indirect immunofluorescence confirmed seropositivity in all needle-infected mice. In contrast, mice exposed to infected mosquitoes, did not succumb to the disease, develop any symptoms or seropositivity.

Conclusions: Our data suggest that different routes of RVFV infection have a major impact on the outcome of experimental disease in mice. Further studies are required to test why mice show resistant to RVFV through mosquito bites,

and how this system may be modified to allow studying local CD8+ T cell responses and memory in mosquito-exposed livestock and humans.

THE ANTIVIRAL CAPACITY OF HUMAN MONOCYTE-DERIVED DENDRITIC CELLS IS MODULATED BY BUTYRATE

Krisztián Bene, Szábit Al-Taani, Márta Tóth, Anett Mázló, Éva Rajnavölgyi
Department of Immunology, University of Debrecen, Hungary

Introduction: Dendritic cells (DCs) are essential part of the innate immunity and play an essential role in mediating adaptive immune responses against microbes. The resident gut microbiota is able to modulate the outcome of antiviral immune responses at mucosal surfaces by producing short-chain fatty-acids such as butyrate. The butyrate has beneficial effects on different physiological systems in the host including fluid balance, immunity, cell proliferation and differentiation. These effects proceed through the activation of fatty acid receptors that results in inhibition of histone deacetylases. We aimed to study the antiviral responses of monocyte-derived DCs (moDCs) differentiated in the presence or absence of butyrate followed by the activation with synthetic viral RNA.

Methods: Human monocytes were isolated from leukocyte-enriched buffy coats and differentiated to moDCs in serum free AIM-V medium for three days in the presence of GM-CSF, IL-4 and in the presence or absence of butyrate. We activated the resting moDCs with 10 μg/ml poly(I:C) for 24 hours followed by the removal of supernatants and harvesting the cells for quantitative real time PCR analysis and flow cytometry. The concentration of produced cytokines and chemokines was measured by ELISA. To examine the T cell activating capacity of the moDCs we used ELISPOT assay and flow cytometry.

Results: CD14+ Monocytes express fatty acid receptors for butyrate. The butyrate inhibited the cell surface expression level of CD1a and CD1c glycolipid receptors while that of DC-SIGN, CD14 and CD1d remained unaffected. We also found that in the presence of butyrate the protein level of molecules mediating type I interferon and pro-inflammatory cytokine secretion was modified by SB. Furthermore, we established that butyrate decreased the Th1 polarizing capacity of poly(I:C)-stimulated moDCs while did not affect the priming of cytotoxic T cells.

Summary: The short-chain fatty acid butyrate proves the indirect effects of the gut microbiota on educating local innate immune cells and affects the antiviral capacity of moDCs.

THE EFFECT OF SOLUBLE FACTORS PRODUCED BY TUMOR CELLS ON THE DIFFERENTIATION OF DENDRITIC CELLS

Ramóna Bíró-Kovács, Anett Türk-Mázló, Éva Rajnavölgyi
Department of Immunology; University of Debrecen Faculty of Medicine, Hungary

Introduction: Dendritic cells functioning as professional antigen presenting cells have potential to alter the developing immune responses through the modulation of T cell polarization. In the last few years increasing number of studies have published highlighting the regulatory effects of environmental factors including cytokines, chemokines and metabolits in the differentiation of dendritic cells ensuring the immune homeostasis. The balance between the immune reaction and tolerance could be overwhelmed in case of tumors. Our goal was the characterization of monocyte derived dendritic cells (moDCs) differentiated in the presence of conditioned media (CM) collected from different tumor cell lines.

Methods: To approach this purpose tumor cell lines (MDA, CCRF, Raji, HT-29, MDA, HeLa, WM-35) were cultured in RPMI media and after 48 hours the conditioned media (CM) was collected. Freshly isolated CD14+ monocytes were cultured in the presence of tumor derived factors containing media completed with interleukin (IL)-4 and granulocyte-monocyte colony stimulating factor (GM-CSF). On the day fourth moDCs were characterized by flow cytometry and their cytokine production was measured by ELISA. Our aim was to compare the ability of conditioned moDCs to drive the T cells polarization performed by using flow cytometry.

Results: According to our results the CM collected from different tumor cell lines able to modulate the differentiation of moDCs resulting various changes in the phenotype and function of dendritic cells. moDCs differentiated in WM35-CM can be characterized with up-regulated expression of CD103, co-inhibitory CTLA-4, PD-L1 and co-stimulatory molecule CD86. Interestingly, the expression of CD1a molecule on moDCs was inhibited in all cases but the appearance of CD1d molecule was significantly enhanced by WM35 tumor cell line released factors. moDCs generated in the presence of tumor-derived CM expressed CD11b and CD11c molecules but at lower levels. moDCs differentiated in HeLa-, WM35-, CCRF- or HT29- derived CM, were able to induce the production of IL-4 and IL-17 by allogenic T lymphocytes while the moDCs generated in the presence of HeLa or MDA tumor cell line factors were able to increase the IFNγ production by T cells.

Conclusion: According to our findings we can state that different types of tumors could alter the differentiation of moDCs leading to the generation of dendritic cells associated with different phenotype and functions. Based on our results we can confirm the importance of the personalized therapies in case of different types of tumors. The characterization of dendritic cells could provide useful information in the anti-cancer cures resulting more effective anti-tumor responses.

CORONIN-1A IN PSORIASIS

Renáta Bozó[1], Lilla Erdei[1], Judit Danis[2], Beáta Szilvia Bolla[1], Zsuzsanna Bata-Csörgő[1, 2], Lajos Kemény[1, 2], Gergely Groma[1, 2]
[1]University of Szeged, Department of Dermatology and Allergology, Szeged, Hungary
[2]MTA-SZTE Dermatological Research Group

Introduction: Coronin-1A is an actin binding protein that regulates different T-cell functions including activation, homeostasis, survival and migration. We performed a large scale proteomic analysis, where the psoriatic non-lesional (NL), lesional (L) and the healthy (H) skin proteome were compared. The protein expression of CORO1A was higher in the NL and the highest in the L skin compared to H controls. CORO1A is known to plays an important role in activated T-cell dependent immune disorders. Thus, we aimed to investigate the involvement of CORO1A in psoriasis.

Methods: Expressional differences of CORO1A were validated in three different skin samples (H, NL and L) by Western blot analysis and cell types positive for CORO1A in skin were determined by immunofluorescence staining. The expression of CORO1A was also examined in low and in high Ca^{2+} cultured keratinocytes by RT-PCR and Western blot. The role of CORO1A in keratinocytes was examined by siRNA mediated gene silencing.

Results: Western blot and immunofluorescence staining analysis of CORO1A confirmed our proteomic results. CORO1A expression was the highest in CD3+ cells, Langerhans cells showed a lower intensity and the lowest staining was detected in keratinocytes in all investigated skin samples. Among keratinocytes cells in the basal layers often showed a more intense CORO1A staining than cells in the differentiated layers. Similarly, in culture, Ca^{2+} induced differentiation of keratinocytes led to the decrease of CORO1A levels compared to Ca^{2+} untreated cells. CORO1A gene silencing compromised the motility of keratinocytes without any obvious influence on morphology or proliferation rate.

Conclusion: Our results indicate that the expression of the immune cell regulator CORO1A is elevated in both NL and L skin similarly to other immune mediated diseases. In psoriasis the minor and a more extensive infiltration of immune cells in the NL and L skin respectively, is likely to be the major reason for the differential CORO1A expression detected by our analysis. However, we also show that some non-professional immune cells like keratinocytes also express CORO1A, similarly to professional immune cells, although at a lower level. In contrast to T cells, silencing of CORO1A did not affect the proliferation and cell survival of keratinocytes but their cellular motility was compromised. Apart from the already described role of CORO1A in the regulation of T cell functions, an altered motility of keratinocytes may additionally contribute to the characteristic epidermal changes in the psoriatic plaques.

CHARACTERIZATION OF THE LYMPHATIC VASCULATURE IN ATHEROSCLEROSIS

Carolin Christ[1, 2] and Zoltán Jakus[1, 2]
[1]Department of Physiology, Semmelweis University School of Medicine, Budapest, Hungary
[2]MTA-SE „Lendület" Lymphatic Physiology Research Group of the Hungarian Academy of Sciences and the Semmelweis University, Budapest, Hungary

Introduction: Lymphatic vessels are present in the adventitia of the arterial wall, including the aorta, but the physiological and pathophysiological role of these vessels is not fully understood. Recently, it has been shown, that lymphatic vessels play a role in reverse cholesterol transport, in which cholesterol is removed from peripheral tissues and transported to the liver for excretion. Therefore, reverse cholesterol transport mediated by the lymphatic vessels might be an important modulator of the development of atherosclerosis. In this study we aimed to characterize the morphology and growth of the lymphatic vasculature in atherosclerosis.

Methods: $Prox1^{GFP}$ and $Flt4^{YFP}$ lymphatic reporter mice were used to monitor lymphatic growth in the wall of the great vessels. In parallel, $Ldlr^{-/-}$ and $ApoE^{-/-}$ mice were used to characterize the lymphatic morphology and growth in atherosclerosis. For these experiments, age- and gender-matched littermates were set on a high-fat or control diet and their aortas were isolated after 18-22 weeks. To analyze the lymphatic vasculature and plaque formation in atherosclerosis, paraffin-based histology was used followed by regular staining (e.g. H&E) and immunostaining of lymphatic and other markers. Tissue clearing followed by immunostaining of lymphatic endothelial cell markers was also performed to visualize the lymphatic vasculature. The lipid deposit in the aortic wall was stained by an Oil-Red-O staining.

Results: In our experiments we demonstrated the presence of lymphatic vessels in the adventitia of the aorta. We found several lymphatic vessels in connection to the thoracic and lumbar aorta, but less lymphatic structures in the aortic arch. Both knockout mouse models ($Ldlr^{-/-}$ and $ApoE^{-/-}$) developed severe atherosclerosis after 18-22 weeks on a high-fat diet, and our experiments indicated the presence of the largest atherosclerotic plaques in the aortic arch. The atherosclerotic mice on high-fat diet showed an increased number of lymphatic vessels in the adventitia of the arterial wall in comparison to the mice on control diet.

Conclusion: Our results suggest the possible role of the lymphatic vasculature in the modulation of the development of atherosclerosis. In our current experiments we are using genetic loss of function and gain of function approaches to block and stimulate lymphatic growth to define the role of the lymphatics in the pathogenesis of atherosclerosis. Revealing the role of lymphatic function in the pathogenesis of atheosclerosis may lead to the development of novel therapeutic approaches in the future.

A HIGHLY EFFICIENT IMMUNE RESPONSE IN THE INVASIVE ZAPRIONUS INDIANUS

Gyöngyi Cinege[1], Zita Lerner[1], Bálint Soós[1], Ildikó Kristó[2], Attila L. Kovács[3], Renáta Tóth[1], Gábor Juhász[3], Zoltán Hegedűs[4], Éva Kurucz[1], István Andó[1]
[1]Immunology Unit, Institute of Genetics, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary
[2]Developmental Genetics Unit, Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary
[3]Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest, Hungary
[4]Laboratory of Bioinformatics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

Introduction: The cellular immunity of insects involves a coordinated expression of effector functions as the phagocytosis of microbes, and the encapsulation of bigger invaders. Different hemocyte subpopulations are responsible to exert these cellular immune functions. We characterize the blood cell types of the invasive Zaprionus indianus species from Drosophilidae and study their role in the cellular immune responses.

Methods: Maintenance of several parasitoid wasps and Z. indianus; development of monoclonal antibodies to hemocyte subsets; Western blot; immune induction and infection assays of Z. indianus larvae; preparation of FITC labeled bacteria; phagocytosis assays; indirect immunofluorescence assays; FACS analysis; electron microscopy.

Results: We characterized the blood cells of Z. indianus of the vittiger subgroup of Drosophilidae. We observed that plasmatocytes take up Gram-positive and -negative bacteria and a novel hemocyte subset, the multinucleated giant hemocytes (MGH), encapsulate parasitoid wasp eggs, but do not take up bacteria by phagocytosis. The MGH fulfill the role of the lamellocytes described in Drosophila melanogaster. MGH are formed with cell fusion in the circulation and also in the lymph gland. We carried out detailed cytological and developmental studies and compared the development of these cells with the encapsulating cells of D. melanogaster and Drosophila ananassae. Moreover electron microscopic analysis of the MGHs revealed a unique ultrastructure of this hemocyte type.

Conclusion: Z. indianus uses MGH for the encapsulation of large foreign particles. These cells are extremely efficient in the elimination of parasites and have unique developmental and ultrastructural features, not observed so far in any other organism.

Acknowledgements: This research was supported by grants from the Hungarian National Science Foundation, NKFI K128762, NKFI K120142, NKFI K120140, GINOP-2.3.2-15-2016-00001 and GINOP-2.3.2-15-2016-00035.

POSSIBLE MECHANISMS REDUCING SYMPTOMES OF ARTHRITIS IN ARHGAP25 KNOCKOUT MICE

Roland Csépányi-Kömi, Domonkos Czárán, Réka Pusztai, Péter Sasvári, Ana Redrado Osta, Tim Svanya, Petra Lévay, Éva Wisniewski, Krisztina Ella, Ágnes Sűdy, Erzsébet Ligeti
Semmelweis University, Department of Physiology, Budapest, Hungary

Introduction: ARHGAP25 is a leukocyte specific GTP-ase activating protein (GAP) characterized by our research group. Due to its reduction of Rac activity it has a central role in regulating different neutrophilic functions including phagocytosis, superoxide production and transendothelial migration as we showed in earlier studies. Moreover, lack of ARHGAP25 caused a remarkable decrease in the signs of inflammation (e.g. ankle thickness, clinical score and function of the limbs) in mice that suffered in rheumatoid arthritis. In arthritic, ARHGAP25 knockout mice, we observed reduced neutrophil infiltration into ankle joints and in these neutrophils, filamentary actin level was decreased. In this study, we investigated the possible reasons which may lead to reduced arthritic symptoms in ARHGAP25 knockout mice.

Methods: In our experiments we used the K/BxN serum transfer arthritis mouse model. Bone marrow chimeras were generated with lethal irradiation (11.5 Gy) of recipient animals (CD45.1+, CD45.2–) followed by i.v. injection of Arhgap25–/– (KO) or Arhgap25+/+ (WT) bone marrow cell suspensions (CD45.1–, CD45.2+), or the 1:1 mixture of KO and WT cells (in case of mixed bone marrow chimeras). This generates wild type mice containing only transgenic hematopoietic cells. Efficiency of repopulation of hematopoietic compartment was determined by FACSCalibur flow cytometer analyzing the expression of CD45.1 and CD45.2. Bone marrow chimeras were used 4–8 weeks after transplantation. Measurement of superoxide production was carried out using the Cytochrome C reduction assay.

Results: After arthritic serum treatment, bone marrow chimeras carrying KO hematopoietic cells showed decreased signs of arthritis (ankle thickness, clinical score and function of the limbs) compared to those, which carry WT hematopoietic cells. Using mixed bone marrow chimeras, we revealed, that cell-autonomous migration of KO neutrophils was increased compared to WT, however, in full knockout animals, we observed decreased infiltration of KO neutrophils into ankle joints. Investigating the superoxide production on immune-complex surface, we did not find any significant differences between KO and WT bone marrow derived neutrophils.

Conclusion: Our previous observation that ARHGAP25 deletion mitigates the symptoms of rheumatoid arthritis, cannot be explained by the altered migration, or superoxide production of ARHGAP25 knockout neutrophils. In further experiments, we turn to the examination of possibly changed cyto-

kine environment or altered macrophage functions in the KO animals to reveal the role of ARHGAP25 in rheumatoid arthritis.

Funding: NKFIH K108382119236, Hungary

FUNCTIONAL CHARACTERIZATION OF DISEASE ASSOCIATED VARIANTS OF HUMAN COMPLEMENT FACTOR H-RELATED PROTEIN 5

Marcell Cserhalmi[1], Barbara Uzonyi[1], Dorottya Csuka[2], Katalin Uray[3], Attila Iliás[1], Zoltán Prohászka[2], Mihály Józsi[1]
[1]Department of Immunology, Eötvös Loránd University, Budapest, Hungary
[2]3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary
[3]MTA-ELTE Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Eötvös Loránd University, Budapest, Hungary

Background: Complement is a major humoral arm of innate immunity that plays important roles in the protection against infections, regulation of inflammation and disposal of immune complexes and cellular waste. Dysregulation of the complement alternative pathway is involved in the pathogenesis of several diseases, including the kidney diseases atypical hemolytic uremic syndrome (aHUS) and C3 glomerulopathy (C3G). Factor H is a main inhibitor of the alternative pathway; however, the role of the factor H-related FHR proteins is less characterized. FHR5 consists of 9 complement control protein (CCP) domains that are homologous to CCPs 6-7, 10-14 and 19-20 of FH. FHR5 was described to bind C-reactive protein, pentraxin 3 and C3b. Our aim was to map the ligand binding sites in FHR5 and functionally characterize two known FHR5 mutations, FHR5 G278S and R356H.

Methods: Wild type and mutant FHR5 were expressed in insect cells. Recombinant FHR5 mutants were analyzed and compared with the wild type protein for C3b binding by surface plasmon resonance assay. Binding studies were also performed by ELISA. Fifteen amino acid long, overlapping peptides of FHR5 CCPs 3-9 and mutant peptides were synthesized in duplicates on Mimotopes NCP gears and analyzed for ligand binding in pin ELISA.

Results: Surface plasmon resonance experiments showed that FHR5 G278S and R356H association (k_a) to C3b was decreased, but FHR5 R356H dissociation (k_d) from C3b was slower, while that of G278S was faster than the wild type FHR5. Similar results were obtained by ELISA, indicating weaker C3b binding by FHR5 G278S. Epitope mapping revealed that C3b binding to FHR5 peptides with K144N, V170M, N178S was increased and binding of monomeric C-reactive protein (mCRP) to the R356H and M514R mutant peptides was decreased compared to wild type peptides.

Conclusion: Altogether, our results identified amino acid residues in FHR5 that are involved in C3b and mCRP binding. We identified altered interaction with C3b for two FHR5 mutant proteins by surface plasmon resonance and reduced mCRP binding to several mutant FHR5 peptides. The data suggest a possible role of FHR5 and these ligand interactions in the pathogenesis of aHUS and C3G.

Supported by the Kidneeds Foundation (Iowa, US), the Hungarian Academy of Sciences (Lendület program, grant nr. LP-2012/43), the Institutional Excellence Program of the Ministry of Human Capacities of Hungary, KMOP-4.2.1/B-10-2011, and (to D.Cs.) NKFIH grant PD116119.

ASSESSMENT OF THE C3b- AND iC3b-BINDING ABILITY OF CFHR5 VARIANTS

Dorottya Csuka[1], Nóra Garam[1], Nóra Veszeli[1], Mihály Józsi[2], Marcell Cserhalmi[2], Barbara Uzonyi[2], Michael Rudnicki[3], Gere Sunder-Plassmann[4], Alice Schmidt[4], George S Reusz[5], Zoltán Prohászka[1]
[1]3rd Department of Internal Medicine, Semmelweis University, Budapest
[2]Complement Research Group, Department of Immunology, Eötvös Loránd University, Budapest, Hungary
[3]Department of Internal Medicine IV - Nephrology and Hypertension, Medical University Innsbruck, Innsbruck
[4]Medical University of Vienna, Department of Medicine III, Division of Nephrology and Dialysis, Vienna
[5]1st Department of Pediatrics, Semmelweis University, Budapest

Introduction: Atypical hemolytic uremic syndrome (aHUS) and C3-glomerulopathies (C3G) are related to the dysregulation of the alternative complement pathway in the majority of cases, which might result from mutations of certain (complement) genes. Having a weak complement regulatory activity, CFHR5 competes for C3b binding with factor H, but scarce data is available on its physiological functions. Thus, we intended to study the C3b- and iC3b-binding ability of CFHR5 variants identified in patients with aHUS or C3G.

Methods: Altogether 89 C3G patients and 108 aHUS patients were screened for mutations in CFHR5 by Sanger-sequencing. After measuring the CFHR5 levels in the patients' sera, an in-house ELISA was developed for quantifying the C3b- and iC3b-binding ability of the different CFHR5 variants. In brief, plates were coated with purified human C3b or iC3b fragments. Thereafter, serum samples of subjects with or without CFHR5 mutations were added (1 h, 37 °C) and the CFHR5 bound to C3b or iC3b was detected using monoclonal mouse anti-human CFHR5 antibody.

Results: Altogether 17 different CFHR5 variations were identified in 22 aHUS and in 23 C3G patients and of them, several patients carried the P46S (n=7), V110A (n=7), K144N (n=4), G278S (n=4) and R356H (n=14) variations. A weaker binding of the R356H variant (median: 45.2%) compared to the wild-type CFHR5 protein was detected, and an even weaker binding was observed for the G278S mutant (24.5%).

Two patients carried a hypothesized CFHR2-CFHR5 hybrid protein that exhibited an increased binding to the C3b fragments. When coating the plates with iC3b, similar results were obtained.

Conclusions: Our study showed that serum level of CFHR5 strongly influences its binding capacity to C3b or iC3b, and the binding ability is further altered in case of specific CFHR5 mutations that may have a functional role for the initiation of renal injury in aHUS or in C3G, especially in those cases, where no additional mutation was identified in the other disease-associated genes.

Support: National Research Fund of Hungary (OTKA PD116119) and Bolyai János Research Fellowship (2015-2018).

INVESTIGATION OF PROTEIN BIOMARKERS FOR HUMAN GLIOBLASTOMA MULTIFORME

Gabriella Dobra[1], Matyas Bukva[1], Almos Klekner[2], Zoltan Szabo[3], Bella Bruszel[3], Krisztina Buzas[1, 4]
[1]Laboratory of Microscopic Image Analysis and Machine Learning, Institute of Biochemistry, Biological Research Centre of Hungarian Academy of Sciences, Szeged, Hungary
[2]Department of Neurosurgery, Clinical Centre, University of Debrecen, Debrecen, Hungary
[3]Department of Medical Chemistry, University of Szeged, Hungary.
[4]Department of Oral Biology and Experimental Dental Research, Faculty of Dentistry, University of Szeged, Szeged, Hungary

Introduction: Glioblastoma multiforme (GBM) is the most common malignant disease of the central nervous system. Its prognosis is unfavorable, and the median overall survival of patients is 16 to 24 months. One of the important goals of oncology is to develop biomarkers that can be identified through simple noninvasive or less invasive methods with the potential to identify cancer risk, possibility to diagnose early and utility in accurate grading and treatment monitoring. Serum is a rich source of biomarkers, and it can be obtained by a less invasive procedure. The aim of this study was to identify protein molecules that can serve as potential serum biomarker for the clinical management of glioma patients.

Methods: Based on the literature, we created a list of feasible GBM and invasion related protein biomarkers. Preliminary tests on GBM tissue samples were performed using mass spectrometry to identify the selected proteins. Six GBM and five control serum samples were pooled and depleted from the top 12 abundant protein. Expression levels were measured by mass spectrometry to pre-select the 20 investigated molecules. To determine the differences in the levels of the selected proteins, the GBM and the normal control serum samples were individually analyzed by Western blot analysis.

Results: In this research, 20 molecules were selected for the analysis including proteolytic enzymes, extracellular matrix proteins, cell adhesion molecules, cytoskeletal components and glia specific elements. We found considerable difference between the analyzed samples for some of the 20 proteins. We identified three cancer-related molecules as upregulated proteins in GBM serum samples compared to the healthy controls.

Discussion: During the last decade large number of serum biomarkers has been identified. However, their individual usage is limited, their reproducibility between studies is unclear, and none have been validated for clinical use. The systematic use of biomarkers could be used to form a framework for better prediction, prognosis and treatment selection. Our results bring some protein molecules into focus that can be used for the noninvasive diagnosis of GBM.

TYROSINE KINASE PATHWAYS IN MONOSODIUM-URATE CRYSTAL-INDUCED INFLAMMATORY RESPONSES

Krisztina Futosi, Tamás Németh, Attila Mócsai
Department of Physiology, Semmelweis University School of Medicine, Budapest, Hungary and MTA-SE "Lendület" Inflammation Physiology Research Group of the Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary

Background: Deposition of monosodium urate (MSU) crystals in the joints or other tissues is a hallmark in the pathogenesis of gout. The urate crystal-induced inflammation is known to be mediated mainly by neutrophils besides monocytes and macrophages. Although MSU crystal-mediated signal transduction is in the focus of recent investigations, the molecular mechanism is only partially characterized.

Objectives: In this study, we investigated the role of Src family kinases in MSU crystal-induced in vitro activation of primary murine and human neutrophils and their in vivo significance in experimental model of gout.

Methods: Bone marrow isolated neutrophils from wild type and triple Src family kinases-deficient ($Hck^{-/-}Fgr^{-/-}Lyn^{-/-}$) mice or vehicle and Src-inhibitor treated human neutrophils were stimulated with different concentration of MSU crystals. The superoxide production of the cells was measured by a luminometric assay, while the supernatants of MSU crystal activated cells were analyzed by using various enzyme-linked immunosorbent assay (ELISA) kits. The phagocytosis of the urate crystals by neutrophils was followed by videomicroscopy and flow cytometry. Gouty arthritis was induced by injection of MSU crystals into the hind paws of the experimental mice and was assessed by ankle thickness measurements and detection of the synovial cytokine levels by ELISA.

Results: The MSU crystal-induced superoxide release, cytokine production and the crystal-phagocytosis were abrogated in Src family kinases-deficient murine or in Src-inhibitor trea-

ted human neutrophils. In contrast to wild type animals, Src family kinases-deficient mice showed significantly decreased paw swelling and neutrophil accumulation at the site of inflammation. In line with this, the synovial levels of interleukin-1β and CXCL2 were also strongly reduced in Src family kinases-deficient mice compared to wild type animals.

Conclusions: Src family kinases play an indispensable role in MSU crystal-induced superoxide and cytokine production as well as crystal-phagocytosis of neutrophils. The fact, that Src family kinases-deficient mice are partially protected from crystal-induced inflammatory reactions indicate the important role of these kinases in in vivo gouty arthritis. Identification of these key players in urate crystal-induced intracellular signaling pathways in neutrophils leads to a better understanding of the pathogenesis of gout.

Support: This project is supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences and K.F. is a recipient of the Bolyai+ Fellowship (ÚNKP-18-4).

MONOCLONAL ANTIBODIES FOR THE CHARACTERIZATION OF HEMOCYTE SUBPOPULATIONS IN THE HONEY BEE, APIS MELLIFERA

Erika Gábor[1], Gyöngyi Cinege[1], Gábor Csordás[1], Tibor Török[2], Katalin Folkl-Medzihradszky[1], Zsuzsanna Darula[1], Éva Kurucz[1], István Andó[1]
[1]Biological Research Centre of the Hungarian Academy of Sciences, Szeged
[2]Department of Genetics, University of Szeged, Szeged

Introduction: The honey bee, Apis mellifera, is a social insect which lives in highly structured colonies. In a colony there are three casts: the worker, the drone and the queen. Nowadays, the bee specific parasites and pathogens and the pesticides used in the agriculture are endangering beekeeping and making great ecological and economical losses worldwide. As a social insect honey bee has communal and individual defense against these negative effects. The individual defense is the immune system, which involves the humoral and the cell-mediated responses. In insects the blood cells, so called hemocytes, are responsible for the cell mediated immune reactions, by phagocytosis, encapsulation and producing antimicrobial peptides. Until now the hemocytes of the honey bee were described only by morphological and lectin binding characteristics, the mechanisms of cellular immunity are not yet understood in details due to limited information about the hemocyte subtypes, their functions and differentiation. The aim of our research is to develop a toolkit to identify immunological markers by monoclonal antibodies for developmental, cast specific and functional subsets of the hemocytes.

Methods: 'In vivo' and 'in vitro' immunological and molecular technics were used, such as: hybridoma production, immunofluorescence and immunohistochemical staining, Western-blot, LC-MS/MS analysis, RNA interference and FACS.

Results: We developed monoclonal antibodies against honey bee hemocytes which distinguished three main hemocyte types. The oenocytes are responsible for the melanization, the granulocytes are able to phagocytose microorganisms and the plasmatocytes can form cell-aggregates around particles which are too large to be taken up by phagocytosis. The expression of the molecular markers is changing through developmental changes, but it is the same between the three casts. We have identified the honey bee hemolectin produced by the plasmatocytes.

Conclusion: The hemocyte marker panel allows to study the cell-mediated immunity of the honey bee and could be used also for the identification and characterization of hemocyte lineages and hematopoietic tissues.

Support comes from NKFI K 120140, GINOP-2.3.2-15-2016-00001 and GINOP-2.3.2-15-2016-00035.

MADCAM-1 ADDRESSIN IS DISPENSABLE FOR PERIPHERAL LYMPH NODE ACTIVITY IN MICE.

Fanni Gábris[1], Zoltán Kellermayer[1], Angela Schippers[2], Norbert Wagner[2], Péter Balogh[1]
[1]Department of Immunology and Biotechnology, University of Pécs;
[2]University Hospital RWTH Aachen

Introduction: The development of peripheral lymph nodes is dependent on recruitment of α4β7-expressing lymphoid tissue inducer (LTi) cells to MAdCAM-1+ stromal anlagen. MAdCAM-1 is also expressed in follicular dendritic cells (FDCs) during germinal center (GC) reaction. We have previously shown that peripheral lymphoid tissues develop with a normal structure in MAdCAM-1−/− mice. In this work we investigated the function of peripheral lymph nodes upon subcutaneous immunization. We also analyzed the number of RORγt+ LTi cells in different secondary lymphoid tissues at various postnatal ages and examined their addressin expression pattern.

Materials and methods: We used C57BL/6 and MAdCAM-1-deficient mice throughout our work. Adult mice received ovalbumin (OVA) with CFA in their left footpads on day 0 and day 14 and were sacrificed on day 21. Anti-OVA IgG was measured from serum with ELISA. The GC response was analyzed in popliteal lymph nodes with immunofluorescence using antibodies against CD21 to identify FDCs and lectin-histochemistry against Peanut agglutinin to identify GCs. Numbers of ILC3 (CD45+ CD3- RORγt+ Thy1+) and LTi cells (CD45+ CD3− RORγt+ Thy1+ CD4+) were determined from inguinal, brachial, axillary, popliteal, and mesenteric lymph nodes from 1 week old, 2 week old, 4 week old and adult mice.

Results: Upon immunization with ovalbumin we observed that anti-OVA IgG levels did not differ between MAdCAM-1−/− and C57Bl/6 control sera. In both genotypes the ratio of CD21+PNA+ GCs increased in popliteal lymph nodes compared to contralateral and untreated popliteal lymph nodes and

did not differ significantly between the two mouse strains. In the early postnatal period, number of ILC3 and LTi cells was generally higher in all examined peripheral lymphoid tissues in the absence of MAdCAM-1.

Conclusions: In MAdCAM-1–/– mice peripheral lymph nodes developed and functioned similarly to normal, raising the possibility that ILC3/LTi cells utilize other addressins to home to developing lymph node anlagen in the absence of MAdCAM-1. However, the increased number of these cells might be a result of an imperfect switch to other homing mechanisms. Further research into MAdCAM-1-independent lymphoid tissue formation may be relevant to understanding ectopic lymphoid tissue development in chronic inflamma-tory conditions.

CIRCULATING EXOSOMAL MIRNA PROFILE OF LIFESTYLE INTERVENTION

Kitti Garai[1, 2], Krisztina Banfai[1, 2], Zoltan Adam[3], Adam Gyebrovszki[3], Emese Katai[4], Tamas Nagy[2, 4], Judit E. Pongracz[1, 2], Marta Wilhelm[3], Krisztian Kvell[1, 2]
[1]Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Pecs
[2]Szentagothai Research Center, University of Pecs
[3]Department of Sport Sciences and Physical Education, Faculty of Science, University of Pecs
[4]Department of Laboratory Medicine, University of Pecs

Introduction: Regular physical activity is known to delay senescence-related decline, but lifestyle-related immune and molecular alterations due to physical inactivity in younger population are not well described yet. Previous results suggest that exosomes and small vesicles are released into circulation with exercise and may be involved in exercise-mediated adaptation processes. However, the long-term impacts of regular physical activity on exosome miRNA in young and healthy, but previously inactive individuals have not been addressed thoroughly.

Methods: Our study aimed to examine the effects of a 6-month long lifestyle intervention program on physiological parameters, exosomal miRNA and cytokine profile. Physically inactive, but otherwise healthy young individuals were subjected to 1h training three times a week. Exosomal miRNA was isolated from serum samples before lifestyle intervention, after 3 months and 6 months of regular physical activity. Exosome miRNA samples were evaluated with NanoString miRNA platform.

Results: Six month-long training improved the aerobic capacity of each participating individual. Moreover, we could detect significant changes in several blood parameters including glucose, insuline, cholesterol and cortisol levels. Improvement in the health status of individuals was also supported by the decrease of C reactive protein (CRP) levels and inflammatory cytokines. Exosome miRNA profiling showed a significant number of microRNAs being altered in response to 6 months of regular physical activity.

Summary/Conclusion: A profuse number of exosomes are present in human serum that show significant change in response to long-term regular physical activity. Our exosomal miRNA analysis revealed a chronic disease-preventing molecular pattern with unique epidemiological and global health significance.

Funding: Research was funded by ÚNKP 2018/2019 to KG and Bolyai+ 2018/2019 to KK.

VALIDATION OF PATHOGENIC PATTERNS IN A NOVEL COHORT OF PATIENTS WITH MEMBRANOPROLIFERATIVE GLOMERULONPEHRITIS (MPGN) BY A CLUSTER ANALYSIS

Nóra Garam[1], Zoltán Prohászka[1], Michael Rudnicki[2], Christof Aigner[3], Adrian Catalin Lungu[4], Jana Reiterova[5], Tanja Kersnik Levart[6], Ágnes Haris[7], Ágnes Hartmann[8], György Reusz[9], Kata Kelen[9], Csaba Bereczki[10], Dorottya Csuka[1]
[1]Research Laboratory, IIIrd Department of Internal Medicine, and MTA-SE Research Group of Immunology and Hematology, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary
[2]Dept. of Internal Medicine IV – Nephrology and Hypertension, Medical University Innsbruck, Innsbruck, Austria
[3]Division of Nephrology and Dialysis, Department of Medicine III, Medical University of Vienna, Vienna, Austria
[4]Fundeni Clinical Institute, Pediatric Nephrology Department, Bucharest, Romania
[5]Department of Nephrology, First Faculty of Medicine, Charles University and General Teaching Hospital, Prague, Czech Republic
[6]University Medical Centre Ljubljana, Division of Pediatrics, Department of Pediatric Nephrology
[7]Department of Nerhrology, Szent Margit Hospital, Budapest, Hungary
[8]Department of Pediatrics, University of Pécs, Pécs, Hungary
[9]1st Department of Pediatrics Semmelweis University, Budapest, Hungary
[10]Department of Pediatrics, University of Szeged, Szeged, Hungary

Introduction: Membranoproliferative glomerulonephritis (MPGN) includes – beside of immuncomplex glomerulonephritis (ICGN) – and C3 glomerulopathy (C3GP). The immunfluorescence microscopy shows two magnitude stronger C3 staining than any other immunoreactant. At the background of the disease acquired and genetic factors could be identified but in most of the cases none of them could be found. Our aim was to identify new pathogenic factor and with the analysis of the patients clinical and histological data get new insights in this pathology.

Methods: 92 patients with MPGN were included in the study. We collected the patients' clinical and histological data and we performed the complement testes such as activation, regulators level, antibodies and genetic results. We perfor-

med new methods for the detection of C4 nephritic factor (C4NeF). We performed cluster analysis to confirm results of an Italian study.

Results: 4 clusters were generated with Ward method. High level of sC5-b9, low C3 levels, few crescent formation and young age at onset (15.6±7.9 years) was characteristic for cluster 1, whereas for cluster 2 strong IgG staining, low C3 levels and high prevalence of nephritic syndrome at disease onset was observed. Low plasma sC5-b9 level and high prevalence of likely pathogenic variants (LPV) was present in cluster 3, and patients with late onset of the disease (47.24±12.6 years), near-normal C3 level and low prevalence of LPV and/or C3Nef were in cluster 4. 9 C4NeF positive patients were included in the study from whom 7 were classified into cluster 1.

Conclusion: Our results confirm the main findings of the original cluster analysis and indicate that the observed distinct pathogenic patterns replicate in our cohort. Further investigations are necessary to analyze the distinct biological and pathogenic processes in these groups of patients.

Support: This work was supported by the Hungarian Scientific Research Fund (116119).

IMMUNE MEDIATED SKIN INFLAMMATION IS SIMILAR IN SCALP AND SKIN PSORIASIS

Krisztián Gáspár[1, 2], Adrienn Jenei[1, 2], Ahmed Khasawneh[1, 2], Zsolt Dajnoki[1, 2], Barbara Medgyesi[1, 2], Zoltán Hendrik[3], Gábor Méhes[3], Andrea Szegedi[1, 2], Anikó Kapitány[1, 2]2
[1]Division of Dermatological Allergology
[2]Department of Dermatology
[3]Department of Pathology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Introduction: Scalp psoriasis shows variable clinical features, moreover its treatment often possesses great challenge. It is unknown whether the immune mechanisms of scalp psoriasis, localized on oily-sebaceous gland rich (SGR) area, are different from that of psoriasis in other skin area. Since earlier we have found distinct immune and barrier features on healthy SGR skin compared to sebaceous gland poor (SGP) regions, we aimed to compare, whether scalp psoriasis, developing on SGR skin, shares similar immunological mechanisms as psoriasis in other (on SGP) skin regions or they also differ depending on their localization.

Methods: Skin samples from psoriatic scalp and from other skin regions were obtained and the number of immune cells and the expression of different Th17 related chemokines (CCL2, CCL20), antimicrobial peptides (AMPs, S100A7/8/9, LCN2) and barrier molecules (AHR, FLG, LOR, KRT17) were investigated by immunohistochemistry or qPCR.

Results: We have found no significant differences in the amount of $CD4^+$ T cells and $CD11c^+$ mDC, while significantly elevated $CD1a^+$ Langerhans cell count were detected in scalp psoriasis compared to psoriasis in other skin area. Regarding the expression of Th17 related AMPs, chemokines and also barrier molecules no significant differences were found between the investigated two groups, except for 2 molecules (CCL20 and LCN2), which showed significantly elevated levels in scalp samples.

Conclusion: According to our results the immune characteristics of psoriasis are identical on the different skin areas and independent from the initial immune characteristics of the disease affected region. Although the formulation of the local therapy can be different on scalp and other skin areas, but the active ingredients of both local and systemic treatment are suggested to be similar.

Supports: NKFIH K-128250, GINOP-2.3.2-15-2016-00050 and EFOP-3.6.1-16-2016-00022 projects. The project is co-financed by the European Union and the European Regional Development Fund and the European Social Fund. AK is recipient of the János Bolyai research scholarship of the Hungarian Academy of Sciences.

ABNORMAL TYPE VII COLLAGEN PROTEIN EXPRESSION IN NON-LESIONAL PSORIATIC SKIN

Barbara Gubán[1], Renáta Bozó[1], Judit Danis[1], Nikolett Miklovics[1], Balázs Koncz[1], Róbert Kui[1], István Németh[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]MTA-SZTE Dermatological Research Group, Szeged, Hungary
[3]University of Szeged, Department of Medical Genetics, Szeged, Hungary

Introduction: The healthy looking non-lesional (NL) skin already carries alterations that can contribute to the manifestation of psoriasis. These include extracellular matrix defects that have been observed in psoriatic NL skin, such as the disruption of laminin networks leading to impaired keratinocyte adhesion to the basement membrane. We aimed to further examine basement membrane alterations by focusing on the type VII collagen in NL skin of psoriatic patients.

Methods: Samples were collected from 12 patients, from lesional skin and from non-lesional skin from the edge of the lesion (non-lesional near) and from at least 6 cm far from the lesion (non-lesional far) and from 6 healthy volunteers for the experiments. Healthy, NL psoriatic and lesional psoriatic skin were immunostained for type VII collagen and STAT3. The expression of the type VII collagen mRNA and protein was examined in fibroblasts and keratinocytes by RT-qPCR and western blot, respectively. Type VII collagen pathway was investigated in cultured fibroblasts – derived from NL and healthy skin – by inhibiting STAT3 signaling.

Results: Type VII collagen is uniformly expressed in psoriatic lesional and in healthy skin, but not in NL skin. High PASI score patients were characterized by intensive type VII

collagen staining in the lesional and NL skin. In contrast, low PASI score patients showed nearly non-detectable type VII collagen in NL skin. The decrease of type VII collagen expression in NL skin corresponds to the distance from the lesion. Next, we determined type VII collagen mRNA expression in two major characteristic cell types of healthy and NL skin. Cultured fibroblast from NL skin showed reduced type VII collagen expression compared to the healthy fibroblast while there was no change observed in keratinocytes. The expression of type VII collagen was negatively regulated by activated STAT3 in healthy fibroblast, while upregulated in fibroblast derived from NL psoriatic skin. We demonstrated a high degree of STAT3 activation both in NL and lesional psoriatic skin but not in the healthy skin.

Conclusions: In the psoriatic NL skin, changes in basal membrane protein expressions could create a damaged, unstable dermal-epidermal junction zone that may manifest in a chronic inflammatory phenotype.

A NEW THEORY OF THE THYMIC STRUCTURE

Krisztina H.-Minkó[1], Ildikó Bódi[1], Emese Huszár[1], Nándor Nagy[1], Sophie Creuzet[2] and Imre Oláh[1]
[1]Semmelweis University, Faculty of Medicine, Department of Anatomy, Histology and Embryology, Budapest, Hungary
[2]Paris-Saclay Institute of Neuroscience, Paris, France

The thymus gland develops from three different sources. The thymic epithelial anlage is a derivative of the foregut endoderm. The branching epithelial cords of thymic primordium grows into the surrounding mesenchyme of neural crest (NC) origin. This epithelial-mesenchyme primordium is colonized by hemopoietic cells. The blood vessels reach the thymic tissue from the capsule and interlobular trabecules, the latter developing from the NC cells. The epithelial framework of thymus consists of reticular epithelial cells (RECs). The anti-cytokeratin immunostaining identifies two sub-compartments in the medulla. One is continuous with the cortical epithelial cells, which expresses keratin (keratin positive network-KPN) and the other does not show keratin staining (keratin negative area-KNA) and is devoid of RECs. The connective tissue space of interlobular septae is continuous with KNA, that is "a dilation" of the septae and the supporting tissue of the KNA is identical with the septae, which may indicate the common NC origin of KNA and septae.

The immunologically competent T cells from the KPN have to enter to the KNA, where the blood vessels and thymic dendritic cells are localized. The hemopoietic cells (T-cells, B-cells, dendritic cells) show a topographical arrangement in medulla, which indicates some cell-sorting mechanism at the KNA and KPN border.

According to our hypothesis, NC cells invade not only the capsule and septae of the thymus, but make up the KNA in the medulla. The precise origin of the KNA is unknown, so our aim is to show its NC origin and characterize the changes in thymic development of cranial NC ablated and transplanted chicken embryos and to obtain deeper knowledge about the exact structure of the functional cortico-medullary border, which selects the T-cells.

EXAMINATION OF THE EFFECT OF IMMUNOTHERAPY TO SKIN SENSITIZATION WITH ATOPY PATCH TEST

Krisztina Hajdu[1, 2, 3], Krisztián Gáspár[1, 2, 3], Zsolt Dajnoki[1, 2], Anikó Kapitány[1, 2], Andrea Szegedi[1, 2]
[1]Division of Dermatological Allergology
[2]Department of Dermatology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[3]these two authors are equally contributed to the work

Introduction & Objectives: Atopic dermatitis (AD) is one of the most common inflammatory skin diseases. To prove skin sensitization in AD atopy patch test (APT) is a suitable test. The effect of allergen specific immunotherapy (ASIT) in AD is still controversial and there is no data whether ASIT can change skin sensitization. The aim of this study was to examine the clinical and histological alteration of APT reaction in AD patients sensitized only with house dust mite (HDM) after 6 months of ASIT.

Materials & Methods: 14 mild and moderate AD patients were selected into the study who were only mono-sensitized to HDM and had allergic rhinitis with perennial clinical symptoms. All patients received local therapy for AD (moisturizer, low-medium potency local steroids). 7 patients were also treated with ASIT (sublingual immunotherapy to HDM). At the start of the study and in 6 months APT was evaluated. Scoring Atopic Dermatitis (SCORAD) of every patient was also measured. Biopsies were taken from APT at baseline (n=7), in 6 months of ASIT (n=4), besides biopsies from chronic lesional AD skin (n=6), chronic non-lesional AD skin (n=6), and healthy dry skin (n=6) were also harvested. Immunohistochemical (IHC) stainings of the skin samples were made. Measurement of filaggrin, thymus stromal lymphopoietin (TSLP), CD4, interferon-gamma (IFN-G), interleukin (IL)-13, IL-10, CD11c, CD83, CCL-17, IgE expression were evaluated and compared in skin.

Results: After 6 months APTs became negative of each patient received ASIT (Treated group), in contrast to patients who received only local therapy (Control group) of whom APT remained positive in all occasions. Objective SCORAD values reduced significantly in the Treated group compared to the Control group. With IHC the post-ASIT negative APTs showed similar FLG expression levels as healthy dry skin. Expressions of TSLP, CD4, IFN-G, IL-13, IL-10, CD11c, CD83, CCL-17, IgE were decreased in the negative APT compared to the positive APT and chronic lesional skin.

Conclusions: Skin sensitization could be the first step in the development of allergic rhinitis and bronchial asthma in

AD. Negative APT reaction after ASIT could indicate that the sensitization to the allergen may no longer exist.

Supports: NKFIH K-128250, GINOP-2.3.2-15-2016-00050 and EFOP-3.6.1-16-2016-00022 projects. The project is co-financed by the European Union and the European Regional Development Fund and the European Social Fund. AK is recipient of the János Bolyai research scholarship of the Hungarian Academy of Sciences.

HISTONE ACETYLATION AFFECTS MONOCYTE-DERIVED DENDRITIC CELLS PROPERTIES

Hajnalka Emese Halász, Erzsébet Nagyné Kovács, Péter Gogolák
Department of Immunology, Faculty of Medicine,
University of Debrecen

Human dendritic cells (DCs) are professional antigen-presenting cells with varied roles in immune responses. Previous studies have shown that monoctyes can differentiate in vitro to $CD1a^-$ and $CD1a^+$ DCs in different ratio. This two population of DCs has diverse cytokine secretion and distinct functions in T-cell activation. The expression of CD1a molecule is influenced by many environmental factors which presumably involve epigenetic regulations. Generally, the acetylation of histones results an open and transcriptionally accessible chromatin structure which is regulated by histone acetyltransferase (HAT) and histone deacetylase (HDAC) enzymes. In this study our aim is to observe and understand the effects of histone acetylation on the properties of monocyte-derived DCs (moDCs).

Freshly isolated CD14+ monocytes were cultured and differentiated to DCs in AIMV medium. During the differentiation the cells were treated with HAT activator, HAT inhibitor and HDAC inhibitor. Immature DCs were activated at day 3 of culture. Phenotyping of resting and activated moDCs was performed by flow cytometry, the concentration of secreted cytokines was measured by ELISA.

Our results show that the activation of HAT increases $CD1a^+/CD1a^-$ moDC ratio while HAT inhibitor obstructed the expression of CD1a molecule. This kind of inhibition has a disadvantageous effect on DC's viability too. In contrary to our expectations the treatment with HDAC inhibitor results CD1a- DC population in higher proportion. The acetylation modifying agents have effects on activated moDCs too. In case of HAT activator treatment, we noticed changes in CD83, CD86 expression. Interestingly, HDAC inhibitor-treated DCs produced TNF in higher concentration after LPS activation.

Epigenetic modifications, involving the modification of histone acetylation could provide additional useful tools for influencing the immune response and for shaping dendritic cell based therapies in the future. A notably observation of our research is that HAT activator can increase the $CD1a^+$ moDCs ratio, although other investigated environmental factors are more likely to induce the appearance of $CD1a^-$ phenotype. Inhibiting HDAC resulted the development of $CD1a^-$ moDCs which suggests that some genomic region needs down-regulation by deacetylation for a normal CD1a expression. Our results confirm the observation of other studies that HDAC inhibitors cannot be always useful as anti-inflammatory drugs because in some circumstances they can favor the inflammation.

INTEGRITY AND FUNCTION OF THE SESSILE TISSUE IN DROSOPHILA MELANOGASTER

Viktor Honti[1], Gergely István Varga[1], Gábor Csordás[1],
Szilárd Szikora[2], Ferenc Jankovics[2], Éva Kurucz[1], István Andó[1]
[1]HAS BRC, Institute of Genetics, Immunology Unit
[2]HAS BRC, Institute of Genetics, Developmental Genetics Unit

Introduction: Similarly to their vertebrate counterparts, hemocytes, the blood cells of Drosophila melanogaster, differentiate in multiple waves and are located in separate compartments. Besides the long-studied lymph gland and the circulation, the sessile tissue plays an important role in the larval hematopoiesis. Although genetic studies elucidated a complex regulatory network within the lymph gland, less data are available on factors that maintain the integrity and ensure the function of the sessile tissue. Besides the isolation of these factors, our goal is to understand the regulatory mechanisms underlying sessile tissue function.

Methods: We used high resolution in vivo confocal videomicroscopy and a special transgenic system that enables tracing and cell fate transformation of entire hemocyte lineages (Honti et al., 2010). Hemocytes were characterized by the expression patterns of our specific immunological markers and transgenic reporters (Kurucz et al., 2003, 2007; Honti et al., 2009). Larvae were analyzed with in vivo confocal videomicroscopy and immunofluorescence (Csordás et al., 2014). Hemocyte dynamics was analyzed with imaging softwares.

Results: We observed that circulating hemocytes continuously enter and quit the sessile tissue. We identified the phagocytosis receptor Eater as a major mediator in the attachment of hemocytes to the wall of the hemocoel (Bretscher et al., 2015). We could not detect dynamic changes or disintegration of the sessile tissue upon lamellocyte differentiation following immune induction or in tumorous mutants.

Conclusions: Unlike the lymph gland, the sessile tissue is in dynamic steady state with the circulation. The integrity of the sessile tissue and the differentiation of lamellocytes are regulated independently. Our results indicate that hematopoietic compartments interact with each other during the regulatory events of effector hemocyte differentiation.

Acknowledgement: This research was supported by grants from the Hungarian Science Foundation, OTKA grants NN 118207 (IA), PD 115534 (HV), and GINOP-2.3.2-15-2016-00001 (IA). This research was supported by the European Union and the State of Hungary, co-financed by the European

Social Fund in the framework of TÁMOP 4.2.4.A/2-11-1-2012-0001 'National Excellence Program' (for VH and GIV).

SIMULTANEOUS DETERMINATION OF HUMAN PLASMA SERINE PROTEASES COMPLEXED WITH C1-INHIBITOR

Erika Kajdácsi[1], Anna Koncz[1], Nóra Veszeli[2, 4], Dominik Gulyás[1], Veronika Makó[4], László Cervenak[1], Péter Gál[2], József Dobó[2], Henriette Farkas[1, 2], Lilian Varga[1, 2]
[1]3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary
[2]Hungarian Angioedema Reference Center, 3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary
[3]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary
[4]MTA-SE Research Group of Immunology and Hematology, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary

The C1-inhibitor (C1-INH) is an important regulator of complement, coagulation, fibrinolytic and contact systems. The quantity of enzyme/C1-INH complexes in the blood is proportional to the level of the in vivo activation of various cascade-like plasma enzyme systems. Parallel determination of C1-INH-containing activation complexes would be important to understand the regulation of plasma enzyme cascades in hereditary angioedema with C1-INH deficiency. We aimed to develop a set of sensitive, quantitative, sandwich ELISA assays for the determination of FXI/C1-INH, FXII/C1-INH, C1s/C1-INH, C1r/C1-INH, MASP-1/C1-INH, MASP-2/C1-INH and thrombin/C1-INH complexes.

For developing ELISA assays, commercially available plasma-derived C1-INH, active FXI, thrombin (TR) and FXII, as well as active recombinant C1s, C1r, MASP-1 and MASP-2 were used to generate the complexes required for the standards. The ELISA assays were tested of blood samples from healthy volunteers.

All enzymes formed complexes with C1-INH in vitro. In each developed sandwich-ELISA assay, the detection threshold was less than 0.01% of the normal plasma concentration of the enzyme. Intra- and inter-assay variations were less than 20%. Based on the concentrations of C1-INH complexes with FXI-, FXII , C1s-, C1r-, MASP-1-, MASP-2- and TR measured in four healthy volunteers, the medians of in vivo generated enzyme/inhibitor complex concentrations were 0.070, 0.005, 2.821, 1.861, 0.026, 0.007 and 0.054% of that of the mean C1-INH concentration, respectively.

We successfully developed a set of ELISAs for the sensitive determination of various enzyme/C1-INH complexes, which makes the simultaneous investigation of C1-INH-regulated activation systems possible. Based on our measurements in healthy subjects, C1-INH dominantly forms complexes with C1s and C1r in vivo.

These ELISA methods might prove suitable for studying the pathomechanism of hereditary angioedema with C1-INH deficiency.

Supported by OTKA 112110

COMPLEMENT FACTOR H FAMILY PROTEINS INFLUENCE MONOCYTE AND NEUTROPHIL CELL FUNCTIONS

Éva Kárpáti, Andrea E. Schneider, Noémi Sándor, Dávid Hajnal, Mihály Józsi
Complement Research Group, Department of Immunology, Eötvös Loránd University, Budapest, Hungary

Factor H (FH) is the major inhibitor of the alternative complement activation pathway in body fluids and when bound to cells through acceptor molecules (e.g. sialic acids) or soluble host ligands (e.g. pentraxins). In addition, FH can bind to different cell types via receptors (such as the complement receptors CR3 and CR4), and affect various functions of neutrophil granulocytes and monocytes, innate immune cells that are important players in inflammation. The human FH protein family also includes factor H-like protein-1 (FHL-1), a splice variant derived from the CFH gene, and five FH-related (FHR) proteins, which are composed of varying numbers of complement control protein domains homologous to those of FH, but are functionally less characterized. Recent data indicate that, in contrast to the inhibitor FH, FHR proteins promote complement activation. Our aim was to study whether similar to FH other FH family members and a recombinant mini-FH construct influence various functions of human monocytes and neutrophil granulocytes.

Monocytes were isolated by magnetic cell separation and neutrophil granulocytes by dextran sedimentation. Binding of the proteins to the cell surface was detected by flow cytometry and Western blot. Neutrophil extracellular traps (NETs) were induced by PMA treatment and extracellular DNA was labeled with nucleic acid dye and quantified with a fluorescent microplate reader. Visualization of NETs and determination of cell spreading was carried out using confocal laser scanning microscopy. The amount of the cytokines produced in response to stimulation with the different proteins was measured by ELISA.

Similar to FH, FHR-1, FHR-5, mini-FH and FHL-1 bound to neutrophils and monocytes. Significantly increased spreading was observed on immobilized FHR-1, FHL-1 and mini-FH in the case of neutrophils, and on FHR-1 and mini-FH in the case of monocytes compared to BSA control. FHR-5 and FHL-1 had an inhibitory effect on the generation of NETs compared to the PMA-treated samples. As previously described, immobilized FH is able to induce IL-8 production by neutrophils; we found that also immobilized mini-FH enhanced IL-8 and IL-1β production.

These data support an additional role of FH family proteins, beyond their canonical role in complement, as modu-

lators of cellular functions. Furthermore, the artificial complement inhibitor mini-FH also affects cell activation that should be taken into account when considering its potential therapeutical application.

Supported by the Hungarian Academy of Sciences (grant nr. LP2012-43), the National Research, Development and Innovation Fund (grants K 109055 and K 125219), and the Kidneeds Foundation (Iowa, US).

REAL-TIME MONITORING OF INTEGRATED CELLULAR RESPONSES OF PRIMARY B CELLS UPON SIMULTANEOUS RECEPTOR ENGAGEMENT

Kristóf Kliment[1], Bernadett Mácsik-Valent[1, 3], Beatrix Péter[2], Enikő Farkas[2], Inna Székács[2], Róbert Horváth[2], Anna Erdei[1, 3], István Kurucz[1]

[1]MTA-ELTE Immunology Research Group, Eotvos University, Budapest, Hungary

[2]Hungarian Academy of Sciences, Centre for Energy Research, Institute for Technical Physics and Materials Science, Nanobiosensors Group, Budapest, Hungary

[3]Department of Immunology, Eotvos University, Budapest, Hungary

Introduction: Our group previously applied a resonant waveguide grating (RWG) based optical biosensor to measure the integrated cellular responses in primary human tonsil B cells by monitoring the dynamic mass redistribution (DMR) of cellular contents triggered by different external stimuli. Dose-dependent, specific DMR responses triggered by BCR induced B cell activation were successfully measured; and based on these results our aim was to apply this technique for the tracking of DMR responses upon simultaneous receptor engagement of primary B cells.

Methods: Epic BenchTop (EPIC BT) system was employed. Human B cells were isolated from tonsils the day before the experiment, the cells were seeded on 384 well biosensor microplates. The B cell receptor was ligated with anti-human IgG/A/M preparations. Simultaneous receptor engagement (BCR+FcgRIIb and BCR+CR1) was also attempted (using anti-human IgM + heat aggr. human IgG or heat aggr. human C3). The specificity of the response was verified with the application of irrelevant immunoglobulins and downstream inhibitors of the BCR activation pathway, as well as high concentrations of inert protein HSA. The shift of reflected (resonant) wavelength relative to the baseline value was recorded in picometres (Δ pm) and was used for the calculations.

Results: EPIC BT biosensor was successfully applied to monitor integrated cellular response of human primary B cells upon simultaneous receptor engagement. BCR-induced B cell activation was not changed by the presence of indifferent protein (HSA), but attenuated – dose-dependently – in the presence of FcγRIIb ligands and (although in a highly donor-dependent way) CR1 ligands as well.

Conclusion: In vivo cellular response is an integrated process balanced by simultaneous activator and inhibitor stimuli. Conventional cell based assays usually focus on the measurement of certain selected components of the cellular response, label-free methods enable non-invasive real time detection of complex cellular changes. EPIC BT optical biosensor was applied to examine the effect of simultaneous stimulation of BCR+FcgRIIb or BCR+CR1. Here we demonstrated that FcγRIIb- and CR1-regulated, dose-dependent attenuation of the BCR-dependent DMR responses can be detected. While the inhibitory pathway – by which FcγRIIb interrupts BCR-induced B cell activation – is mostly deciphered, the exact mechanisms of CR1-mediated inhibition are not yet understood.

NEUTROPHILIC GRANULOCYTES RELEASE FUNCTIONALLY DIFFERENT SUBSETS OF EXTRACELLULAR VESICLES

Ferenc Kolonics[1], Ákos Lorincz[1], Veronika Farkas[2], Ádám Farkas[2], Erzsébet Ligeti[1]

[1]Department of Physiology, Semmelweis University

[2]Department of Medical Biochemistry, emmelweis University

Background: Extracellular vesicles (EVs) are known for their capability of transferring biologically active molecules from their cell of origin. Our previous results show that, depending on their activation state, neutrophilic granulocytes (PMN) can release EVs with or without antibacterial properties. Several groups reported both pro- and anti-inflammatory effects of PMN derived EVs produced upon different stimuli. In this study we investigated under comparative conditions the thrombo- and immunomodulatory effects of three different well-characterized PMN derived EV populations.

Methods: Human PMN were incubated in unstimulated conditions for 24 h. Other PMN were stimulated with opsonized particles or left non-activated for 20 min. Cells were eliminated and medium-size EVs were obtained by two-step centrifugation and filtration. EVs derived from these three different conditions (from activated cells – aEV, spontaneously produced – sEV, from apoptotic cells – apoEV) were co-incubated with PMN, monocytes, lymphocytes or pooled human plasma. We evaluated the uptake of the vesicles and their effect on phagocytosis, cell migration, superoxide production and coagulation.

Results: Both sEVs and aEVs were taken up by the investigated cell types. Neither the kinetics nor the maximal capacity of PMN phagocytosis was affected by the EVs. aEVs seem to slightly enhance the migratory potential of PMN as opposed to sEVs. Superoxide production of PMN was enhanced by aEVs, diminished by sEVs and right-shifted by apoEVs. apoEVs showed a strong procoagulant effect in recalcified plasma both in the presence and absence of thromboplastin (TP), while sEVs only enhanced coagulation in the absence of TP and aEVs did not have any effect on coagulation.

Summary/Conclusion: Our data show that human PMN release different EV populations, which can have different and specific effects on various physiological processes depending on the conditions during their release from the cells.

Funding: NKFIH K119236, Hungary

HLA PROMISCUITY AND PEPTIDE PRESENTATION OF CANCER CELLS

Balázs Koncz[1], Gergő Mihály Balogh[1], Leó Asztalos[1], Lajos Kemény[1, 2], Balázs Papp[3], Csaba Pál[3], Máté Manczinger[1, 2, 3]
[1]SZTE ÁOK Bőrgyógyászati és Allergológiai Klinika, Szeged
[2]MTA-SZTE Dermatológiai Kutatócsoport, Szeged
[3]Szegedi Biológiai Kutatóközpont, Biokémiai Intézet, Szintetikus és Rendszerbiológiai Egység, Szeged

Introduction: Human leukocyte antigen (HLA) molecules are essential for the immune recognition and subsequent killing of neoplastic cells by the immune system., Tumor antigens must be presented in an HLA-restricted manner to be recognized by T-cell receptors. HLA molecules are highly polymorphic. Different variants (alleles) have different peptide-binding promiscuity, which is the number of different peptide sequences bound by a given allele.

We hypothesized that allele promiscuity has an important role in the presentation of mutant tumor peptides and, thus, in antitumor immunity.

Methods: We collected immunopeptidomics data of 91 tumor cell lines and tumor samples with known HLA genotype. We determined promiscuity of HLA alleles using bioinformatic tools. We calculated the sequence diversity of peptides eluted from HLA molecules of each cell line.

Results: Promiscuous HLA-A and B, but not HLA-C alleles were associated with the presentation of more diverse peptide sequences.

Conclusion: Promiscuity of HLA molecules has a significant effect on the diversity of peptides presented on the surface of cancer cells. Consequently, HLA promiscuity may play an important role in antitumor immunity.

EFFECT OF BGP-15 ON THE INFLAMMATORY RESPONSES OF LPS-INDUCED HUMAN MACROPHAGES

Gergő E. Kovács, Elek Kocsis, Ahmad Alathsan, Szilvia Benkő
Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Introduction: BGP-15 is a chemical compound with a ring structure containing two heterocyclic rings which are connected through a hydroxylamine, and this molecule does not have toxic effect on the human body. This structure ensures the effect of BGP-15 thus it changes the fluidity of the membrane. BGP-15 has many therapeutic uses, like in diabetes mellitus or in cardiovascular diseases. It induces the expression of heat shock protein 70 (HSP70), also it has cytoprotective effects. Earlier study showed that oxidative stress elevates the calcium level in mitochondria and it leads to mitochondrial damage. But the BGP-15 is able to protect the membrane against oxidative damage. However, the role of this compound in inflammatory responses has not been investigated yet.

Aims: Our aim was to study the effect of BGP15 on LPS-induced human monocyte-derived macrophages from the perspective of HSP70 induction, cytokine production and other cellular changes.

Methods: We used monocyte-derived human macrophages and measured cytokine production by ELISA method. The expression level of specific mRNA was determined by qPCR. The HSP70 protein presence and induction was measured by western blot.

Results: Our results suggest that BGP-15 has effect on the inflammatory responses of human macrophages like changing the cytokine production and signaling pathways. In our future plan we would like to investigate how the molecular mechanism of BGP-15 effect cellular and immunological functions.

Support: The work was supported in part by OTKA-K109429 (to S.B.)

PREDICTING THE ONSET OF RHEUMATOID ARTHRITIS VIA ANTIBODY REPERTOIRE SEQUENCING

Kristóf György Kovács[1], Bence Szikora[1], Anita Marx[1], Orsolya Pipek[2], István Csabai[2], Imre Kacskovics[1]
[1]Department of Immunology, Eötvös Loránd University, Budapest, Hungary
[2]Department of Physics of Complex Systems, Eötvös Loránd University, Budapest, Hungary

Introduction: Rheumatoid Arthritis (RA) is a progressive, chronic, inflammatory autoimmune disease. It results not only in severe articular, but also in systemic manifestations, and is prevalent in around 0.8% of adults worldwide. An individual, who is likely to develop RA, is referred to as a so-called at-risk RA-patient, in case of suffering from arthralgia and having elevated serum ACPA and/or RF titres. Nevertheless, some at-risk patients will remain healthy. Therefore, there is an urgent need to identify an accurate and sensitive molecular biomarker, capable of predicting the onset of RA. Several research groups approach this subject by analysing the B-cell diversity via Next-Generation Sequencing (NGS). According to a recent research result (Tak et al., 2017.), the onset of RA is highly likely in those, having several, clonally related sequence bearing B-cells in their peripheral blood. Our goal is to validate the protocol and implement a refined analysis workflow.

Materials and Methods: Our validation experiments require only 3 ml peripheral blood of a patient, collected into special tubes, preserving the nucleic acids of the samples.

After RNA isolation and cDNA synthesis, an IgG heavy chain cDNA library is generated. Then, after adapter ligation and quality control, the samples get sequenced via Illumina MiSeq NGS platform. Raw reads are processed by MIGEC software, and the reads are uploaded to the IMGT database. Then, using the IMGT/HighV-QUEST tool, productive antibody sequences are identified. The resulted lists are analysed bioinformatically, in order to determine certain characteristics of the repertoires, such as the number of dominant clones (i.e. particular antibody sequences, which are expanded beyond 0.5% of the total repertoire).

Results and Discussion: So far, we have analysed the antibody repertoire of 14 RA-patients and 16 healthy volunteers. We have identified antibody repertoires, consisting of several dominant clones in case of both RA-patients and healthy volunteers. Furthermore, we have also found repertoires in both cases, which contained zero dominant clones. We are planning to analyse the repertoires of further individuals, and even of at-risk RA-patients. We are going to fine-tune the applied methods, in order to establish a robust molecular biomarker research (based on the protocol above), with which the early diagnosis and prognosis of RA become considerably more accurate and reliable.

Acknowledgements: Project no. FIEK_16-1-2016-0005 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the FIEK_16 funding scheme.

A NEW APPROACH OF TREATMENT DURING ERYTHEMA MARGINATUM IN HEREDITARY ANGIOEDEMA

Kinga Viktória Kőhalmi, György Ledniczky, György Temesszentandrási, Nóra Veszeli, Lilian Varga, Henriette Farkas
Hungarian Angioedema Reference Center, 3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary

Introduction: Hereditary angioedema (HAE) attacks in patients with HAE with C1-inhibitor deficiency (C1-INH-HAE) may be preceded by erythema marginatum (EM). According to the data of the Erythema Marginatum Questionnaire (EMQ), 65/165 Hungarian C1-INH-HAE patients (39.4%) from 43 families had EM in their life. Moreover, HAE attack can be prevented by the proper prophylactic treatment administered during EM.

Methods: Our aim is to introduce safe and effective novel prophylactic treatments during EM to prevent the formation of HAE attacks.

Results: According to the data of EMQ, we selected eight patients who frequently had EM. We instructed these patients to administer plasma-derived C1-inhibitor concentrate (pdC1-INH) [Patient #1 127 vials on 127 occasions, Patient #2 17 vials on 17 occasions, Patient #3 127 vials on 127 occasions, Patient #4 135 vials on 45 occasions, Patient #5 78 vials on 39 occasions], or recombinant C1-inhibitor concentrate (rhC1-INH) [Patient #1 2 vials on 2 occasions, Patient #2 12 vials on 12 occasions, Patient #3 11 vials on 11 occasions, Patient #6 1 vial on 1 occasion], or icatibant [Patient #1 2 syringes on 2 occasions; Patient #6 1 syringe on 1 occasion], or to increase the dose of danazol (in case of two patients), as soon as possible after the onset of EM, in order to prevent the development of HAE attack. Surprisingly, no HAE attacks developed in any patients when these medicinal products were administered during EM.

Conclusions: As novel prophylactic treatments in C1-INH-HAE, pdC1-INH, rhC1-INH, icatibant and danazol administered during EM may be effective, individual therapeutic options in those patients whose HAE attacks are often preceded by EM. These therapeutic strategies, involving drug administration in the lowest effective doses, administered only when EM occurred is supposed to be the most cost-effective therapeutic option to improve the quality of life of these patients.

Disclosure of potential conflict of interest: This study was supported by ÚNKP-17-3-III-SE-13 New National Excellence Program of the Ministry of Human Capacities, National Scientific Research Fund (OTKA) 124557, and the Pharming Group NV.

ANALYSIS OF INTRAPERITONEAL HOMING OF THYMOCYTES IN IMMUNODEFICIENT MICE

Reka Kugyelka, Lilla Prenek, Katalin Olasz, Timea Berki, Peter Balogh, Ferenc Boldizsar

The zeta chain-associated protein of 70 kDa (ZAP-70) plays a key role in T cell differentiation and signalling, in its absence the development of thymocytes is blocked in the double positive stage. Thus, ZAP-70 homozygous knockout (ZAP-70$^{-/-}$) mice have no mature T cells in their peripheral lymphoid organs and blood. Previously we have shown that the adoptive transfer of wild-type thymocytes reconstitutes this immunodeficiency. Donor-originated thymocytes appeared and the thymus of ZAP-70$^{-/-}$ mice and gave rise to mature T cells in the periphery. We have found the intraperitoneal (i.p.) route of administration to be the most efficient, so we investigated the mechanism of lymphocyte trafficking after i.p. injections.

We used a single i.p. injection to deliver CFSE-labeled donor thymocytes to ZAP-70$^{-/-}$ recipients and to wild-type (BALB/c) mice to compare the effect of immundeficiency on the homing of thymocytes. We sacrificed animals after various time points and investigated the cellular composition of omentum, peripheral and mesenteric lymph nodes, thymus and spleen. In the mesentery and omentum we analysed the localization of CFSE^{+} donor cells; we also investigated

We have found that after i.p. injection donor thymocytes leave the peritoneum and form aggregates already after 24 hours in the mesentery along lymphoid vessels and in the omentum, however no donor-originated cells were visible in

peripheral and mesenteric lymph nodes. No CFSE$^+$ cells were found in the thymus 3 days after transfer, which is in line with our previous findings, where donor-cells appeared only 7-10 days after the transfer. The aggregates found in the omentum are most likeley located in milky spots, and based on their surface markers they were mostly composed of double negative thymocytes. Our results suggest that the omentum is an important location for thymocyte entry from the peritoneum in both wild-type and immunocompromised mice, as well.

To gain further insight into the peritoneal trafficking we plan to investigate the possible alterations in trafficking after i.p. injection under different contitions (inflammation, stromal deficiency).

Funding: OTKA K101493; EFOP-3.6.1.-16-2016-00004; GINOP 2.3.2-15-2016-00050; KA-2015-23; BO/00086/12/5; 716/180/2014/KIF; KA-POSTDOK-12-05

COMPARATIVE ANALYSIS OF THE MULTINUCLEATED GIANT HEMOCYTES IN TWO DROSOPHILA SPECIES

Zita Lerner[1], Gyöngyi Cinege[1], Attila L. Kovács[2], Gábor Juhász[1, 2], Éva Kurucz[1], István Andó[1]
[1]Immunology Unit, Institute of Genetics BRC HAS, Szeged
[2]Department of Anatomy, Cell and Developmental Biology, Eötvös Lóránd University, Budapest

Introduction: Insects developed an effective innate immune response–consisting of cellular and humoral elements-to successfully combat infections. In various Drosophila species, special immune cells, the hemocytes eliminate microbes and parasites. In the melanogaster subgroup, three functionally different subsets, the phagocytic plasmatocytes, the melanizing crystal cells and the encapsulating lamellocytes were identified as the effectors of cellular immunity. In a comprehensive analysis, we identified a novel blood cell type in the species of the ananassae subgroup and also in its distant relatives, in the species of Zaprionus genus, which we named Multinucleated Giant Hemocytes (MGH). In the present study we analyzed the function, the formation and the structure of the MGHs in two representative species, the Drosophila ananassae and Zaprionus indianus.

Methods: We developed D. ananassae and Z. indianus cell-specific monoclonal antibodies to define differentiation of the MGHs, upon immune induction with parasitic wasp. To study the formation and the movement of the giant cells we carried out time lapse video analysis. We also investigated the cytoplasmic structure of MGHs with electron microscopy.

Results: We observed that MGHs of D. ananassae appear in the circulation of the larvae after infestation with parasitoid wasps, and differentiate from phagocytic plasmatocytes to capsule forming terminally differentiated cells. MGHs of Z. indianus are present in the circulation and also in the central hematopoietic organ, the lymph gland. We monitored the differentiation of these cells and revealed substantial differences among the studied species. Time lapse video revealed that, MGHs of both species are motile cells and are able to project filopodia. According to the electron microscopic analysis, the MGHs are multinuclear cells and have a special ultrastructure with so far undetected compartmentalization. The organization of the subcellular components suggest active metabolism, secration and vesicular transport.

Conclusions: The MGHs are important effector cells of the cell-mediated immune response of different Drosophila species. They have a unique structure, which could provide a basis for the very effective immune response and isolation of the intruders. Their formation and differentiation show substantial differences in the studied two species. On the basis of our studies we suggest that multinucleation is a more common phenomenon in the anti-parasitic immune responses than it was assumed before. As MGHs are similar to mammalian multinuclear giant cells, we believe that they can provide a powerful model to understand the process of granuloma formation in vertebrates.

Acknowledgements: This research was supported by grants from the Hungarian National Science Foundation, NKFI K128762, NKFI K120142, NKFI K120140, GINOP-2.3.2-15-2016-00001 and GINOP-2.3.2-15-2016-00035.

THE ROLE OF CR3 (CD11B/CD18) AND CR4 (CD11C/CD18) IN ADHERENCE UNDER PHYSIOLOGICAL AND INFLAMMATORY CONDITIONS

Szilvia Lukácsi[1, 2], Tamás Gerecsei[3, 4], Barbara Francz[5], Bálint Szabó[3], Róbert Horváth[4], Anna Erdei[1, 2], Zsuzsa Bajtay[1, 2]
[1]Department of Immunology, Eötvös Loránd University, Budapest, Hungary
[2]MTA-ELTE Immunology Research Group, Eötvös Loránd University, Budapest, Hungary
[3]Department of Biological Physics, Eötvös University, Budapest, Hungary
[4]Nanobiosensorics "Lendület" Group, Institute of Technical Physics and Material Sciences,
Centre for Energy Research, Hungarian Academy of Sciences, Budapest, Hungary
[5]CellSorter Company for Innovations, Budapest, Hungary

CR3 and CR4 belong to the family of β_2-integrins and play an important role in phagocytosis, cellular adherence and migration. CR3 and CR4 are generally expected to mediate similar functions due to their structural homology and overlapping ligand specificity. Previously we proved that CR4 is dominant in the adhesion of monocytes, monocyte derived macrophages (MDMs) and monocyte derived dendritic cells (MDDCs) to fibrinogen under physiological conditions. Here we studied the expression of CR3 and CR4 and their participation in adherence to fibrinogen in inflammatory conditions induced by LPS.

The expression of CR3 and CR4 was monitored by flow cytometry at different time points during the LPS induced cell activation. Cell adhesion to fibrinogen was evaluated using a state-of-the-art biophysical method, namely the force of cell attachment was measured with a computer controlled micropipette using vacuum assisted fluid flow.

Comparing the amount of CR3 and CR4 on the cell surface we found that LPS treatment alters their expression differently on MDMs and MDDCs. Whereas on MDMs the expression of both CR3 and CR4 decreases (44%±4 and 64%±17, respectively compared to non-treated control cells), on MDDCs CR4 shows a significantly elevated level (up to 147%±34), in contrast to the diminished expression of CR3 (73%±10). Applying the micropipette, we observed a reduced adhesion force in the case of the LPS treated cells that was more pronounced in MDDCs than in MDMs. Using blocking antibodies we proved that adherence to fibrinogen is dominated by CD11c in both physiological and inflammatory conditions.

EFFECT OF COMPLEMENT RECEPTOR TYPE 1 (CD35) ON TLR AND TLR+BCR DEPENDENT FUNCTIONS OF HUMAN B CELLS

Bernadett Mácsik-Valent[1, 2], Mariann Kremlitzka[3], László Fazekas[1], Anna Erdei[1, 2]
[1]Department of Immunology, Eötvös Loránd University, Budapest, Hungary
[2]MTA-ELTE Immunology Research Group, Department of Immunology, Eötvös Loránd University, Budapest, Hungary
[3]Present address: Division of Medical Protein Chemistry, Department of Translational Medicine, Lund University, Malmö, Sweden

Although it is well accepted that separate activation of the complement system and Toll-like receptors (TLRs) is involved in the initiation and regulation of the adaptive immune response, much less is known about the modulation of various B cell functions by the simultaneous activation of these two systems. Therefore we investigated how engagement of complement receptor type 1 (CR1) influences the activation of human B cells, induced by TLR7 and TLR9 with or without a BCR stimulus.

Resting tonsillar B cells were activated via BCR by a suboptimal dose of $F(ab')_2$ anti-human IgG/M/A and via TLR7 and TLR9 by synthetic activators. The stimuli were applied either separately or simultaneously in the presence or absence of the natural ligand of CR1, a multimeric "C3b-like C3". The effect of CR1 clustering was assessed on the expression of activation markers (flow cytometry), cytokine secretion (ELISA), proliferation (3H-thymidine incorporation) and antibody production (ELISPOT).

We show that CR1 clustering significantly and dose dependently reduces the TLR9-induced activation of tonsillar B cells, but has no effect on the TLR7-induced functions. The enhanced response to the simultaneous engagement of TLR7 or TLR9 and BCR was also significantly inhibited by CR1 clustering.

Our data demonstrate that engagement of CR1 downregulates the TLR9-induced B cell functions but does not influence the TLR7 mediated processes. Interestingly however, when B cells are simultaneously triggered via BCR+TLR7 or BCR+TLR9, clustering of CR1inhibits the B cell response. We assume that CR1 exerts its inhibitory effect by acting on signalling molecules linked to both BCR and TLR9 in human B cells.

MONITORING MULTIPLE MYELOMA USING NEXT-GENERATION SEQUENCING OF THE V(D)J GENES FROM CIRCULATING MYELOMA CELLS AND DERIVED DNA

Anita Marx[1], Bence Szikora[1], Orsolya Pipek[2], István Csabai[2], Ferenc Uher[3], Gábor Mikala[3], István Vályi-Nagy[3], Imre Kacskovics[1]
[1]Department of Immunology, Eötvös Loránd University, Budapest
[2]Department of Physics of Complex Systems, Eötvös Loránd University, Budapest
[3]South-Pest Hospital Centre – National Institute for Infectology and Haematology

Multiple myeloma is one of the many hematological malignancies and is caused by malignant antibody secreting plasma cells in the bone marrow, where they proliferate uncontrollably. Patients who received chemotherapy are monitored constantly after their treatment to discover if they develop minimal residual disease (MRD). That is, the treatment did not eradicate all cancer cells from the body and patients still have low levels of myeloma cells. It is therefore crucial to detect these survival tumor cells in time, which means we need methods with high sensitivity. Recently, in addition to multiparametric flow cytometry and allele-specific oligonucleotide qPCR, next-generation sequencing (NGS) offers an even more sensitive method to detect MRD.

Next-generation sequencing is a high throughput method, capable of analyzing millions of sequences in a massively parallel manner. Immunoglobulin DNA sequences identify individual B cells like a fingerprint because each B cell expresses unique rearranged gene segments, the V(D)J region. These numerous B cells give rise to different B cell clones constituting the B cell repertoire, whose composition change in time and during immune challenges and malignancies. Thus, we can analyze the B cell repertoire (IgSeq) of myeloma patients and monitor their plasma cell statuses by NGS technology and bioinformatical data evaluation first from their bone marrow, and after identifying the tumor clone ('fingerprint') we can track their statuses from their blood, supporting therapy decisions – hence the need for the high sensitivity of NGS.

We isolated genomic DNA from bone marrow and blood

mononuclear cells and cell-free DNA from plasma samples of myeloma patients. Then we prepared DNA libraries and sequenced on Illumina MiSeq platform. We processed the error-corrected sequences using the IMGT and then we done the bioinformatical analysis of the resulting clones and sequences. We determined the sequence of the myeloma clone in the bone marrow sample and we also found that sequence ('fingerprint') in the blood samples. In collaboration with the South-Pest Hospital Centre, we plan to set up our method by analyzing 30 patients' samples.

Project no. FIEK_16-1-2016-0005 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the FIEK_16 funding scheme.

FACTOR H-RELATED PROTEIN 3 (FHR3) DEREGULATES COMPLEMENT ON PENTRAXINS, DNA AND MALONDIALDEHYDE EPITOPES

Alexandra Tünde Matola[1], Ádám István Csincsi[1], Barbara Uzonyi[1], Marcell Cserhalmi[1], Richard Pouw[2], Agustín Tortajada[3]3, Santiago Rodríguez de Córdoba[3], Diana Wouters[2], Mihály Józsi[1]
[1]Department of Immunology, Eötvös Loránd University, Budapest, Hungary
[2]Department of Immunopathology, Sanquin Research and Landsteiner laboratory of the Academic Medical Center, University of Amsterdam, the Netherlands
[3]Department of Cellular and Molecular Medicine, Centro de Investigaciones Biológicas and Ciber de Enfermedades Raras, Madrid, Spain

As a humoral effector arm of innate immunity, the complement system can effectively protect the body against pathogens and altered self structures. Inadequate regulation of complement activation can cause inflammation and several diseases. One of the complement regulatory proteins is factor H (FH), which inhibits the alternative complement pathway at the level of the central component C3. In addition to FH, the human FH protein family includes FH-like protein 1 (FHL1), which is an alternative splice product of the CFH gene, and five FH-related (FHR) proteins. FHL1 is also an inhibitor of the alternative pathway, but FHRs lack the complement regulatory domains of FH. In contrast to FH, their function is poorly characterized. In this study, we investigated the potential interaction of FHR3 with disease-relevant ligands, such as pentraxins, DNA and malondialdehyde (MDA) epitopes, and the role of the previously described C3b binding by FHR3.

Recombinant FHR3 and FHL1 were expressed in insect cells. Binding of FHR3 to pentraxins, DNA and MDA-BSA was analyzed in ELISA. Complement activation was measured by deposition of C3 fragments from human serum. Convertase activity was measured by cleavage of C3 and release of C3a.

We found that FHR3, similar to FHR1, FHR4 and FHR5, supported the assembly of an active C3 convertase enzyme of the alternative pathway. FHR3 interacted with the acute phase proteins C-reactive protein (CRP) and pentraxin 3 (PTX3) and, when bound to these pentraxins, enhanced complement activation. FHR3 also bound to ligands exposed by dying cells, such as DNA and MDA epitopes, and competed for binding with the complement regulators FHL1 and FH. DNA-bound FHR3 caused alternative pathway activation.

In summary, we identify novel ligands, such as pentraxins, DNA and MDA epitopes for FHR3 that are present under inflammatory conditions. Our results support a role for FHR3 as an enhancer of complement activation by acting as a complement deregulator via competition with the inhibitory proteins FH and FHL1, as well as by directly enhancing complement activation on these newly identified ligands. These data may provide an explanation for the protective role of FHR3 deficiency in age-related macular degeneration.

Supported by the National Research, Development and Innovation Fund (grant K 109055).

SEPTIC INJURY TRIGGERS IMMUNE RESPONSE IN *DROSOPHILA MELANOGASTER* AGAINST FUNGAL PATHOGENS

Navodita Maurice[1], Tamás Papp[2], Gábor Nagy[2], István Andó[1], Éva Kurucz[1]
[1]Biological Research Centre, Hungarian Academy of Sciences, Szeged
[2]MTA-SZTE "Momentum" Fungal Pathogenecity Mechanisms Research Group, Szeged, Hungary

Introduction: *Drosophila melanogaster* relies on multiple innate defense reactions against bacterial and fungal infections, involving the production of the highly efficient antimicrobial peptides. The production of the antimicrobial peptides (AMPs) is regulated by two signaling pathways namely, the *Toll* pathway activated by fungi and Gram-positive bacteria and the *Imd* pathway activated by Gram-negative bacteria. In addition, *JAK/STAT* and *JNK* pathways are also involved. The virulence of the intruders is a major factor in the final outcome of the infection. Since, the virulence of bacterial and fungal pathogens is governed by many factors, including the structure of their complex cell walls, our major interest underlies in studying the cell wall-host interaction along with different fungal pathogens that evoke an immune response. The population of immunocompromised individuals is increasing at an alarming rate, therefore, we have focused our study on analyzing *Drosophila* immune response against different *Mucor fungi*, responsible for causing Mucormycosis (zygomycosis) in the immunocompromised hosts.

Methods: We have induced 'septic injury' in flies having mutations in components of the different immune pathways (Toll and Imd) (Kari et al., 2013). We by cutting the tarsi of their fore-legs (this method was generated and has already been tested in our lab). These flies were introduced on the

vials with Mucor spore infected yeast extract media. Non-injured flies were introduced in the same way on the vials with Mucor spore infected yeast extract media and were used as control. Survival of the flies was traced for 5, 29, 53 and 77 hours.

Results: Our results show that Mucor spores can penetrate the body cavity of the fly through the site of the septic injury induced by cutting the tarsi of the forelegs and therefore, can initiate an immune response. We have found marked differences in the sensitivity and resistance of the mutant flies of the Toll and Imd pathways induced with septic injury when compared with the non-injured flies taken as control.

Conclusion: This 'septic injury' approach (Kari et al., 2013) appears as an efficient tool for the induction of infection in Drosophila melanogaster and is capable of monitoring the efficiency of an immune response. Our studies have shown that Mucor spores can enter the gut of the Toll and Imd pathway mutant flies with septic injury and can trigger immune response. Imd and Toll pathway mutant flies show differences in the levels of sensitivity and resistance.

Reference: Kari B, Zsámboki J, Honti V, Csordás G, Márkus R, Andó I, Kurucz É. A novel method for the identification of factors involved in host pathogen interactions in Drosophila melanogaster. J Immunol Methods 2013; 76–82.

This work was supported by grants from the Hungarian Science Foundation:GINOP-2.3.2-15-2016-00035.

THE POSSIBLE ROLE OF PD-1/PD-L1 COINHIBITORY PATHWAY IN THE MATERNO-FETAL INTERFACE

Mátyás Meggyes[1, 2], Brigitta Szigeti[1], Éva Mikó[1, 2] László Szereday[1, 2]
[1]University of Pécs, Medical School, Department of Medical Microbiology and Immunology
[2]University of Pécs Szentágothai Research Centre

Introduction: Numerous study examined the PD-1/PD-L1 interaction and their critical role in maintaining peripheral immune tolerance. Nevertheless much less study has been published in the context of this checkpoint inhibition during pregnancy. The aim of this present study was to examine the contribution of PD-1/PD-L1 immune-checkpoint pathway to maternal immunotolerance mechanisms.

Method of study: 13 healthy pregnant women and 10 non-pregnant controls were involved in this study. Peripheral blood mononuclear cells and decidual immune cells were isolated from peripheral blood and from decidual tissues. After the characterization of different immune cell subsets, fluorochrome-conjugated monoclonal antibodies were used to measure the expression level of PD-1, PD-L1, NKG2D and CD107a molecules by flow cytometry.

Results: Significant alternations in the percentage of decidual immune cell subsets were revealed compared to the periphery. Elevated PD-1 expression by decidual CD8+ T, CD4+ T, and NKT-like cells were also detected accompanied by the increased PD-L1 expression by decidual CD4+ T, Treg, NKT-like and NK^{dim} cell subset compared to the peripheral blood. Cytotoxic potential by the PD-1 negative decidual immune cells was significantly higher compared to the periphery. At the same time cytotoxicity by the decidual PD-1+ CD8+ T cells was significantly lower compared with the peripheral counterpart. An activation receptor NKG2D expression was decreased by the PD-1+ CD8+ T subset in the first trimester compared to the non-pregnant condition, but the expression level of the decidual counterpart was significantly elevated compared to the periphery. The cytotoxic potential of decidual PD-1/NKG2D++ CD8+ T cells was significantly decreased compared to the peripheral subsets.

Conclusions: Our findings demonstrate the complexity of the activating and inhibitory immunological mechanisms at the materno-fetal interface. Based on our results we assume, that the PD-1/PD-L1 pathway might have a novel role in the maintenance of immunological environment at the materno-fetal interface.

This work was supported by grants from the National Research, Development and Innovation Office (NKFIH K119529), PTE ÁOK-KA-2018/07.

COMPLEMENT CLASSICAL PATHWAY MONITORING IN BLOOD AND URINE FOR THE DETECTION OF ANTIBODY MEDIATED REJECTION IN KIDNEY TRANSPLANT RECIPIENTS

Blanka Mező[1], Andreas Heilos[2], Georg Böhmig[3], Farsad Eskandary[3], Markus Wahrmann[3], Gregor Bond[3], Philip F. Halloran[4], Krisztina Rusai[2**] and Zoltán Prohászka[1**]
[1]Research Laboratory, IIIrd Department of Internal Medicine, and MTA-SE Research Group of Immunology and Hematology, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary
[2]Department of Pediatrics and Adolescent Medicine, Medical University Vienna, Vienna, Austria
[3]Division of Nephrology and Dialysis, Department of Medicine III, Medical University Vienna, Vienna, Austria
[4]Alberta Transplant Applied Genomics Centre, ATAGC, University of Alberta, Edmonton, AB, Canada

Introduction: Antibody mediated rejection (ABMR) is a severe clinical problem, which is the major immunological cause of kidney transplant failure. According to current knowledge, late ABMR is classically caused by the development of donor specific antibodies (DSA) and the classical pathway (CP) of the complement system believed to contribute to tissue damage. The aim of study was to determine whether monitoring the CP allows for improved prediction of ABMR in kidney transplant recipients.

Methods: In the present study, 741 long-term transplant patients (>6 months post-transplantation; Vienna transplant unit) were subjected to cross sectional ABMR screening in the context of a prospective international trial (BORTEJECT). Our

analysis was focused on 83 DSA-positive recipients subjected to protocol biopsy, who had available data for IgG mean fluorescence intensity (MFI) and complement fixation and adequate material for complement screening. The activity of CP and levels of complement proteins (C1q, C3a, SC5b-9, C4d and C5a) were measured by enzyme-linked immunosorbent assay (ELISA) using EDTA plasma and/or urine samples.

Results: Among 83 DSA-positive patients, 47 (56.6%) ABMR+ and 21 (25.3%) C4d+ recipients were identified. Although no significance difference in plasma and urine parameters were observed between AMBR+ or C4d+ and nonrejecting or C4d-negative patients ($p>0.05$), we found a highly significant ($p<0.0001$) positive correlation between the protein/creatinin ratio and the urinary levels of complement activation products (C4d, r=0.48; C3a, r=0.45; C5a, r=0.66; SC5b-9, r=0.45).

Conclusion: Monitoring the complement classical pathway in blood and urine failed to have an independent diagnostic advantage and did not improve the prediction of ABMR either alone or combined with antibody characteristics. Never-theless, detection of increscent complement activation products in urine associated with higher protein/creatinin ratio and worse renal function.

This work was supported by the National Research Fund of Hungary (NN110909).

DIVERSE FUNCTIONS OF CR3 (CD11B/CD18) AND CR4 (CD11C/CD18) β_2-INTEGRINS EXPRESSED BY HUMAN B LYMPHOCYTES

Zsuzsa Nagy-Baló[1], Szilvia Lukácsi[1, 2], Bernadett Mácsik-Valent[1, 2], Zsuzsa Bajtay[1, 2], Anna Erdei[1, 2]
[1]Department of Immunology, Eötvös Loránd University, Budapest, Hungary
[2]MTA-ELTE Immunology Research Group, Eötvös Loránd University, Budapest, Hungary

Introduction: CR3 and CR4 are known for long to participate in adhesion and migration of myeloid cells, while the expression and function of these β_2-integrins on human B lymphocytes has not been extensively studied yet. CR3 and CR4 are not widely expressed on normal human B cells, however they can be detected on particular subpopulations and after activation. CR3 and CR4 are also known to be expressed in various B cell malignancies, including B cell chronic lymphocytic leukaemia (CLL), but their function and their possible contribution to the pathomechanism is unexplained so far. The aim of the present study is to investigate the expression and role of CR3 and CR4 on human B lymphocytes. We also intend to clear up the function of these two receptors on B cells of CLL patients.

Methods: B cells were isolated from the blood of 3 patients with CLL by negative selection. CLL B cells were then cultured in the presence of IL-2 and the $(ab')_2$ fragment of goat anti-human IgG+A+M antibody, which provide survival signal to the cells. CR3 and CR4 expression was detected by flow cytometry. Adhesion of the BJAB B cell line and CLL B cells was measured by confocal microscopy. Migration of Daudi cells and CLL B cells towards the chemoattractant SDF-1 was measured by transwell assay. Proliferation of CLL B cells on PLL-PEG blocked as well as on fibrinogen coated (and PLL-PEG blocked) surfaces was assessed by measuring ^{3}H-thymidine incorporation.

Results: Our results obtained with human B cell lines demonstrate that both CR3 and CR4 play a role in migration, however CR4 dominates adhesion to fibrinogen over CR3. Investigating these receptors on the B cells of CLL patients we confirmed that they can be expressed on CLL B cells and showed that CR4 plays important role in adhesion to fibrinogen and migration towards SDF-1. Measuring the proliferation of CLL B cells cultured on different surfaces we found that blocking with PLL-PEG rather lowered proliferation, while fibrinogen-coated surfaces increased the proliferation of CLL B cells of one patient.

Conclusion: Adhesion and migration are important functions of these malignant cells to reach the protective niches of the bone marrow, where they get survival signals, proliferation stimuli and resistance to drugs. Because CR4 participates in the adhesion to fibrinogen and migration of CLL B cells towards SDF-1, it might be an important contributor of disease progression in CLL patients. This assumption is further strengthened by our finding that adhesion of the activated CLL B cells enhances proliferation.

MASP-1 POTENTIATES THE BRADYKININ MEDIATED PERMEABILITY OF THE ENDOTHELIAL CELLS

Zsuzsanna Németh[1], Márta Lídia Debreczeni[1], Erika Kajdácsi[1], Zoltán Doleschall[2]2, József Dobó[3], Péter Gál[3], László Cervenak[1]
[1]3rd Department of Internal Medicine, Semmelweis University, Budapest
[2]Department of Pathogenetics, National Institute of Oncology, Budapest
[3]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest

Increased vascular permeability and uncontrolled complement activation plays and important role in the pathomechanism of many life-threatening diseases such as hereditary angioedema (HAE). The main reason of the HAE attacks is the extreme production of bradykinin in the lack of C1-inhibitor function (which normally regulates the activation of early complement components). We have previously shown that during these attacks, endothelial cells become activated. The mannan-binding lectin-associated serine protease-1 (MASP-1) is the most abundant enzyme of the lectin pathway of complement activation and able to activate endothelial cells. MASP-1 is naturally inhibited by C1-inhibitor, so we wanted

to further investigate the impact of MASP-1 on endothelial permeability.

We used confluent layer of human umbilical vein endothelial cells (HUVECs). The cells were treated with 0.6 μM MASP-1 for 2 hours and mRNA was isolated from MASP-1 treated and untreated HUVECs. Agilent Microarray was utilized to assess gene expression and quantitative real time PCR to validate the results. Intracellular Ca^{2+} mobilization assay were performed with fluorescence microscopy after 0,6 μM MASP-1 pretreatment for 24 hours.

We have previously demonstrated that MASP-1 can directly increase endothelial permeability. The whole transcriptome microarray analysis showed that MASP-1 significantly changed the expression of 25 permeability-related genes. From these, 16 genes were up- and 9 down-regulated. Most importantly, MASP-1 up-regulated the expression of the bradykinin B2 receptor. Using RT-PCR we validated this result. By treating the HUVECs with bradykinin, we detected the intracellular Ca^{2+} increase, one of the first signaling steps of GPRCs. In concert with the findings at mRNA level, we demonstrated that MASP-1 pretreatment of the HUVECs resulted an increased Ca^{2+} mobilization to bradykinin.

Taken together these new results and our previous knowledge, we can hypothesize that complement MASP-1 can play a role in the pathogenesis of HAE attacks. On one hand MASP-1 can directly increase the permeability of endothelial cells and is able to cleave high-molecular weight kininogen to bradykinin. On the other hand, MASP-1 up-regulates the expression of bradykinin B2 receptor and therefore potentiates the permeability response to bradykinin. These present results propose MASP-1 as a potential target in HAE therapy.

CHARACTERIZATION OF NEUTROPHILS GENERATED IN VITRO FROM HOXB8-TRANSDUCED MYELOID PROGENITOR CELLS

Anita Orosz, Attila Mócsai
Department of Physiology, Semmelweis University School of Medicine and SE-MTA „Lendület" Inflammation Physiology Research Group, Budapest, Hungary

Background: Acute inflammation and neutrophil granulocytes have long been mentioned together, however the molecular background of the neutrophils function is still mostly unknown. Therefore we are hoping to uncover these molecular mechanisms using ex vivo generated neutrophil cells from the so called SCF ER-Hoxb8 progenitors. These bone marrow-derived progenitors are retrovirally transduced to express the Hoxb8 transcription factor in the presence of β-estrogen, in order to keep them in progenitor state for long periods of time. From them, unlimited amounts of neutrophils can be differentiated using certain cytokines.

Materials and methods: Neutrophils were generated via growing progenitors in β-estrogen free medium supplemented with G-CSF. Cell surface markers were detected using flow cytometry. ROS production was measured according to cytochrome-c reduction. Adhesion and migration capabilities were tested using different stimulating agents. Fagocyting properties were measured using fluorescent Staphylococcus aureus and Candida albicans.

Results: CD45+ progenitor cell line can be cultured for long time. Ly6G+ neutrophils start to differentiate in 4 days. 5-6 days old neutrophils show strong adhesion upon PMA and IC stimulation. Hoxb8 neutrophils can also produce ROS upon various stimuli in vitro. Neutrophils appear in the circulation after adoptive transfer of progenitors into lethally irradiated recipient mice. In vivo generated neutrophils can migrate and carry out phagocytosis in inflammatory conditions.

Conclusions: The SCF ER-Hoxb8 cell line can be a great alternative to the common- genetically modified mice-based methods in use to discover the molecular basis of the role of the neutrophils in inflammatory diseases both in vitro and in vivo.

ANALYSIS OF GLUCOCORTICOID-INDUCED APOPTOSIS IN THYMOCYTES AND MATURE T CELLS

Lilla Prenek, Réka Kugyelka, Ferenc Boldizsár, Péter Németh, Tímea Berki
Department of Immunology and Biotechnology, University of Pécs Clinical Center, Pécs, Hungary

Non-genomic effects of glucocorticoids (GC), especially the translocation of the activated GC receptor (GR) to the mitochondria, correlates the GC-induced apoptosis sensitivity of a certain cell type. In our previous work we have found that in double positive (DP) thymocytes, which are very sensitive to GC-induced apoptosis, the activated GR translocated to the mitochondria upon short term, high dose, in vitro dexamethasone (DX) treatment. This phenomenon was accompanied by the decrease of the mitochondrial membrane potential, as an early sign of apoptosis. After this observation we analysed the association of GR with members of the Bcl-2 protein family and we have found that the GR associated with certain members of the Bcl-2 family, which supposedly played important role in the induction of apoptosis. In our other work we have analysed the effect of in vivo DX treatment on the cellular composition of lymphoid organs. We have described that thymic regulatory T cells (tTregs) showed resistance to DX-induced apoptosis compared to CD4+ thymocytes, but peripheral regulatory T cells (pTregs) appeared to have similar sensitivity to DX-induced apoptosis as splenic $CD4^+$ T cells. Based on these previous results we wanted to further analyse the apoptosis of regulatory T cells and $CD4^+$ T cells whether the DX-induced apoptotic sensitivity or the induced apoptotic pathways are different in them.

In our work we analysed the differences and similarities between the DX-induced apoptosis of thymocytes and mature T cells, especially that of Tregs.

Splenocytes and thymocytes were isolated from 4–6-week-old BALB/c mice. The cells were treated with 10^{-6} M DX in vitro for different intervals. After DX treatments Annexin V labelling, kinetics of caspases' activation and mitochondrial membrane potential were analysed with flow cytometry.

DX-induced apoptotic process was different in mature T cells, particularly in Tregs, than in thymocytes. tTregs seemed to be the least sensitive to DX-induced apoptosis. The apoptosis sensitivity of pTregs were similar to $CD4^+$ splenocytes. $CD4^+$ thymocytes were the most sensitive to DX-induced apoptosis. These results can be the consequences of the different non-genomic effects in T cell subpopulations.

Funding: OTKA K105962, K101493, EFOP-3.6.1.-16-2016-00004; GINOP 2.3.2-15-2016-00050.

INHIBITORY POTENTIAL OF ANTI-ADAMTS13 ANTIBODIES IN ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA

György Sinkovits[1], Marienn Réti[2], Zoltán Prohászka[1]
[1]Research Laboratory, 3rd Department of Internal Medicine and MTA-SE Research Group of Immunology and Hematology, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary
[2]Department of Hematology and Stem Cell Transplantation, Szent László Hospital Campus, Central Hospital of Southern Pest, National Institute of Hematology and Infectious Diseases, Budapest, Hungary

Introduction: The acquired form of thrombotic thrombocytopenic purpura (TTP) is an autoimmune disease, in which the underlying ADAMTS13 deficiency is caused by autoantibodies against the protease. Some of the anti-ADAMTS13 antibodies inhibit the activity of the enzyme directly, whereas others facilitate its clearance from the circulation. Most anti-ADAMTS13 autoantibodies belong to the IgG1 and IgG4 subclasses; IgG4 was found to be dominant during and following a relapse of TTP.

The aim of our present study was to investigate the association between the inhibitory potential and IgG subclass of anti-ADAMTS13 autoantibodies.

Patients and methods: We previously determined the relative and absolute amount of anti-ADAMTS13 IgG subclasses with an in-house ELISA method based on a commercial ELISA kit in 101 samples of 81 TTP patients. The inhibitory capacity of 97 of these samples were determined by mixing and incubating the samples with equal amounts of pooled normal human samples, and measuring the ADAMTS13 activity of the mixture by a FRET assay. A low ADAMTS13 activity of the mixed sample indicated a high inhibitory potential of the autoantibodies, and vice versa. The specific inhibitory potentials of anti-ADAMTS13 IgG antibodies were determined in all acute samples with IgG4 proportions below 30% or over 70% (35 samples) and all available remission samples (14 samples). Samples were diluted with PBS to reach an equivalent (25 U/mL) final concentration of anti-ADAMTS13 IgG, and then the mixing activity assay was performed as described above.

Results: We found that the ADAMTS13 activity of the mixed samples indicating the inhibitory capacity was associated with anti-ADAMTS13 IgG4 ($r = -0.473$, $p < 0.0001$) but not with IgG1 ($r = -0.134$, $p = 0.191$) concentrations. The specific inhibitory potentials of IgG4-dominant anti-ADAMTS13 antibodies were higher compared to IgG1-dominant autoantibodies ($p = 0.0012$). There was no difference in the inhibitory potentials of IgG4-dominant samples from different disease stages.

Conclusions: Our results indicate that anti-ADAMTS13 autoantibodies of the IgG4 subclass have higher specific inhibitory potentials compared to those of the IgG1 subclass, irrespective of disease stages. Whether higher inhibitory potentials of IgG4 autoantibodies are attributable to the different properties of the Fc region, to affinity maturation parallel to isotype switching, or to other mechanisms, cannot be clearly determined from these data; additional studies are needed to answer these questions.

Support: Our work was supported by the National Research Fund of Hungary (OTKA K100687).

COMPLEMENT TERMINAL PATHWAY DEFICIENCY IN A PATIENT WITH RECURRENT MENINGITIS

Edina Erzsébet Szabó, Beáta Takács, Dorottya Csuka, Zoltán Prohászka
Semmelweis University, 3rd Department of Internal Medicine, Budapest

The complement system is a key part of innate immunity. The deficiency of terminal pathway components increases susceptibility to infections and their recurrence.

Medical history: A 22 years old female was hospitalized due to two episodes of meningococcal infection (at age of 15 meningitis, at age of 22 sepsis with serogroup C). Workup for complement identified deficiency of classical and alternative pathways, whereas complement factors and regulators were in the reference range. Anti-FH and anti-C1q autoantibodies were negative, raising the possibility of terminal complement pathway deficiency.

Level of complement component 8 (C8) in serum was repeatedly very low (16,5% and 18%, ref: 70-130%). Analysis was extended to family members (parents and 3 brothers) which excluded terminal pathway deficiency. These results raised the suspicion for a complex (including possibly also de novo variation) genetic trait behind the deficiency of our patient. We sent the patient's sample for next-generation sequencing (NGS), and the patient was found to carry two heterozygous mutations in exon 3 and 9 of the C8B gene, both causing the generation of a premature stop codon of the complement C8 protein β-chain, and 3 additional rare, and

multiple common variations. Detailed complement functional analysis and mutational screening of family members was very helpful to make conclusion on functionality of the identified variations.

The complex problem of identifying causative variations after NGS is increasing, and the history of our case highlights the necessity of detailed functional analysis and family screening for patients with rare diseases.

THE EFFECT OF INFLAMMATORY CYTOKINE TNF-α ON NEUTROPHILIC GRANULOCYTE'S EXTRACELLULAR VESICLE PRODUCTION

Viktória Szeifert[1], Ákos M. Lőrincz[1], András Ács[2], László Drahos[2], Erzsébet Ligeti[1]
[1]Department of Physiology, Semmelweis University
[2]MS Proteomic Research Group, Hungarian Academy of Sciences

Introduction: Previously, our group characterized three distinct extracellular vesicle (EV) populations released from human neutrophilic granulocytes (PMN): EVs formed spontaneously (sEVs), upon activation with opsonized particles (aEVs) and during apoptosis (apoEVs). Our primary aim was to examine the effect of TNF-α pre-treatment on production of different types of EVs from the PMNs. Our secondary aim was to examine the effect of the conditions of preparation on the neutrophilic granulocyte's EV formation.

Methods: Medium-size EVs were separated by a two-step centrifugation from PMNs isolated from the peripheral blood of healthy volunteers under different conditions (sterile/non-sterile) and at different time-points (immediately/delayed). We evaluated the EV release based on their count determined by flow cytometry, their protein amount determined by Bradford assay and their protein cargo determined by proteomic analysis.

Results: TNF-α pre-treatment resulted in an increase in sEV release and a decrease in protein-content. This effect was more pronounced on PMNs prepared under sterile conditions, and even higher on older PMNs. TNF-α did not increase the aEV release, the aEV/sEV ratio decreased. TNF-α was not able to substitute the opsonization in case of the non-opsonized particles. The TNF-α pre-treatment slightly increased the ratio of the granule proteins in sEVs, and increased the granule protein content even more in aEVs. Sterile PMN preparation condition could not potentiate further the TNF-αincreased aEV release. The older PMNs could release less EVs, with significantly higher protein-content vesicles and the TNF-α had greater impact on these cells' EV release. However, the ratio of the granule proteins was lower in these vesicles produced by older PMNs.

Conclusions: The effect of TNF-α seems to be highly dependent on the conditions of preparation, state of the cells (sterile or normal preparation; immediate or delayed stimulation), and on the secondary activator such as opsonins.

Funding: NKFIH K119236, Hungary

THE DIVERSITY OF THE HUMORAL IMMUNE RESPONSE OF BFCRN TRANSGENIC MICE

Szikora Bence[1], Anita Marx[1], Péter Károly Jani[2], Orsolya Pipek[3], István Csabai[3], Imre Kacskovics[1, 2]
[1]Department of Immunology, Eötvös Loránd University, Budapest, Hungary
[2]ImmunoGenes Ltd, Budakeszi, Hungary
[3]Department of Physics of Complex Systems, Eötvös Lóránd University, Budapest, Hungary

Introduction: The neonatal Fc receptor (FcRn) plays key roles in IgG and albumin homeostasis, maternal IgG transport, and antigen presentation of IgG-opsonized antigens. Previously, we reported that transgenic (Tg) mice that overexpress bovine FcRn (bFcRn) have augmented T-dependent humoral immune response with increased IgG protection, higher level of antigen-specific antibodies, greater number of antigen-specific B cells, and effective immune response even against weakly immunogenic epitopes. In addition, our research group demonstrated in a microarray-based oligopeptide scanning study that ovalbumin immunization results in greater diversity of immune response at IgM level in bFcRn Tg mice as compared with wt controls. As a next step, we analyzed the diversity of the humoral immune response of bFcRn Tg mice at greater details, using CDR3 spectratyping and Next Generation Sequencing (NGS) analysis.

Materials and Methods: bFcRn transgenic and wild type (wt; BALB/c) mice were immunized with different antigens (ovalbumin, and transfected cells expressing human cell surface molecules), using standard immunization protocols. Three days after the booster injection the animals were sacrificed and the antibody secreting plasma cells (CD138+) or the CD19 and antigen double positive cells in case of OVA were sorted out from the spleen by FACS. RNA was extracted from the cells and cDNA library was made from them. Thereafter, diversity of the heavy chain variable region of IgM and IgG antibodies were analyzed by either CDR3 spectratyping or NGS and evaluated by bioinformatics analysis.

Results and Discussion: We analyzed the diversity of the BCR repertoire of bFcRn Tg and wt mice with different immunization protocols, antigens, cell types and Ig isotypes. Our CDR3 spectratyping and NGS analysis show, that the BCR repertoire of bFcRn Tg animals is significantly more diverse compared to the wild type ones, which supports our previous observations indicating that the FcRn augments the humoral immune response and suggests, that the Tg mice are better choice for monoclonal antibody production.

INVESTIGATING THE ROLE OF PLCγ2 IN AUTOIMMUNE SKIN INFLAMMATION

Kata Szilveszter[1], Tamás Németh[1], Kitti Ajtay[1, 2], Zoltán Jakus[1, 2], Attila Mócsai[1]
[1]Department of Physiology, Semmelweis University School of Medicine, Budapest, Hungary
[2]MTA-SE „Lendület" Lymphatic Physiology Research Group of the Hungarian Academy of Sciences and the Semmelweis University, Budapest, Hungary

Background: Investigating the pathogenesis of autoimmune diseases is important due to their severity and the lack of proper therapies. Epidermolysis bullosa acquisita (EBA) is a human autoimmune skin inflammation caused by autoantibodies against type-VII collagen (C7) which is an essential component of the dermal-epidermal junction. Phospholipase Cγ2 is an important member of immunoreceptor signaling pathways leading to intracellular Ca^{2+} signal causing immune cell activation. Our aim was to study the role of PLγ2 in the effector phase of autoimmune skin inflammation using the mouse model of EBA.

Materials and methods: Rabbits were immunized by a GST fusion protein of the autoantigenic fragment of C7. Total IgG was purified by proteinG affinity chromatography. Purity was checked by SDS-PAGE. In the EBA model wild type and PLCγ2 deficient mice were injected subcutaneously five times with the total amount of 60 mg rabbit IgG. PBS was used as control. The development of the disease was followed for two weeks by measuring and scoring the affected skin area. Histology samples were prepared from ears followed by H&E staining and immunostaining against anti-rabbit IgG. Anti-C7 antibody levels in blood was tested by ELISA.

Results: Wild type mice developed a severe disease after antibody-treatment compared to control-treated animals. We observed alopecia, blisters and ulceration mostly on ears, cheeks and limbs. In contrast, PLCγ2 deficient mice appeared to be protected from the disease even though the amount of circulating anti-C7 antibody was similar to the wild type. Anti-C7 deposition at the dermal-epidermal junction in ear sections were present but dermal immune cell infiltration was strongly reduced in PLCγ2 deficient mice contrary to the wild type.

Conclusions: PLCγ2 deficient mice failed to develop relevant skin inflammation upon anti-C7 treatment compared to wild type animals. However, anti-C7 IgG was present in the circulation during the experiment and according to immunostaining it successfully deposited along the basement membrane in the skin. These results indicate that PLCγ2 have a role in the effector phase of autoimmune skin inflammation.

ORAL TOLERANCE AND REGULATORY T CELL FUNCTION IS IMPAIRED IN INNATE IMMUNE ACTIVATION OF B CELLS IN EARLY DIFFUSE CUTANEOUS SYSTEMIC SCLEROSIS

Vivien Telek[1], Judit Rapp[1], András Bognár[1], Gabriella Nagy[2], Tünde Minier[2], László Czirják[2], Timea Berki[1], Diána Simon[1]
[1]University of Pécs, Clinical Center, Department of Immunology and Biotechnology
[2]University of Pécs, Clinical Center, Department of Immunology and Rheumatology

Objective: Immunological abnormalities including B cell activation with autoantibody and cytokine production is associated with systemic sclerosis (SSc). The underlying processes remain elusive so far, but B cell activation is an early event in the development of SSc. Classical activation downstream of BcR and CD19 co-receptor engagement involves phosphatidylinositol-3 kinases (PI3K) signaling pathway and it also integrates the effects of multiple other co-stimulatory receptors.

Methods: Peripheral blood CD19+ B cells were purified from dcSSc patients and healthy controls. mRNA expression of 92 phosphatidylinositol-3 kinases (PI3K) pathway related genes was measured using a Taqman qPCR array and were validated by individual qPCR. Isolated B cells were activated by crosslinking the BCR in the presence or absence of IL-4. B cells were also stimulated with TLR4 ligands and anti-human CD180.

Results: Our analysis of PI3K pathway in CD19+ peripheral blood B cells from early diffuse cutaneous SSc (dcSSc) patients showed an overexpression of molecules playing part in alternate activation of B cells. Our finding that the expression of both IL-4 receptor and osteopontin (SPP1) are upregulated in B cells of untreated early dcSSc patients support the role of alternate B-cell activation in the pathogenesis of SSc, but they are downregulated upon immunosuppressive therapy. Remarkably, upregulation of TLR4 and complement component 3 and the downregulation of CD180 was not influenced by immunosuppressive treatment suggesting that innate immune molecules may have a role in preserving the activated state of B cells in early dcSSc. Our results also suggest that innate immune activation of B cells show differences in early dcSSc compared to healthy controls.

Conclusion: Analysis of purified B cells from early dcSSc patients revealed that B cells possess molecular changes in PI3K pathway that include innate immune signals. Understanding the involvement of alternate B-cell activation mechanisms in SSc could be essential for finding a clinically useful therapeutic target.

PHYLOGENESIS OF β-CATENIN: IDENTIFICATION AND CHARACTERIZATION IN ADULT, DEVELOPING AND REGENERATING EARTWORMS

Gréta Tolnai[1], Péter Engelmann[1], Ákos Boros[2], Éva Rumpler[3], Bohdana Kokhanyuk[1], László Molnár[3], Péter Németh[1], Kornélia Bodó[1]
[1]Department of Immunology and Biotechnology, Clinical Center, University of Pécs, Pécs, Hungary
[2]Regional Laboratory of Virology, ANTSZ, Baranya County, Institute of State Public Health Service, Pécs, Hungary
[3]Department of Comparative Anatomy and Developmental Biology, Faculty of Sciences, University of Pécs, Hungary

Wnt/β-catenin signaling is a highly conserved pathway throughout evolution. β-catenin (β-cat) plays an essential role in cell proliferation, differentiation, apoptosis, stem cell renewal and immune cell functions. We aimed to isolate and characterize the potential homologue of β-cat in Eisenia andrei earthworms.

We hypothesized the presence of a β-cat homologue in E. andrei due to its evolutionary conserved nature and central function in the cellular homeostasis. Based on the available annelid β-cat sequences conventional RT-PCR was executed to identify a β-cat candidate. The resulted PCR products were sequenced and analyzed. Then, qPCR analyses were performed to measure β-cat levels in adult tissues, embryos and regenerating blastema.

We identified the partial mRNA sequence for β-cat in E. andrei (Ea-β-cat). The cloned cDNA of Ea-β-cat consisted of 518 bp in the coding region. The deduced amino acid sequence comprised of 172 amino acids that fits to the first armadillo repeat. Phylogenetic analyses of Ea-β-cat showed strong similarity among Lophotrochozoan β-cat sequences. Ea-β-cat evidenced ubiquitous, but variable expression in the different Eisenia tissues: high (metanephridia), moderate (ventral nerve cord, ovary) or low (foregut, body wall, vesicular seminalis, coelomocytes). During embryogenesis we observed gradually elevation of Ea-β-cat up to the E4 stage. During anterior and posterior regeneration the patterning mechanisms was rather different.

Wnt-signaling proteins are highly conserved in a variety of organisms, but earthworms provided limited information in this extent. We identified the partial coding sequence of Ea-β-cat mRNA from E. andrei earthworms. Kinetic analysis of Ea-β-cat expression upon pathogen exposure is in progress. Based on our preliminary results Ea-β-cat plays indispensable role in the regular physiological conditions but in tissue renewal and blastema formation.

This work was supported by the Bolyai János Research Scholarship of the Hungarian Academy of Sciences and MedicalSchool Research Foundation, University of Pécs (PTE-ÁOK-KA 2017/04) to EP, GINOP-232-15-2016-00050 and EFOP-361-16-2016-00004.

BIOMARKERS FOR THE EARLY DIAGNOSIS OF LUNG CANCER: C9 EPITOPE VARIABILITY

Ilona Tornyi[1, 2], Annamária Ferenczi[2], József Lázár[2], László Takács[1, 2]
[1]University of Debrecen, Department of Human Genetics
[2]Biosystems International Ltd.

Incidence of lung cancer (LC) is among the three leading cancer types and takes the first place of cancer caused death. Average survival rate at five years is only 16%, and this depends on LC stage at diagnosis. Despite of novel therapies (targeted, immune and biological therapies) survival did not improve significantly since the early 1970-ies. With the help of early discovery of asymptomatic LC via novel biomarkers the survival rate is expected to improve.

A major system of innate immunity is the complement cascade (C), which also works in concert with adaptive immunity. The complement system includes a variety of proteins that circulate in blood in inactive forms. Previously, we identified the soluble form of plasma C9 as a member of a LC specific biomarker panel. We showed that C9 levels (quantity) in blood were significantly higher in LC compared to apparently healthy controls, but we detected this phenomenon exclusively via specific mAb-s reacting with particular epitopes of C9. With the aid of QuantiPlasma™ monoclonal antibody library and the Randox Evidence Investigator System, multiple C9 epitopes were profiled in LC patients and controls. Three epitopes of C9, which are detected by independent C9 specific mAbs behave in different ways in LC. Representation of C9 epitope-1, detected by BSI0581 increases, epitope-2 (BSI0639) decreases, while C epitope-3 (BSI0449) shows no-change.

There are numerous causes of protein variability (alternative promoters, alternative splicing, etc.). The observed phenomenon provides us with an experimental model to study the mechanisms of biomarker protein variability in lung cancer and provides an important application for epitope mapping in early LC diagnosis. Initial experiments addressing C9 protein variability will be presented.

MSCS HAVE THE POTENTIAL TO INDUCE THE GENERATION OF CTLA-4+ HUMAN MODCS

Anett Türk-Mázló[1], Márta Tóth[1], Ramóna Kovács[1], Ágota Apáti[2], Tamás Bíró[1], Krisztián Bene[1], Attila Bácsi[1], Éva Rajnavölgyi[1]
[1]Department of Immunology; University of Debrecen Faculty of Medicine, Hungary
[2]Research Group of Membrane Biology; Hungarian Academy of Sciences and Semmelweis University, Hungary

Introduction: Professional antigen presenting dendritic cells (DCs) are essential players in maintaining and restoring the sensitive balance between tolerance and immunity. The outcome of the communication between the highly plastic

DCs and their cellular interaction partners is determined by the developmental status and the functional activities of DCs as well as the available combination of immunomodulatory cytokines and lipid mediators. Mesenchymal stem cell like (MSCl) cells are a relevant cell population having potent ability to regulate the immune responses through the modulation of DC differentiation.

Methods: Wee co-cultured moDCs with MSCl cells for three days at a ratio of 5:1. After 4 days the moDCs were separated from the MSCl cells by the lectin receptor CD209. The separated moDCs were characterized by the expression of phenotypic and activation markers by using flow cytometry and cytokine production measured by ELISA. The polarisation of T cell response was monitored by ELISPOT and flow cytometry.

Results: In this study we revealed a mechanism by which MSCs contribute to the generation of co-inhibitory Cytotoxic T-Lymphocyte-Associated protein-4 (CTLA-4) and Programmed Death-Ligand 1 (PD-L1) expressing DCs derived from monocytes (moDCs) associated with inhibited expression of group I CD1 molecules and with up-regulated appearance of co-stimulatory and MHCII molecules. moDCs can be re-programmed by MSCs and are able to enhance the number of IL-10 and IL-17 producing T cells on a CTLA-4 dependent manner. We also identified the molecular mediators produced by MSCs which can modify the differentiation of moDC. The development and functions of moDCs are regulated by master transcription factors including PPARγ and RARα which receptors can be ligated by MSC-derived lipids such as all-trans retinoic acid (ATRA). We found that the selective blockade of ATRA production in MSCs as well as the inhibition of PPARγ and RARα activity in moDCs could restore the differentiation program of moDCs.

Conclusion: Collectively, we demonstrated that the cell surface expression level of CTLA-4 on moDCs could be induced by a changed lipid microenvironment by MSCls on a PPARγ and RARα dependent manner and the modulation of moDC development by MSCl polarizes IL-10 and IL-17 producing T cells on a CTLA-4 dependent manner. These results can be associated with clinical benefits in case of pathogenic conditions such as autoimmunity and cancer.

Support: The Project is Supported by the ÚNKP-18-3 New National Excellence Program of the Ministry of Human Capacities.

CHARACTERIZATION OF COMPLEMENT FACTOR H AUTOANTIBODIES IN NEUROMYELITIS OPTICA

Barbara Uzonyi[1], Zsóka Szabó[1], Eszter Trojnár[2], Satu Hyvärinen[3], Helle H. Nielsen[4], Katalin Uray[5], Anna Erdei[1], T. Sakari Jokiranta[3], Zoltán Prohászka[2], Zsolt Illes[4, 6], Mihály Józsi[1]

[1]Department of Immunology, Eötvös Loránd University, Budapest

[2]Research Laboratory, 3rd Department of Internal Medicine, Semmelweis University, Budapest

[3]Department of Bacteriology and Immunology, University of Helsinki

[4]Department of Neurology, University of Southern Denmark, Odense

[5]MTA-ELTE Research Group of Peptide Chemistry, Eötvös L. University, Budapest

[6]Division of Clinical and Experimental Neuroimmunology, University of Pécs

Neuromyelitis optica spectrum disorder (NMO-SD) is an autoimmune inflammatory disease of the central nervous system (CNS). NMO-SD is characterized by pathogenic, complement-activating autoantibodies against aquaporin 4 (AQP4), the main water channel in the CNS, and by lesions in the CNS containing complement activation products. Additional autoantibodies are frequently associated with NMO-SD. Since complement is implicated in the pathogenesis of NMO-SD, our aim was to evaluate the presence of autoantibodies against the complement regulator factor H (FH) in the serum of NMO patients.

Serum samples of 45 NMO-SD patients, all seropositive for AQP4 autoantibodies, were screened by ELISA. FH-specific mAbs, recombinant FH fragments, FH-related protein 1 (FHR-1) and synthetic peptides were used in ELISA to map autoantibody binding sites. Serum FHR-1 was analyzed by Western blot. FH–autoantibody complexes in sera were analyzed after IgG precipitation and Western blott. Avidity of the antibodies was measured in ELISA using NaSCN as a chaotropic salt. Inhibition of C3b binding to FH domains 19-20 was measured by ELISA.

4 out of 45 patients (~9%) had FH autoantibodies of IgG isotype. IgA was detected in one sample, also containing IgG FH autoantibody, and all samples were negative for IgM. FH autoantibody titres were low in three and high in one of the positive sera. The avidity indexes calculated for the FH autoantibodies were low. FH–IgG complexes formed in vivo were detected in the IgG fractions. The autoantibodies bound to FH domains 19-20, and also recognized FHR-1. Two autoantibodies bound within domain 19, one in domain 20 and one in both domains. In line with that the epitope within domain 19 is exposed under reducing conditions, autoantibody binding to reduced FH was stronger in those samples. FH autoantibodies inhibited binding of C3b to FH, similar to pathogenic FH autoantibodies associated with atypical hemolytic uremic

syndrome (aHUS). In contrast to the majority of FH autoantibody-positive aHUS patients, these NMO-SD patients did not lack FHR-1.

Our results identify FH autoantibodies in NMO-SD and suggest that generation of antibodies against complement components among other autoantibodies may contribute to the complement-mediated damage in NMO-SD.

HEADCASE, A NOVEL REGULATOR OF HEMOCYTE DIFFERENTIATION IN *DROSOPHILA MELANOGASTER*

Gergely I. B. Varga[1], Gábor Csordás[1], Ferenc Jankovics[1], Tamás Lukácsovich[2], Gyöngyi Cinege[1], Rita Sinka[3], Éva Kurucz[1], István Andó[1*] and Viktor Honti[1*]

[1]Institute of Genetics, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

[2]University of Zurich, Zürich

[3]Department of Genetics, Faculty of Science and Informatics, University of Szeged, Hungary

[*]Joint corresponding authors

Introduction: One of the main criteria of the effective immune system is the precise regulation of blood cell development. In the fruit fly, Drosophila melanogaster, the differentiation of the immune cells, the hemocytes – the phagocytic plasmatocytes, the melanizing crystal cells and the encapsulating lamellocytes – is controlled by a complex network of signaling pathways. We isolated the ortholog of the human tumor suppressor HECA, headcase (hdc), which is expressed in one of the immune compartments of the larva, the lymph gland. Headcase is downregulated in hemocytes that leave the organ upon immune induction, suggesting that it may act as a regulator of hemocyte differentiation.

Methods: P-element conversion and remobilization screens were carried out to generate a hdc-Gal4 driver and an amorphic hdc allele. Gal4-UAS system was applied to study the expression pattern of the factor throughout the development of the larva and to silence hdc by RNA interference. A candidate misexpression – and silencing screen was performed to identify the potential interaction partners of Hdc. The hematopoietic compartments were analyzed with fluorescent and in vivo confocal microscopy.

Results: In the lymph gland of the second instar larva, hdc expression was detected in the blood cell progenitors (MZ), in the differentiated hemocytes (CZ) and in the hematopoietic niche (PSC), however the frequency of the cells expressing hdc dramatically reduced by the end of the larval development. In hdc mutant larvae, spontaneous lamellocyte differentiation was observed. By silencing hdc with various hemocyte specific drivers, we mapped the focus of the mutation to the PSC and the MZ of the lymph gland. In a PSC-specific misexpression screen, we identified the JAK/STAT, the Decaplentaplegic (Dpp) and the Hedgehog (Hh) pathways as potential interacting partners of Hdc.

Conclusions: Our results show that Hdc is a key regulator of lamellocyte differentiation via its interaction with the JAK/STAT, the Dpp and the Hh pathways.

Acknowledgement: Our work is supported by OTKA NK-101730 (IA), PD-115534 (VH) and GINOP-2.3.2-15-2016-00001 grants. The research of V. Honti and G. I. Varga was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.4.A/ 2-11/1-2012-0001 'National Excellence Program'.

NOVEL IMIDAZO[1,2-B]PYRAZOLE-7-CARBOXAMIDES INDUCE APOPTOSIS IN HUMAN LEUKEMIA CELLS AT NANOMOLAR CONCENTRATIONS

Vanessza Laura Végi[1, 3], József Á. Balog[1], András Demjén[3], László Jr. Hackler[3], Iván Kanizsai[3], László G. Puskás[1, 3], Gábor J. Szebeni[1, 2]

[1]Laboratory of Functional Genomics, Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

[2]Department of Physiology, Anatomy and Neuroscience, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

[3]Avidin Ltd., Szeged, Hungary

Leukemia, the malignancy of the haematopoietic system accounts for 10 % of cancer cases with poor overall survival rate in adults warning high unmet medical need for the development of novel therapeutics. The library of 78 novel imidazo[1,2-b]pyrazole-7-carboxamides has been recently synthetized and published by Avidin Ltd. for anti-tumor activity. Further development resulted in more active compounds against leukemia cells reported herein.

Eight original imidazo[1,2-b]pyrazole-7-carboxamides has been tested for cytotoxic activity against five leukemia cell lines: acute promyelocytic leukemia (HL-60), acute monocytic leukemia (THP-1), acute T-lymphoblastic leukemia (MOLT-4), biphenotypic B myelomonocytic leukemia (MV-4-11) and chronic myelogenous leukemia (K562), and human primary fibroblasts as healthy control cells in vitro. Inhibitory concentration 50 (IC_{50}) has been determined for each cell type by the resazurin viability assay upon 72h treatment. Apoptosis has been studied by flow cytometry by Annexin V and propidium iodide staining after 24h of treatment. The expression of genes associated with drug induced cytotoxicity has been studied by quantitative real-time PCR after treatment with 40 nM and 200 nM compounds for 6, 12 and 24 hours.

Seven compounds of eight hampered the viability of all five leukemia cell lines with different potential. Optimization of structure activity relationship resulted in the following IC_{50} values for the most effective lead compound DU385: 16.54 nM, 27.24 nM and 32.25 nM on HL-60, MOLT-4, MV-4-11, respectively. Human primary fibroblasts were resistant in the

applied concentration range (12.3 nM – 3 μM). Four compounds under 100 nM IC_{50} value have been selected for apoptotic studies on the most sensitive three cell lines (HL-60, MOLT-4, Mv-4-11). Flow cytometry confirmed the absence of necrosis and revealed 60% late apoptotic population for MV-4-11 and 60% early apoptotic population for HL-60 suggesting that MV-4-11 cells are most sensitive to drug candidates. MOLT-4 cells showed only about 20% of total apoptotic population indicating that lymphoid leukemias are less sensitive than myeloid leukemia cells. Toxicogenomic study of the most active DU385 on the most sensitive Mv-4-11 cells dissected an early and a late response upon treatment. Genes were significantly downregulated as an early (6h) or midterm response (12h): EGR1, ATF3, FGF21, SLC21A3, LGALS1, LBR, ALOX5AP, TXN, ANXA2, GADD153, SOD1, UBC, BCL2A1, HSPA1A, CALR. Genes were significantly upregulated after 24h treatment: GDF15, ATF3, FGF21, SLC21A3, LBR, ALOX5AP, BCL2A1.

Novel imidazo[1,2-b]pyrazole-7-carboxamides induced apoptosis in human leukemia cells at nanomolar concentrations.

Funding: GJSz was supported by János Bolyai Research Scholarship (BO/00139/17/8) and Bolyai+ Research Scholarship (UNKP-18-4-SZTE-73).

IDENTIFICATION OF A FHR3 HYBRID PROTEIN IN A PATIENT WITH FAMILIAL FORM OF C3-GLOMERULONEPHRITIS

Nora Veszeli[1], Barbara Uzonyi[2], Nora Garam[1], Simona Stancu[3], Adrian Catalin Lungu[4], Mihály Józsi[2], Zoltán Prohászka[1], Dorottya Csuka[1]

[1]Research Laboratory, IIIrd Department of Internal Medicine, and MTA-SE Research Group of Immunology and Hematology, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary

[2]Complement Research Group, Department of Immunology, ELTE Eötvös Loránd University, Budapest, Hungary

[3]Carol Davila University of Medicine, Bucharest, Romania

[4]Fundeni Clinical Institute, Pediatric Nephrology Department, Bucharest, Romania

Introduction: C3 glomerulopathy (C3G) is an ultra-rare complement-mediated renal disease characterized histologically by the predominance of C3 deposition within the glomeruli. Familial cases of C3G are uncommon and offer exceptional insight into the genetic drivers of complement dysregulation. As part of the genetic diagnostic workup of a C3-glomerulonephritis (C3GN) patient, multiplex ligation-dependent probe amplification (MLPA) assay covering the complement factor H-related (CFHR) region was performed, and a genomic rearrangement was identified. The deletion in the CFHR3-1 region raised the possibility that an unusual hybrid protein may exist in the patient.

Methods: We obtained blood samples from 13 individuals from the patient's family. MLPA assay were performed with SALSA MLPA probemix P236-A3 kit (MRC-Holland, the Netherlands), designed to detect deletions or duplications in CFH, CFHR1, CFHR2, CFHR3 and CFHR5. We performed Western blot (WB) analyses with the serum of the patient as well as the involved family members using a panel of antibodies against FHR proteins. We designed a genomic PCR to verify the presence of the CFHR3-1 hybrid gene.

Results: WB analyses using monoclonal anti-FHR3, monoclonal anti-FH and monoclonal anti-FHR1 antibodies verified that the patient has no normal FHR3 protein but has a hybrid protein at 44-46 kDa consisting of FHR3 SCRs 1-2 and probably all five SCR domains of FHR1. The mother of the patient and her first degree cousin, both of them having end-stage renal disease, showed an identical FHR3 hybrid protein on WB. Three children in the family also showed the FHR3 hybrid protein on WB. We confirmed the presence of the CFHR3-1 hybrid gene in the family members who express the FHR3 hybrid, whereas no amplicon was generated in individuals with either two copy of the CFHR3 and CFHR1 genes or with homozygous CFHR3-CFHR1 deletion.

Conclusion: Based on our results the presence of the CFHR3-1 hybrid gene in all affected family members can be a unique cause of the disease in this family. We could not demonstrate complete segregation with the disease because three children carry the CFHR3-1 hybrid gene, however follow-up of them is ongoing because of their young age.

Support: National Research Fund of Hungary (OTKA PD116119).

SYK/CARD9 DEPENDENT IMMUNITY TO CANDIDA PARAPSILOSIS

Erik Zajta[1], Katalin Csonka[1], Adél Tóth[1], Janka Zsófia Csepregi[2], Anita Orosz[2], Tamás Németh[2], Csaba Vágvölgyi[1], Attila Mócsai[2], Attila Gácser[1, 3]

[1]Department of Microbiology, University of Szeged, Szeged, Hungary

[2]MTA-SE 'Lendület' Inflammation Research Group, Department of Physiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary

[3]MTA-SZTE 'Lendület' „Mycobiome" Research Group

C-type lectin receptors signal through Syk and CARD9. Several major fungal pathogens activate this route in innate immune cells. Candida parapsilosis is a regular cause of candidaemia and threatens especially neonates. Nevertheless, little is known about the immunological pathways triggered by C. parapsilosis infections. We aimed to examine the role of Syk and CARD9 in experimental C. parapsilosis infections.

We generated bone marrow chimeras with wild type (WT), Syk- or CARD9-deficient hematopoietic systems. Peritoneal macrophages and bone marrow derived macrophages were cultured and infected with C. parapsilosis or C. albicans. Supernatants were analysed for cytokine content. Phagocy-

tosis of C. parapsilosis and subsequent phagosomal acidification in macrophages were assessed by FACS. Mice were infected intravenously with C. parapsilosis or C. albicans and fungal burden was determined from organs and blood. PAS and HE stained histological sections from kidneys were prepared and examined for fungal presence and signs of inflammation. IL-1β was measured from kidney homogenates.

The absence of Syk or CARD9 in macrophages led to reduced proinflammatory cytokine secretion upon both C. albicans and C. parapsilosis infections. There was no difference in phagocytic activity between WT and CARD9 KO macrophages. Syk KO macrophages phagocytosed both yeast species less efficiently than WT cells. Ongoing experiments also seem to suggest impaired phagosomal acidification in Syk KO macrophages. Both Syk- and CARD9-deficient mice were highly susceptible to C. albicans. Syk- and CARD9-deficient mice were only slightly more sensitive to systemic C. parapsilosis infection than WT mice. Abundant C. albicans hyphae and leukocyte infiltrates were detected in the kidneys of C. albicans infected Syk- and CARD9-deficient mice. Fungal presence was scarce and strong inflammation was not seen in sections made from kidneys with all other experimental conditions. High production of IL-1β was measured in the kidneys of C. albicans infected Syk- and CARD9-deficient animals in contrast to C. parapsilosis infected ones.

Our data revealed that both Syk and CARD9 influence CC. parapsilosis induced host responses in vitro. However, unlike in the case of C. albicans, these signalling molecules are not as crucial players in the control of systemic C. parapsilosis infection as seen in C. albicans infection. This project was funded by NKFIH K123952 and GINOP-2.3.2- 15-2016- 00015.

INTERACTION OF KERATINOCYTES WITH THE SKIN COMMENSALIST CANDIDA PARAPSILOSIS AND PATHOGENIC CANDIDA ALBICANS

Erik Zajta[1], Emese Halmos[1], Máté Csikós[1], Lilla Erdei[2], Kornélia Szabó[3], Csaba Vágvölgyi[1], Lajos Kemény[2, 3], Attila Gácser[1, 4]
[1]Department of Microbiology, University of Szeged, Szeged, Hungary
[2]Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary
[3]MTA-SZTE Dermatological Research Group, Szeged, Hungary
[4]MTA-SZTE 'Lendület' „Mycobiome" Research Group

Candida infections are globally prevalent and are a threat to e.g. low weight neonates and immunocompromised patients. Besides severe deep-seated infections, Candida species can also lead to cutaneous infections. Candida parapsilosis is a commensalist on the human skin and rarely leads to disease there. In contrast, Candida albicans is not an element of the normal flora of the human skin, yet is the most predominant etiological agent of cutaneous candidiasis. We aimed to examine the interaction of the more pathogenic Candida albicans and the less harmful Candida parapsilosis with human keratinocytes.

We infected HaCaT and HPK-KER cell lines with Candida albicans (WO1 and SC5314 strains) and Candida parapsilosis (Clib214 and GA1 strains). We determined LDH activity from supernatants of infected keratinocytes and measured cytokine production from supernatants with ELISA. We used fluorescently stained yeast cells to investigate the attachment and phagocytic activity of keratinocytes against the two species with imaging flow cytometry.

Candida albicans damaged keratinocytes to a higher extent than Candida parapsilosis. Both Candida species induced the production of IL-6, IL-8 and CCL5 cytokines in a cell line and strain dependent manner. No detectable IL-1β, TNF-α and IL-10 was produced by Candida infected keratinocytes. Cells of both Candida species were able to adhere to keratinocytes and we observed occasional phagocytosis of both yeasts by skin cells. However, yeast-keratinocyte association was very sparse in both cases.

This project may help to better understand skin immunity to pathogenic and commensalist microbes. This project was funded by GINOP-2.3.2- 15-2016- 00015.

SIMPONI (golimumab) 50 mg oldatos injekció előretöltött injekciós tollban (1x), ill. előretöltött fecskendőben (1x) (fogyasztói ára: 318 740 Ft; tb-támogatás: 100%, tételes elszámolás alá eső készítmény a 70/2011. (XII. 23.) és a 2/2012. (I. 3.) NEFMI rendeletek alapján [esetleges változások: www.neak.gov.hu]). **Javallatok**: **1.** *Rheumatoid arthritis (RA):* metotrexáttal (MTX) kombinálva: közepesen súlyos/súlyos aktív RA kezelésére felnőtteknél, ha a betegség lefolyását módosító rheuma elleni gyógyszerekkel (DMARD-ok, beleértve a MTX-ot is) a terápiás válasz nem volt megfelelő; súlyos, aktív, progresszív RA kezelésére korábban MTX-tal nem kezelt felnőtteknél. **2.** Polyarticularis juvenilis idiopathiás arthritis (pJIA) kezelésére MTX-tal kombinálva a korábbi MTX-kezelésre nem megfelelően reagáló, legalább 40 kg testtömegű gyermekeknél. **3.** Aktív és progresszív arthritis psoriatica (APs) kezelésére felnőtteknél monoterápiában v. MTX-tal kombinálva, ha a korábbi DMARD-ok hatása nem volt megfelelő. **4.** Axiális spondyloarthritis: súlyos, aktív spondylitis ankylopoetica (SA) kezelésére felnőtteknél, akik nem reagáltak megfelelően a hagyományos kezelésre; a gyulladás objektív tüneteit mutató (melyet emelkedett C-reaktív protein szint (CRP) és/vagy MRI vizsgálati eredmény igazol) súlyos, aktív, nem-radiológiai axiális spondyloarthritis (nr-axiális SpA) kezelésére felnőtteknél, amennyiben az előzetesen alkalmazott nem szteroid gyulladásgátló szerrel (NSAID) történt kezelés hatása nem volt megfelelő, vagy NSAID intolerancia áll fenn. **5.** Középsúlyos-súlyos colitis ulcerosa (CU) kezelésére felnőtteknél, akik nem reagáltak megfelelően a hagyományos kezelésre, beleértve a kortikoszteroidokat, a 6-merkaptopurint v. az azatioprint is, vagy ezeket a kezeléseket nem tolerálták, ill. azokkal szemben orvosi ellenjavallat áll fenn. **Ellenjavallatok**: a készítmény hatóanyagával v. bármely segédanyagával szembeni túlérzékenység. Aktív tuberkulózis (tbc) v. egyéb súlyos infekció, pl. szepszis, opportunista fertőzések. Közepesen súlyos v. súlyos szívelégtelenség (NYHA III/IV stádium). **Adagolás és alkalmazás**: a kezelést csak RA, pJIA, APs, SA, nr-axiális SpA vagy CU diagnosztizálásában és kezelésében jártas szakorvos kezdeményezheti és felügyelheti. A SIMPONI-val kezelt betegeknek egy Betegtájékoztató kártyát kell kapniuk. A SIMPONI subcutan alkalmazandó. Adagja RA, pJIA (legalább 40 kg testtömegű gyermekeknek), APs, SA, nr-axiális SpA esetén havonta 50 mg, a hónapnak ugyanazon a napján adva, RA és pJIA esetén MTX-tal kombinálva. CU esetén (80 kg-nál kisebb testtömegű betegeknek): a kezdő adag 200 mg, melyet a 2. héten 100 mg követ. A megfelelően reagáló betegeknél 50 mg-ot kell alkalmazni a 6. héten, majd azután 4 hetente. A nem megfelelően reagáló betegeknél kedvező hatású lehet, ha továbbra is 100 mg-ot kapnak a 6. héten, majd azután 4 hetente. CU esetén (80 kg vagy ennél nagyobb testtömegű betegeknek): a kezdő adag 200 mg, melyet a 2. héten, majd azután 4 hetente 100 mg követ. A fenntartó kezelés során a kortikoszteroidok fokozatosan leépíthetőek a klinikai gyakorlati útmutatóknak megfelelően. A klinikai válasz rendszerint 12–14 hét (3-4 dózis) után jelentkezik. Ha ebben az időszakban nem mutatkozik semmilyen terápiás előny, a kezelés folytatását át kell gondolni. **Figyelmeztetések**: *Infekciók:* a kezelés előtt, alatt és befejezése után akár 5 hónapig gondosan figyelni kell az infekciókra, beleértve a tbc-t is. A TNF-gátlót kapók fogékonyabbak a súlyos fertőzésekre. A kezelés előtt vizsgálni kell az aktív v. inaktív (látens) tbc és a HBV-fertőzés meglétét. Aktív tbc: SIMPONI-kezelést tilos kezdeni. Látens tbc: megfelelő tbc elleni terápiát kell kezdeni a SIMPONI-kezelés megkezdése előtt. A SIMPONI-kezelés alatt gondosan ellenőrizni kell az aktív tbc-re utaló panaszokat és tüneteket. SIMPONI-kezelést igénylő, HBV-t hordozó betegek: a kezelés alatt és befejezése után több hónapig gondosan figyelni kell a reaktiválódás jeleire. A HBV reaktiválódásának megelőzésére TNF-gátlóval egyidejűleg adott antivirális terápia hatásossága nem ismert. A HBV reaktiválódása esetén a SIMPONI-kezelést meg kell szakítani, és hatásos antivirális terápiát, megfelelő szupportív kezelést kell kezdeni. *Rosszindulatú daganatok és lymphoproliferatív betegségek:* TNF-gátlókkal kezelteknél a lymphomák, leukaemia v. egyéb malignitások kifejlődésének kockázata nem zárható ki. TNF-gátló-kezelés kezdését gondosan mérlegelni kell, ha a kórelőzményben rosszindulatú daganat szerepel, ill. megfontolandó a kezelés folytatása, ha malignitás alakul ki. A hepatosplenicus T-sejtes lymphoma kialakulásának kockázata TNF-gátlókkal kezelt betegek esetében nem zárható ki: az AZA v. 6-MP és a SIMPONI kombinációjának potenciális veszélye megfontolást igényel. Azon betegeknél, akiknek kórtörténetében vastagbél dysplasia v. carcinoma szerepel, vagy akiknél fokozott ezek kialakulásának veszélye, rendszeres időközönként szűrővizsgálatokat kell végezni a kezelés megkezdése előtt, valamint a betegség fennállásának ideje alatt, ill. akiknél újonnan diagnosztizáltak dysplasiat, át kell gondolni, hogy a terápia folytatható-e. A TNF-gátlók alkalmazása körültekintést igényel COPD-s, valamint a malignitások szempontjából az erős dohányzás miatt magasabb kockázatú betegek esetén. Időszakos bőrvizsgálat javasolt, különösen a bőrrák kockázati tényezőivel rendelkező betegeknél. *Pangásos szívelégtelenség:* körültekintően kell alkalmazni enyhe szívelégtelenségben (NYHA I/II). *Neurológiai események:* demyelinisatiós kórkép esetén a kezelés előtt gondosan mérlegelni kell annak előnyeit és kockázatait. Ha ezek a kórképek kialakulnak, mérlegelni kell a kezelés leállítását. *Sebészeti beavatkozások:* tervezésükkor figyelembe kell venni a hosszú felezési időt. A kezelés alatt műtendő betegnél figyelni kell a fertőzések kialakulására, és meg kell tenni a megfelelő intézkedéseket. *Autoimmun folyamatok:* lupus-szerű szindrómára utaló tünetek és kétszálú DNS elleni antitest-pozitivitás esetén a kezelést abba kell hagyni. *Hematológiai reakciók:* a kezelés leállítását megerősített, jelentős haematológiai eltérésnél meg kell fontolni. *Váltás biológiai DMARD-ok között:* figyelni kell a fertőzések kialakulásának jeleire. *Allergiás reakciók:* súlyos allergiás reakció esetén a SIMPONI adását azonnal be kell fejezni, és megfelelő kezelést kell kezdeni. *Latex szenzitivitás:* az injekciós toll tűjének védőkupakja latex tartalmú, az arra érzékenyeknél allergiás reakciót válthat ki. **Különleges betegcsoportok**: idősek (≥65 év) kezelését körültekintően kell végezni, kiemelt figyelmet fordítva a fertőzések előfordulására. Beszűkült vese- v. májműködésű betegeknél nem végeztek specifikus vizsgálatokat. Beszűkült májműködésű betegeknek óvatosan kell adni. Gyermekgyógyászati betegeknél javasolt, hogy a SIMPONI-kezelés megkezdése előtt a hatályos védőoltási irányelveknek megfelelően megkapjanak minden védőoltást. 18 évesnél fiatalabb betegeken a pJIA-tól eltérő indikációkra vonatkozóan nem igazolták biztonságosságát és hatásosságát. **Interakciók**: SIMPONI és anakinra, abatacept v. egyéb biológiai terápiák kombinált alkalmazása nem ajánlott. A SIMPONI-val egyidejűleg nem adhatók élő vakcinák, ill. terápiás alkalmazású fertőző ágensek alkalmazása nem javasolt. Egyidejű MTX-kezelés: az adatok nem utalnak arra, hogy akár a SIMPONI, akár a MTX adagjának megváltoztatására lenne szükség. **Terhesség és szoptatás**: terhes nőknél nem ajánlott. Fogamzóképes nőknek hatékony fogamzásgátlást kell alkalmazniuk, és azt folytatni kell az utolsó adagtól számítva min. 6 hónapig. A kezelés alatt és az utolsó adagtól számítva min. 6 hónapig nem szabad szoptatni. **Főbb mellékhatások (≥1%)**: felső légúti infekciók (nasopharyngitis, pharyngitis, laryngitis, rhinitis), bakteriális fertőzés (cellulitis), alsó légúti fertőzés (pneumonia), vírusfertőzés (influenza, herpes), bronchitis, sinusitis, felületes gombafertőzések, abscessus, leukopenia, neutropenia, anaemia, allergiás reakciók (bronchospasmus, hypersensitivitás, urticaria), autoantitest-pozitivitás, depresszió, álmatlanság, szédülés, fejfájás, paraesthesia, hypertensio, asthma és a kapcsolódó tünetek (sípoló légzés, bronchialis hyperactivitas), dyspepsia, gastrointestinalis és abdominalis fájdalom, hányinger, gastrointestinalis gyulladásos megbetegedések (gastritis, colitis), stomatitis, ALT/AST-emelkedés, pruritus, kiütés, alopecia, dermatitis, láz, asthenia, az injekció beadási helyén jelentkező reakciók (erythema, urticaria, induratio, fájdalom, véraláfutás, viszketés, irritáció, paraesthesia), mellkasi discomfort, csonttörések. A gyermekgyógyászati vizsgálatban (pJIA) a jelentett nemkívánatos események típusa és gyakorisága általában hasonló volt a felnőttek bevonásával végzett RA vizsgálatokban megfigyeltekéhez.

Felírás előtt, kérjük, olvassa el a teljes alkalmazási előírást, különös tekintettel a javallatokra (4.1), az adagolásra (4.2), az ellenjavallatokra (4.3) és a figyelmeztetésekre (4.4)! (02/07/2018 EMEA/H/C/992/II/79).

Referenciák: 1. Deodhar A, et al. Ann Rheum Dis. 2015; 74(4): 757–761. **2.** Kavanaugh A, et al. Ann Rheum Dis. 2014; 73(9): 1689–1694.**3.** Keystone EC et al. J Rheumatol. 2016;43:298-306.

MSD
MSD Pharma Hungary Kft.
1095 Budapest, Lechner Ödön fasor 8.,
Telefon: 06-1-888-5300, Fax: 06-1-888-5388, hungary_msd@merck.com

A dokumentum lezárásának ideje: 2018. július 13.
RHEU-1230281-0028

Tocilizumab: mi az új a Nap alatt?

Szamosi Szilvia, Szűcs Gabriella, Szekanecz Zoltán

Debreceni Egyetem Általános Orvostudományi Kar, Belgyógyászati Intézet, Reumatológiai Tanszék, Debrecen

Az elmúlt két évben nagy mennyiségű új adat jelent meg az IL-6-receptor-gátló tocilizumab (TCZ) hatásairól rheumatoid arthritisben (RA), juvenilis idiopathiás arthritisben (JIA) és óriássejtes arteritisben (GCA), azaz a TCZ-kezelés indikációit képező betegségekben. Több klinikai vizsgálatban nyert megerősítést a TCZ hatékonysága és biztonságossága intravénás vagy subcutan, methotrexattal (MTX) kombinációban vagy monoterápiában alkalmazva. RA-ban igen hatékony a korai betegségben. Számos adat szerint a TCZ kedvezően befolyásolja a betegek által jelentett mutatókat, a munkaképességet és az életminőséget. Lassítja a radiológiai progressziót, és, MRI-vizsgálatok alapján, csökkenti a synovitist. JIA-ban javíthatja a növekedést. Igen jó a TCZ-hez való adherencia. GCA-ban kiemelkedő hatékonyságú. A klinikai remisszióban levőknél is fennmaradhat szubklinikus gyulladás, ezért a TCZ-kezelést tartósan folytatni kell. A TCZ-kezelés mellett sok információt kapunk az IL-6 patogenetikai szerepéről, hiszen a TCZ befolyásolja az adipokineket, az extracelluláris mátrix molekulákat, csökkenti a gyulladásos angiogenesist és csontvesztést. A terápiás hatást több biomarker (pl. IL-6, sIL-6R, mátrixmolekulák) jelezheti. A TCZ immunogenitása nagyon alacsony. A társbetegségek tekintetében az ENTRACTE és más vizsgálatok alapján a TCZ valószínűleg nem növeli a cardiovascularis rizikót. Kedvezően befolyásolja a szénhidrát-anyagcserét, a fáradtságot, a szekunder osteoporosist és ritkán okoz súlyos májfunkciós eltéréseket. Összességében a TCZ mindegyik indikációjában igen hatékony.

Kulcsszavak: rheumatoid arthritis, juvenilis idiopathiás arthritis, óriássejtes arteritis, interleukin-6, tocilizumab, kortikoszteroid, cardiovascularis, immunogenitás

TOCILIZUMAB: WHAT IS NEW UNDER THE SUN?

During the last two years, a great amount of new data has been published with respect to the effects of the IL-6 receptor inhibitor tocilizumab (TCZ) in rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA) and giant-cell arteritis (GCA). These are the approved indications for TCZ. Numerous clinical trials supported the efficacy and safety of TCZ both in intravenous or subcutaneous forms, both in combination with methotrexate (MTX) or in monotherapy. In RA, it is highly effective in early disease. TCZ favourably influences patient-reported outcomes, work productivity and quality of life. TCZ slows radiographic progression and, due to MRI studies, suppresses synovitis. In JIA, TCZ may stimulate growth. There is good adherence to TCZ. In GCA, TCZ exerts extraordinary efficacy. However, in clinical remission, subclinical inflammation may persist, therefore, sustained TCZ therapy is needed. TCZ treatment enlightens numerous aspects of the action of IL-6. TCZ interferes with adipokine release and extracellular matrix molecules, inhibits inflammatory angiogenesis and bone destruction. There are some biomarkers of therapeutic efficacy (e.g. IL-6, sIL-6R, matrix molecules). TCZ has very low immunogenicity. With respect to comorbidities, ENTRACTE and other studies suggest that TCZ does not increase cardiovascular risk. TCZ favourably affects carbohydrate metabolism, osteoporosis, fatigue and only very rarely causes severe liver toxicity. In summary, TCZ is efficacious in all indications.

Keywords: rheumatoid arthritis, juvenile idiopathic arthritis, giant-cell arteritis, interleukin 6, tocilizumab, corticosteroid, cardiovascular, immunogenicity

Bevezetés

A célzott terápia nagy áttörést jelentett az arthritises és autoimmun betegek kezelésében [1, 2]. Az EULAR legfrissebb, 2016-os, rheumatoid arthritisre (RA) [3], illetve korai arthritisre [4] vonatkozó ajánlásai pontosan meghatározzák a célzott terápia bevezetésének feltételeit és a kezelés folyamatát.

Az interleukin-6 (IL-6) proinflammatorikus citokin, mely központi szerepet játszik az arthritist kísérő gyulladásos fo-

lyamatokban, de részt vesz a szisztémás tünetek kialakulásában is [5]. Az IL-6 központi szerepét korábban e lap hasábjain már áttekintettük [6]. Jelenleg egy IL-6–receptor- (IL-6R-) gátló, a tocilizumab (TCZ), törzskönyvezett és hazánkban támogatott is az RA kezelésére. A TCZ-t korábban törzskönyvezték a polyarticularis (pJIA) [7] és szisztémás (sJIA) juvenilis idiopathiás arthritis kezelésére is [8]. Legutóbb 2016-ban foglaltuk össze az Immunológiai Szemle hasábjain a TCZ-vel kapcsolatos friss adatokat [9].

A reumatológiában és immunológiában a kutatás és fejlesztés rohamléptekkel halad. Ez vonatkozik a TCZ-re is, amellyel kapcsolatban évente száznyi új közlemény jelenik meg. A legfontosabb a már említett, 2016-2017-ben publikált friss RA [3] és korai RA ajánlás [4]. Emellett időközben, miután egyértelműen igazolódott a TCZ hatékonysága és biztonságossága óriássejtes arteritisben (giant cell arteritis, GCA) [10, 11], a szert 2017-ben Európában, így hazánkban is törzskönyvezték a GCA kezelésére [12]. Ezért úgy gondoltuk, áttekintjük a TCZ-vel kapcsolatban az elmúlt két évben megjelent érdekesebb és fontosabb információkat (1. táblázat).

A tocilizumabterápia a nemzetközi ajánlásokban

Az EULAR 2016-ban újította meg az RA hagyományos és célzott betegségmódosító szerekkel való kezelésére vonatkozó ajánlásait. Az ajánlás megerősítette, hogy a TCZ alkalmazható methotrexat- (MTX-) hatástalanság után első vonalban köz-

1. táblázat. **Főbb új adatok a tocilizumabkezelés hatásairól***

Kategória	Betegség	Hatás
Klinikai hatékonyság	RA	– hatékonyság korai RA-ban (U-Act-Early) – kombináció utáni MTX-elhagyás után a hatékonyság fennmarad – javulnak a PRO-k – javul az életminőség – javul a munkahelyi produktivitás
	pJIA, sJIA	– a növekedési retardáció ellen hat (pJIA) – fokozott perzisztencia (sJIA)
	GCA	– kiemelkedő hatékonyság, klinikai remisszió közel 100%-ban
Képalkotás	RA	– röntgenen a radiológiai progresszió lassul – MRI-n a synovitis gyulladásos foka csökken
	GCA	– klinikai remisszió esetén szubklinikus gyulladás maradhat fent az érfalban (MRA, PET-CT)
Patogenezis megismerése	RA	– adipokinek: chemerin csökken, adiponektin nő – sejtadhézió, mátrixmolekulákra hat – csökken a gyulladásos angiogenesis – csökken a gyulladásos csontvesztés – helyreállítja a B-sejt citokinegyensúlyt – csökkenti a C3, C4 komplementfaktorokat
Biomarkerek (hatékonyság)	RA	– ACPA-státusztól függetlenül hatékony – kiinduláskor magas IL-6 és alacsony sIL-6R esetén jobb hatékonyság – hatás összefügg bizonyos mátrixkomponensekkel
Immunogenitás	RA	– gyógyszerellenes antitest nagyon ritka
Társbetegségek	RA	– nem fokozza a CV kockázatot (ENTRACTE, más vizsgálatok) – csökkenti az NT-proBNP-szintet – csökkenti a proatherothromboticus profilt – hiperkatabolizmus miatt emeli a lipidszinteket (lipidparadoxon) – csökkenti a $HgbA_{1c}$-t – csökkenti a fáradtságot – lassítja/megállítja a csontvesztést – ritkán okoz súlyos májfunkciós eltérést – ritkán okoz súlyos infekciót – hepatitis B-n átesetteknek is adható – valószínűleg nem fokozza a daganatok kockázatát

*A rövidítések magyarázatát lásd a szövegben

vetlenül, vagy más hatásmechanizmusú biológiai terápia után másod- és harmadvonalban is. Ami újdonság, hogy az ajánlás 9. pontja kiemeli, hogy bár a biologikumokat MTX-szel kombinálva javasolt adni, azon betegekben, akikben MTX nem adható, a TCZ és a szintetikus célzott szerek (JAK-gátlók) előnyt élveznek más biologikumokkal szemben, azonban jelenleg a legtöbb klinikai bizonyíték a TCZ-vel van [3]. Az új korai RA ajánlás hasonló elveket követ, a TCZ-re vonatkozó egyéb megfontolásokra nem tér ki [4].

Bár GCA-ra vonatkozóan hivatalos terápiás ajánlás nem jelent meg, meg kell említenünk, hogy az EULAR munkacsoport, Dejaco és mtsai [13] 2018-ban közölte a GCA képalkotó diagnosztikájára vonatkozó ajánlását, amelynek fontos relevanciája van a TCZ kezelésre nézve. Összesen 12 pontban fogalmazták meg ajánlásaikat. GCA-ban az ultrahangot javasolják első lépésben, a koponya ereinek arteritise esetén az MRI nem ajánlott. A PET-CT alternatív lehetőség. A képalkotás segíthet a gyulladásos aktivitás monitorozásában, a terápia követésében [13].

Klinikai vizsgálatok: közelebb az IL-6-gátlás hatásmechanizmusához

Rheumatoid arthritis

A legtöbb adat a 2016-ban folytatott U-Act-Early klinikai vizsgálat kapcsán jelent meg. Mint két éve már beszámoltunk róla [9], a holland munkacsoport, jelesül Bijlsma és mtsai [14] végezték ezt a korai terápiás ablak elvének megfelelő multicentrikus, randomizált, kontrollált, kettős vak (RCT) vizsgálatot. Ebben korai RA-ban szenvedő betegeket kezeltek a napi gyakorlatnak megfelelően TCZ + MTX kombinációval, illetve TCZ- vagy MTX-monoterápiával. A betegek valóban nagyon korai fázisban voltak, az átlagos betegségtartam 25–27 nap (!) volt. Az elsődleges végpont a remisszió (DAS28 <2,6) volt, amely legalább 24 hétig tartott (tartós remisszió). Amikor ezt a tartós remissziót elérték, a TCZ-t, illetve MTX-et leépítették, majd elhagyták. A betegeket 60, sőt 120 hétig utánkövetve, a TCZ + MTX kombinációt kapók 85%-a, a TCZ-monoterápián lévők 80%-a, az MTX-monoterápiával kezeltek 40%-a volt remisszióban [14]. Ezt követően további elemzések történtek a U-Act-Early tanulmányban. Teitsma és mtsai [15] a beteg által jelentett kimeneteli mutatók (patient reported outcome; PRO) vonatkozásában azt találták, hogy az életminőségi indexek (SF-36 fizikai komponens, EQ-5D) szignifikánsan jobban javultak bármely TCZ-stratégia (TCZ + MTX vagy TCZ-monoterápia) mellett, mint MTX esetén [15].

A U-Act-Early vizsgálatot is végző munkacsoport, Teitsma és mtsai [16] elvégezték a TCZ mono- és kombinációs terápia metaanalízisét is. Összesen 13 RCT-t választottak be, a metaanalízist 6679 kezelt betegen végezték. Amikor a kombinált TCZ- és a TCZ-monoterápiát vetették össz, kismértékben több beteg került remisszióba (DAS28 <2,6) (RR 1,21; 95% CI: 1,09–1,36), illetve többen teljesítették az ACR50 választ (RR 1,14; 1,03–1,26) a kombinációs kezelés mellett. Ezzel együtt azonban a súlyos mellékhatások (SAE) aránya is szignifikánsan magasabb volt (RR 1,40; 1,03–1,92) a kombináció mellett. Emellett azt is igazolták, hogy szintetikus DMARD (csDMARD-) kezelés mellett, amennyiben a célt nem érik el, mindenképpen statisztikailag hatékonyabb bármelyik TCZ-stratégiára (monoterápia vagy kombináció) váltani, mint a csDMARD-kezelésen maradni [16]. Ami a radiológiai progressziót illeti, az erosiók tekintetében két év után a progresszió lassabb volt a TCZ + MTX és a TCZ-karokon a MTX-karhoz képest. Az ízületi rés szűkület tekintetében nem volt különbség a három kar között. A progressziólassítás elsősorban a lábakon volt kifejezett. A progressziót nem mutató betegek aránya is szignifikánsan magasabb volt TCZ-kezelés mellett az MTX-karhoz képest mind 52, mind 104 hét után [17]. A U-Act-Early vizsgálatban elemezték a hatékonysággal összefüggő fehérjebiomarkereket is, amiket később mutatunk be [18].

Ishiguro és mtsai [19] Japánban végezték a FIRST Bio vizsgálatot. Ebben a biológiai terápia naiv betegek TCZ-kezelését elemezték a mindennapi gyakorlatban. Összesen 839 TCZ-vel első vonalban kezelt beteg adatait elemezték. A CDAI remissziós arány 37% volt. A remissziót elérők fiatalabbak voltak, rövidebb betegségtartammal és társbetegségek nélkül. A HAQ-DI legalább 0,5 pontos csökkenése a betegek kétharmadában jelentkezett. Az eleinte kombinációban adott MTX és kortikoszteroid (KS) elhagyása a betegek 19, illetve 34%-ában volt lehetséges a TCZ-kezelés során. A SAE incidenciája 18 esemény/100 betegév volt, a leggyakoribb detektált mellékhatás az infekció [19].

Szólnunk kell a 22 országban lefolytatott TOZURA vizsgálatról is. Choy és mtsai [20] számoltak be e IV. fázisú program eredményeiről. Ebben a nyílt vizsgálatban 1804 RA-s beteg 24 hétig kapott sc. TCZ-t monoterápiában (19,6%) vagy kombinációban (80,4%). A DAS28-We szignifikánsan csökkent. A remissziót vagy ACR20, ACR50, ACR70 választ elérők aránya azonos volt mono- és kombinációs terápia mellett. SAE a betegek 5,8%-ában lépett fel. Ez a tanulmány is igazolta a sc. TCZ hatékonyságát mind MTX-szel kombinálva, mind monoterápiában [20].

A strukturális károsodást vizsgálták Bird és mtsai [21] az AC-CUTE nyílt ausztrál vizsgálatban. Összesen 52 közepesen súlyos vagy súlyos RA-s beteget vontak be és a kezek és lábak röntgen-, valamint a kezek MRI-vizsgálatát végezték el sc. TCZ-kezelés mellett. MRI-n a synovitis szignifikánsan csökkent. A radiológiai progresszió tekintetében nem volt szignifikáns változás. Mindezt klinikai javulás kísérte: 24 hét után a betegek 78%-a ért el DAS28 remissziót [21].

Ebina és mtsai [22] az ANSWER kohorszban hét biologikum retencióját és a kezelés félbeszakításának okait hasonlította össze. Összesen 1037 kezelési eseményt dolgoztak fel. Ennek 44%-ában (455 esemény) kellett az adott szert leál-

lítani. A leállítások hátterében hatástalanság/hatásvesztés (21%), káros mellékhatások (8%), a remisszió elérése (4%) és egyéb okok (11%) álltak. Összességében a retenciós ráta legmagasabb a TCZ és az abatacept esetén volt. A TCZ esetében a hatástalanság miatt ritkábban kellett a kezelést leállítani, mint az infliximab esetében [22].

Fontos kérdés lehet, hogy azoknál, akik TCZ és MTX kombinációt kapnak, az MTX elhagyása után, TCZ-monoterápia esetén fennmarad-e a hatékonyság. Kremer és mtsai [23] vizsgálatában MTX-re nem reagáló RA-s betegek TCZ + MTX kombinációt kaptak. Ezután 24 heti kezelést követően a legalább LDA-t elérők az 52. hétig vagy folytatták a kombinációt, vagy az MTX-et elhagyva TCZ-monoterápiát kaptak. A kezdeti 718 betegből 24 hét után 296-ot tudtak tovább követni. Az MTX abbahagyása nem hozott rosszabb eredményt a betegségaktivitás vonatkozásában (noninferioritás) a kombinációt folytatókhoz képest. A mellékhatások aránya is hasonló volt a két csoportban. A TCZ + MTX kezelés során legalább LDA-t elérőknél tehát az MTX elhagyható [23].

Strand és mtsai [24] TCZ-monoterápia mellett követték a PRO-k alakulását. Két III. fázisú vizsgálat (AMBITION, ADACTA) adatait elemezték. Az AMBITION vizsgálatban a TCZ-monoterápiával kezelt betegekben kifejezettebben javult a funkció (HAQ), fáradtság (FACIT-Fatigue), az életminőség-skála fizikai komponensei (SF-36 PCS) az MTX-szel szemben. Az ADACTA vizsgálatban jobban javult a betegek megítélése, a fájdalom és az SF-36 skála mentális komponensei a TCZ-kezelés mellett adalimumabbal szemben [24]. Ugyanez a munkacsoport TCZ-vel iv. és sc. kezeltekben is összevetette a PRO-kra gyakorolt hatást. Az OPTION, SUMMACTA és BREVACTA vizsgálatok alapján az iv. és sc. beviteli mód szempontjából nincs különbség. A TCZ mindkét esetben szignifikánsan javította a PRO-kat a placebóhoz képest [25].

Tanaka és mtsai [26] a FIRST ACT-SC vizsgálatban a TCZ és a munkaképesség összefüggéseit kutatták. A sc. TCZ hatásait a mindennapi életben vizsgálták japán dolgozók, illetve háztartásbeliek körében. A vizsgálatban 377 RA-s beteget sc. TCZ-vel, 347-et csDMARD-okkal kezeltek. Egyéves kezelés után elemezték az általános munkaképességet. Amíg a teljes munkaképtelenség (OWI) szempontjából nem volt különbség a két csoport között, a munkahelyi produktivitási és aktivitási index (WPAI), valamint az életminőség vonatkozásában a TCZ-vel kezelt betegek jobban teljesítettek [26].

A humán klinikai vizsgálatok alkalmasak arra is, hogy az IL-6 útvonal gátlása révén megértsünk bizonyos patogenetikai, sejtes és molekuláris kérdéseket. Az adipokinek fontos szerepet játszanak az RA kialakulásában [27]. Fioravanti és mtsai [28] 44 beteget kezelt négyhetente 8 mg/kg TCZ-monoterápiával vagy TCZ + MTX kombinációval. Amellett, hogy kiváló klinikai választ észleltek, az egyértelműen proinflammatorikus chemerin szintje csökkent, az antiinflammatorikus adiponektiné nőtt mindkét TCZ-vel kezelt csoportban. A leptin és a rezisztin szintje nem változott [28].

A IV. típusú kollagén a basalis membrán alkotórésze, melynek komoly szerepe van a gyulladást kísérő adhezív folyamatokban [29]. Gudmann és mtsai [30] a TCZ-vel végzett RADIATE és LITHE vizsgálat összesen 900 betegének szérummintáiban a IV. típusú kollagén metabolizmusának markerét (C4M) vizsgálta. A TCZ dózisfüggő módon, 11–40%-kal csökkentette a C4M szérumszintjét. Az ACR20 szerinti válasz esélye összefüggött a C4M-szuppresszió mértékével [30].

A TCZ-terápia az RA-ban megváltozott B-sejt-egyensúlyt is helyreállíthatja. Fleischer és mtsai [31] a TCZ-kezelés B-sejt citokinprofilra gyakorolt hatását elemezték aktív RA-ban. Aktív RA-ban a B-sejtek fokozottan termelik az IL-8 és groα kemokineket. Ezzel együtt számos olyan citokin termelése csökkent, ami a B-sejt receptor (BCR) aktiváció révén termelődik. Így a protektív TRAIL termelődése is csökkent RA-ban. Ezek a patológiás eltérések helyreálltak TCZ-kezelés mellett [31].

RA-ban fokozott angiogenesis figyelhető meg, amely a gyulladásos leukocyták synoviumba való fokozott áramlásában nyilvánul meg [32]. A célzott terápiák csökkenthetik a gyulladásos angiogenesist [33]. Hirohata és mtsai [34] a TCZ és a TNF-gátlók angiogenesisre gyakorolt hatásait vetették össze RA-s betegek synovialis szövetében. Összesen 13 TCZ-, 13 TNF-gátló- és 10 csDMARD-kezelésben részesülő RA-s beteg synoviumát gyűjtötték össze műtétek során. A mikroerek sűrűségét a TCZ-kezelés jobban csökkentette, mint az anti-TNF- vagy csDMARD-kezelés. A TCZ kedvező hatása érvényesült első vonalbeli kezelés, illetve TNF-gátló utáni terápia esetén is [34].

A komplementrendszer az RA patogenezisében is szerepet játszik [35]. Romano és mtsai [36] a betegségben fokozottan termelődő komplementfaktorok és a TCZ-kezelés összefüggéseit vizsgálta. A TCZ-kezelés a C3 és a C4 komplementfehérjék vérszintjét is csökkentette [36].

Juvenilis idiopathiás arthritis

A pJIA növekedési retardációval járhat. Bharucha és mtsai [37] a TCZ növekedésre gyakorolt hatását vizsgálták kétéves kezelés után. Ebbe a III. fázisú vizsgálatba 187 pJIA-s beteget vontak be, akik TCZ-t vagy placebót kaptak. Összességében a magasság növekedett. A betegek 72%-ában volt a magasság nagyobb, mint kiinduláskor. A növekedési ráta is gyorsabb volt TCZ mellett [37].

Bielak és mtsai [38] a német regiszterben (AID-Registry) elemezték a TCZ hatásait sJIA-ban. 2009–2014 között 200 sJIA-s beteget dokumentáltak, akik közül 46 kapott TCZ-kezelést. A klinikai válaszkészséget (nincs klinikai tünet, nincs gyulladásos laboratóriumi eltérés) a betegek 35%-ában észlelték az első 12 hétben. Inaktív betegséget és/vagy remissziót egyéves kezeléssel a betegek 75%-ában sikerült elérni. Mellékhatás 24%-ban, SAE 4%-ban lépett fel [38].

Kearsley-Fleet és mtsai [39] a TCZ és anakinra rövid távú hatásait vetették össze sJIA-ban az angol regiszter adatai

alapján. Összesen 76 sJIA-s beteget kezeltek, 54-et TCZ-vel és 22-t anakinrával. A hatékonyság tekintetében (ACRPedi90, minimális betegségaktivitás)n nem volt jelentős különbség a két szer között. Egy év után a TCZ-n több beteg maradt (89%), mint anakinrán (59%). A TCZ-perzisztencia tehát jobbnak bizonyult [39].

A biologikumok mellett változó gyakorisággal hypersensitivitasi reakció jelentkezhet. Yasuoka és mtsai [40] az allergiás reakciók előfordulását elemezték 40 sJIA-s betegen. Közülük ötben jelentkezett hypersensitivitasi reakció az első és harmadik TCZ-dózis között. Összességében fiatalabb, alacsonyabb és kisebb testsúlyú betegek és kifejezettebb betegségaktivitás esetén gyakoribb az allergiás reakció esélye [40].

Óriássejtes arteritis

Az óriássejtes arteritis (GCA) a leggyakoribb vasculitis, mely 50 évnél idősebbekben jelentkezik. Korábban a kezelést a KS és az MTX jelentették. Kiderült, hogy az IL-6 fontos szerepet játszik a GCA patogenezisében [41]. A közelmúltban klinikai vizsgálatok is igazolták a TCZ hatékonyságát ebben a betegségben [10, 11, 41].

Villiger és mtsai [10] a 2016-ban lezárt II. fázisú vizsgálat eredményeit ismertették, amely GCA-ban a remisszió indukciójára és fenntartására irányult. Ez egy egycentrumos klinikai vizsgálat volt, melyet Bernben végeztek. Összesen 20 beteget választottak be, akik 2 : 1 arányban 8 mg/kg TCZ-t vagy placebót kaptak (13 infúzió 4 hetente 52 hétig). Mindkét csoport kezdetben 1 mg/kg prednisolont kapott, amit egy meghatározott protokoll szerint csökkentettek 0 mg-ig. Az elsődleges végpont a 12. héten remissziót elérők aránya volt. A TCZ-vel kezeltek 85%-a, a placebokaron a betegek 40%-a érte el a remissziót 3 hónap után. Egy éven át tartó relapsusmentességet a betegek 85, illetve 20%-a ért el. SAE a TCZ-karon a betegek 35%-ában, a placebót kapók 50%-ában jelentkezett [10].

Stone és mtsai [11] a III. fázisú GiACTA vizsgálat eredményeit közölték. Ebbe az egyéves vizsgálatba 251 GCA-s beteget vontak be, akik subcutan TCZ-t kaptak 162 mg dózisban hetente vagy kéthetente, illetve placebót. A betegek prednisolont is kaptak, melyet 26 vagy 52 hét alatt leépítettek. Itt az elsődleges végpont az 52 hétig tartó, KS-mentes tartós remisszió volt. A hetente, kéthetente TCZ-t kapó, 26 hetes KS-leépítés, illetve 52 hetes KS-leépítés mellett placebót kapó négy csoportban az egy évig fennmaradó KS-mentes remissziót elérők aránya sorrendben 56%, 53%, 14% és 18% volt. A TCZ csoportokban szignifikánsan alacsonyabb volt a kumulatív KS-dózis. A fenti négy vizsgálati csoportban a SAE gyakorisága sorrendben 15%, 14%, 22% és 25% volt [11].

Samson és mtsai [42] a KS-hez hozzáadott (add-on) TCZ hatásait vizsgálták GCA-ban, prospektív tanulmányban. KS-sel kezelt GCA-s betegekben a KS-t leépítették, majd négy TCZ-infúziót adtak. Összesen 20 beteget vizsgáltak. Minden betegben remissziót értek el a 26. hétig. A betegeket 52 hétig követték, tíz betegben következett be relapsus. Főleg azokban, akiknél kiinduláskor aortitis, igen magas CRP vagy jelentősebb anaemia volt. Ezek azok a betegek tehát, akiket szorosabban kell követni [42].

Ami a diagnosztikát illeti, Reichenbach és mtsai [43] az MR-angiográfia (MRA) értékét vizsgálták GCA-ban. GCA-s betegek vagy TCZ-t vagy placebót kaptak KS mellett egy évig. Annak ellenére, hogy a betegek többsége klinikailag jól reagált a TCZ-kezelésre, az artériák falában észlelhető, gyulladást jelző MR-jel a TCZ-csoportban levő betegek 33%-ában tűnt el teljesen [43]. A szubklinikus gyulladás tehát továbbra is fennmaradhat a GCA kezelése után, ezért klinikai remisszió esetén is folytatni kell a TCZ-kezelést.

Ugyancsak a szubklinikus aktivitás fennmaradására és ezért a TCZ-terápia tartós fenntartására hívja fel a figyelmet az immunfunkciók monitorozására irányuló vizsgálat. Gloor és mtsai [44] GCA-ban TCZ-vel végzett RCT-ben vizsgált számos immuno-inflammatorikus markert, amit egészséges kontrollokkal vetettek össze. A biomarkerek közül az MMP-3, pentraxin-3 és a szolúbilis TNF-receptor-2 a kezelés kezdetén még klinikai remisszió esetén is magasabb vérszintet mutatott. A kezelés vége felé (52. hét) ezek a markerek is nagyrészt eltűntek a vérből. Ezek a vizsgálatok is arra utalnak, hogy a TCZ-kezelést GCA-ban legalább 52 hétig, és azon túl is érdemes folytatni [44].

Az utóbbi vizsgálatok tehát mind arra utalnak, hogy GCA-ban TCZ-vel csaknem minden esetben klinikai remisszió érhető el, de a háttérben megmaradó szubklinikus gyulladás miatt relapsus lehet. A TCZ tartós alkalmazása és a gyulladás szoros monitorozása javasolt.

Laboratóriumi biomarkerprediktorok és immunogenitás

Több biologikum esetében felmerült, hogy az ACPA- (anti-CCP-) vagy reumafaktor- (RF-) státusz (szeropozitivitás vs. -negativitás) összefügg-e a gyógyszer hatékonyságával. Cappelli és mtsai [45] a CORRONA regiszter elemzése során azt találták, hogy a klinikai hatékonyság egyformán mutatkozott az ACPA-szeropozitív és -szeronegatív betegekben [45].

Felmerül, hogy maga az IL-6 lehet-e biomarker. Diaz-Torne és mtsai [46] a szérum IL-6- és szolúbilis IL-6–receptor- (sIL-6R-) szintjét mérték TCZ-vel kezelt RA-s betegekben. Kiderült, hogy a kezelés előtt magasabb keringő IL-6- és alacsony sIL-6R-szint mellett várható a legjobb hatékonyság [46].

A U-Act-Early vizsgálat adatelemzése során Teitsma és mtsai [18] olyan fehérje biomarkereket kerestek, amelyek összefüggnek a későbbi gyógyszermentes remisszió elérésével. A TCZ + MTX, TCZ- és MTX-karon összesen 9, 14, illetve 13 releváns fehérjét találtak. Ezek jelentős része a leukocyta-

aktivációval, a JAK-STAT útvonalakkal függött össze [18]. Ezek a biomarkerek használhatók lehetnek a korai szakban a TCZ-terápia eredményességének előzetes megítélésére.

RA-ban gyulladásos aktivitás mellett megnő a szívelégtelenség aránya [47]. Az RA kezelésével, akár csDMARD, akár biologikumok alkalmazásával, csökkenthető ennek kockázata [47]. Az NT-proBNP a szívelégtelenség ismert laboratóriumi markere, mely emelkedett RA-ban és összefügg a halálozással [48]. Yokoe és mtsai [49] 70, cardiovascularis betegségen át nem esett RA-s beteget kezelt TCZ-vel 24 héten át. Kiinduláskor RA-s betegekben valóban magasabb volt az NT-proBNP–szint az egészséges kontrollokhoz képest. A 24 hetes TCZ-kezelést követően az NT-proBNP szintje 63%-kal csökkent. Az NT-proBNP-csökkenés korrelált a betegségaktivitás csökkenésével [49].

Bay-Jensen és mtsai [50] a LITHE vizsgálat alapján a csontízületi biomarkerek és a TCZ-kezelés összefüggéseit vizsgálták. Különböző típusú kollagénmetabolitokat (C1M, C2M, C3M), CRP-degradátumot, vimentinmetabolitot (VICM), valamint csontmarkereket (oszteokalcin, OC és CTX) vizsgáltak 740 RA-s betegben. A 16 héten belüli korai klinikai választ legjobban a kiindulási CTX/OC arány (a csontreszorpció és -képződés aránya), valamint a C1M, C2M és C3M kollagénmetabolitok szintjének csökkenése jelezte. Összességében a magas csontturnoverű (CTX/OX) és alacsony C2M-szintű betegeknél volt legkifejezettebb a korai terápiás válasz [50]. Korábban említettük, hogy Gudmann és mtsai [30] vizsgálatában a TCZ-re adott terápiás válasz összefüggött a C4M-csökkenés mértékével. Így a kollagénmetabolitok is hasznos biomarkerek lehetnek [30].

Végül, az immunogenitás, azaz a gyógyszerellenes antitestek (anti-drug antibody; ADAb) megjelenése fontos lehet a biologikumok hatásvesztése szempontjából. A legtöbb TNF-gátló esetében jelentős az immunogenitás [51]. Benucci és mtsai [52] a TCZ immunogenitását vizsgálták a mindennapi gyakorlatban. Összesen 126 TCZ-vel kezelt betegben mérték kiinduláskor és 6 hónapos kezelés után a TCZ és az anti-TCZ vérszintjét LISA-TRACKER Duo-val. A fél év után mindössze egyetlen betegben találtak anti-TCZ antitestet [52].

Az immunogenitást Burmester és mtsai [53] is elemezték. Összesen 13 III. fázisú sc. vagy iv. TCZ-vizsgálatban csaknem 9000 beteg több mint 50 ezer mintáját tekintették át. A sc., illetve iv. TCZ-kezelés mellett a betegek 1,5, illetve 1,2%-ában észleltek ADAb-t TCZ ellen. Az immunogenitás mértéke hasonló volt TCZ–monoterápia, illetve TCZ + MTX kombináció mellett. Azon kevés betegben, akikben ADAb termelődött, ez nem mutatott összefüggést mellékhatásokkal, és az ADAb senkiben sem váltott ki TCZ-hatásvesztést [53].

Társbetegségek, biztonságosság

Ismeretes, hogy gyulladásos reumatológiai kórképekben, így RA-ban megnövekedett cardiovascularis (CV) morbiditás és mortalitás észlelhető [54–56]. Mivel a szisztémás gyulladás a fő rizikófaktor, az EULAR CV ajánlása szerint legfontosabb a gyulladás és a mediátorok visszaszorítása [54]. Az IL-6 és más proinflammatorikus citokinek kiemelt szerepet játszanak a gyulladásos, RA-hoz társuló atherosclerosisban [57], Ezért elvileg várható, hogy IL-6-receptor-gátlás mellett nem fokozódik az RA-hoz kapcsolódó CV betegség gyakorisága, súlyossága.

Az elmúlt évek legfontosabb RA-CV jellegű tanulmánya az ENTRACTE. Ennek a tanulmánynak az eredményeit közleményben még nem publikálták, hanem a 2016. évi ACR kongresszuson prezentálták [58]. Giles és mtsai [58] prezentációja alapján ez az első olyan vizsgálat, amelyben RA-s betegekben két biologikum CV rizikóra gyakorolt hatását head-to-head összehasonlításban vizsgálták. A vizsgálat apropóját a korábban említett „lipidparadoxon" adta és az a tény, hogy több biologikum, így a TCZ is emeli a lipidszinteket. Emiatt felmerült, vajon a TCZ fokozza-e a CV rizikót? Olyan aktív RA-s betegeket vizsgáltak, akik legalább egy csDMARD-ra nem reagáltak. Ők havonta 8 mg/kg TCZ-t vagy heti 50 mg etanerceptet kaptak nyílt formában. Összesen több mint 3080 beteget vontak be és 2957 be is fejezte a vizsgálatot. Az átlagos követési idő 3,2 év volt. Korai terápia-félbeszakítás az etanercept, illetve TCZ-csoportban 23, illetve 26%-ban következett be. Összesen 161 súlyos CV esemény (MACE) történt, ebből 83 a TCZ- és 78 az etanerceptkaron. A TCZ így azonos kockázatot hordozott az etanercepttel. A lipidszintek emelkedése, a vártnak megfelelően, kifejezettebb volt a TCZ-vel kezeltekénél, de ez nem nyilvánult meg a CV rizikó fokozódásában [58].

Kim és mtsai [59] a TCZ és a TNF-gátlók CV biztonságosságát vetették össze. Különböző biztosítói adatbázisokban vizsgálták azon RA-s betegeket, akik újonnan kezdtek TNF-gátlót vagy TCZ-t kapni. Összesen 9218 TCZ-vel és 18 810 anti-TNF-fel kezelt beteg adatait elemezték. Kiinduláskor a TCZ-t, illetve TNF-gátlót kezdők 14,3, illetve 13,5%-ának volt már CV betegsége. A követés alatt 125 CV eseményt regisztráltak. A CV rizikó azonos volt a két csoportban [59].

Generali és mtsai [60] az RA-s betegek CV esemény miatti hospitalizálásának körülményeit vizsgálták a TCZ-vel és etanercepttel összefüggésben. Egy olasz egészségbiztosítási adatbázis retrospektív elemzése során az akut CV eseményeket (infarktus, stroke) vették figyelembe a zárójelentések alapján. Összesen 1752 RA-s beteg adatait tekintették át, akik közül 1086 etanercept-, míg 666 TCZ-kezelést kapott. A TCZ nem fokozta a CV rizikót az etanercepttel kezeltekhez képest [60].

Fent már említettük, hogy a TCZ csökkenti az NT-proBNP cardialis biomarker szintjét [49], ami, mivel az NT-proBNP összefügg az RA-s halálozással [48], szintén kedvező a CV társbetegség szempontjából.

Mivel a CV betegségek hátterében proatherothromboticus események állnak, fontos kiemelni, hogy RA-ban megnő a thromboticus kockázat [61]. Ruiz-Limón és mtsai [62] a TCZ prothromboticus profilra gyakorolt hatását kutatták. Összesen 20 beteget kezeltek TCZ-vel 6 hónapig. Az endotheldiszfunk-

ciót lézer-Dopplerrel mérték. TCZ hatására javult az endothelfunkció, csökkent az oxidatív stressz. A TCZ csökkentette a monocyták proatherogen és prothromboticus profilját is [62].

Az RA gyakran társul metabolikus szindrómával, ezen belül diabetes mellitusszal [27]. Otsuka és mtsai [63] a TCZ és a TNF-gátlók glikozilált hemoglobinra ($HgbA_{1c}$) gyakorolt hatását vizsgálták. Összesen 154 anti-TNF- és 67 TCZ-kezelésben részesülő betegnél állt rendelkezésre kiindulási és 3 hónap utáni $HgbA_{1c}$-szint. Kezdetben a két csoportban hasonló volt a glikozilált hemoglobin szintje. Három hónap után a TCZ-csoportban alacsonyabb volt a $HgbA_{1c}$-szint, és ennek csökkenése is nagyobb mértékű volt [63].

Már említettük a lipidparadoxont, amely miatt biológiai terápia mellett emelkedhet az összkoleszterin- és az LDL-C-szint [27]. Ugyancsak ismeretes, hogy az RA hiperkatabolikus állapotot jelent, és aktív RA-ban rheumatoid cachexia alakulhat ki [27]. Robertson és mtsai [64] a TCZ-kezelés melletti LDL-emelkedés hátterét vizsgálták 11 betegen a KALIBRA vizsgálatban. Három iv. TCZ-infúzió adása után emelkedett az összkoleszterin-, LDL-C- és HDL-C-szint is. Mindezt katabolikus állapot kísérte, amely a funkcionális katabolikus ráta (FCR) fokozódásában nyilvánult meg. Összefüggést találtak az FCR és a lipidemelkedés között, ami azt jelzi, hogy a TCZ-kezelés mellett jelentkező hyperlipidaemia is a hiperkatabolizmus, és nem de novo LDL-szintézis következménye [64].

A CV és metabolikus betegségek mellett fontosak a fáradtság és az osteoporosis is. Az RA-hoz mind jelentős fáradtság [65], mind szekunder osteoporosis társul [66], amelyet az RA kezelése javíthat. Az IL-6 szerepe mindkét társbetegség kialakulásában jelentős [65, 66]. Abu-Shakra és mtsai [67] a TCZ obesitasra és csontvesztésre gyakorolt hatását vizsgálták RA-ban. Ebbe a nyílt vizsgálatba 145 beteget választottak be, akik két évig kaptak iv. TCZ-t. Vizsgálták a FACIT fáradtságmérő skálát és a BMD-t. A kezelés hatására a fáradtság fokozatosan csökkent, míg a csigolya- és a combnyak-BMD stabil maradt, holott kezelés nélkül romlott volna. Mindezt kísérte a betegségaktivitás javulása és a hemoglobinszint emelkedése [67].

A gyulladásos csontvesztéssel kapcsolatban Chen és mtsai [68] is végeztek vizsgálatot. Összesen 76 RA-s beteget kezeltek TCZ-vel. Kiinduláskor a DAS28 fordított összefüggést mutatott a lumbalis gerinc és a kétoldali csombnyak T-score-értékeivel. A kétéves TCZ-kezelés hatására a csontreszorpciót jelző CTX marker szintje csökkent, a combnyak-BMD szignifikánsan nőtt [68].

Genovese és mtsai [69] a TCZ-kezelés melletti májfunkciós eltéréseket és hepaticus mellékhatásokat elemezték. Több II., IV. fázisú és nyílt kiterjesztéses TCZ-vizsgálat eredményeit tekintették át. Összesen több mint 4000 RA-s beteg (16 ezer betegévnyi kezelés) került az elemzésbe. A normáltartomány felső határát meghaladó GPT-, illetve GOT-eltérés a betegek 71, illetve 59%-ában mutatkozott. Legalább 3-szoros emelkedés 9, illetve 3%-ban, legalább ötszörös emelkedés 3, illetve 1%-ban alakult ki. A májfunkciós értékek legtöbbször a kezelés első évében emelkedtek. A 3-szorosnál magasabb GOT/GPT emelkedések az esetek 80%-ában normalizálódtak, átlagosan 5,6 hét alatt. A betegek 2,5%-ában kellett a TCZ-kezelést leállítani GOT/GPT emelkedés miatt. Mindösszesen 7 hepaticus SAE történt. Rendszeres májfunkció-ellenőrzés ajánlott [69].

A TCZ és az infekciós kockázat összefüggéseit Morel és mtsai [70] elemezték a francia REGATE regiszter adatai alapján. A csaknem 1500 TCZ-vel kezelt beteg a legnagyobb európai TCZ-regisztert jelenti. A súlyos infekció kockázatát meghatározó tényezők a betegségaktivitás, az együtt szedett leflunomid és a kiindulási abszolút neutrophil granulocyta szám [70].

Tágabb értelemben az is idetartozik, hogy a korábbi infekciók hogyan befolyásolják a TCZ alkalmazhatóságát. Ahn és mtsai [71] korábban hepatitis B-vírus (HBV-) fertőzésen átesett, gyógyult RA-s betegeken vizsgálták a TCZ-kezelés hatásait, különös tekintettel a vírusreaktivációra. A betegeket anti-HBc-státuszuk alapján két csoportra osztották. A 39 beteg kb. egyharmada volt anti-HBc-pozitív. Végeredményben a TCZ-kezelés alatt egyetlen betegben sem észlelték a HBV reaktivációját. Emellett nem volt változás a betegek HBsAg-, anti-HBc- és anti-HBs-státuszában, -titerében sem. A TCZ tehát biztonságosan alkalmazható HBV-fertőzésen átesett RA-s betegekben [71].

Korábban felmerült, hogy a biologikumok fokozzák-e a daganatok rizikóját. Amíg a 2000-es évek elején valóban megjelentek erre vonatkozó adatok, amelyek inkább az alapbetegség aktivitásának hatását emelték ki [72], 2017-ben nagy vizsgálatok alapján is elmondhattuk, hogy a biologikumok általánosságban nem fokozzák a tumorhajlamot. Ez vonatkozik a TNF-gátlókra és más támadáspontú szerekre is [73]. Wadström és mtsai [73] a svéd RA-regiszter 2006–2015 közötti adatait elemezték. Ebben 1798 TCZ-vel kezelt beteg is volt. Összesen 25 féle összehasonlításban elemezve, egyetlen kivétellel (abatacept és nem melanoma bőrrák) semmilyen jelét nem találták, hogy a TNF-gátlók, TCZ, abatacept, rituximab bármelyik daganatféleség kockázatát emelnék [73]. Egy másik friss vizsgálatban Mercer és mtsai [74] külön, 11 európai regiszterben elemezték az invazív melanoma gyakoriságát. A TCZ, a többi biologikumhoz hasonlóan, szignifikánsan nem emelte e daganattípus kockázatát [74].

Összegzés

Az elmúlt két év közleményei alapján a TCZ-kezelés főbb előnyei:

- Igen hatékony korai RA-ban.
- Kedvezően befolyásolja a PRO-kat, a munkaképességet és az életminőséget.
- Lassítja a radiológiai progressziót és, MRI vizsgálatok alapján, csökkenti a synovitist.
- JIA-ban javíthatja a növekedést.

- GCA-ban klinikai remisszió esetén is fennmaradhat szubklinikus gyulladás, ezért tartós TCZ-kezelés javasolt.
- A TCZ immunogenitása nagyon alacsony.
- A TCZ valószínűleg nem növeli a cardiovascularis kockázatot.
- Kedvezően befolyásolja a szénhidrát-anyagcserét, a fáradtságot, a szekunder osteoporosist és ritkán okoz súlyos májfunkciós eltéréseket.

Összességében a TZC igen hatékony RA-ban, pJIA-ban, sJIA-ban és GCA-ban intravénás vagy subcutan adagolással, MTX-szel kombinációban vagy monoterápiában alkalmazva is.

IRODALOM

[1] Hodinka L, Bálint P, Bender T, et l: Az arthritisek kezelése szintetikus és biológiai betegségmódosító gyógyszerekkel. Immunol Szle 2015, VII(1): 4-25.

[2] Smolen JS, Aletaha D, Barton A, et al: Rheumatoid arthritis. Nature reviews Disease primers 2018, 4: 18001.

[3] Smolen JS, Landewe R, Bijlsma J, et al: EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. Ann Rheum Dis 2017, 76(6): 960-977.

[4] Combe B, Landewe R, Daien CI, et al: 2016 update of the EULAR recommendations for the management of early arthritis. Ann Rheum Dis 2017, 76(6): 948-959.

[5] Scott LJ: Tocilizumab: A Review in Rheumatoid Arthritis. Drugs 2017, 77(17): 1865-1879.

[6] Szamosi S, Szekanecz Z: Interleukin-6: a „másik" pluripotens citokin. Immunol Szemle 2010, II(3): 61-64.

[7] Brunner HI, Ruperto N, Zuber Z, et al: Efficacy and safety of tocilizumab in patients with polyarticular-course juvenile idiopathic arthritis: results from a phase 3, randomised, double-blind withdrawal trial. Ann Rheum Dis 2015, 74(6): 1110-1117.

[8] Yokota S, Kishimoto T: Tocilizumab: molecular intervention therapy in children with systemic juvenile idiopathic arthritis. Expert Rev Clin Immunol 2010, 6(5): 735-743.

[9] Szekanecz Z: Az IL-6-receptor-gátlás nagyító alatt. Immunol Szemle 2016, 8: 4-9.

[10] Villiger PM, Adler S, Kuchen S, et al: Tocilizumab for induction and maintenance of remission in giant cell arteritis: a phase 2, randomised, double-blind, placebo-controlled trial. Lancet 2016, 387(10031): 1921-1927.

[11] Stone JH, Tuckwell K, Dimonaco S, et al: Trial of Tocilizumab in Giant-Cell Arteritis. N Engl J Med 2017, 377(4): 317-328.

[12] https://www.roche.com/media/releases/med-cor-2017-09-22b.htm.

[13] Dejaco C, Ramiro S, Duftner C, et al: EULAR recommendations for the use of imaging in large vessel vasculitis in clinical practice. Ann Rheum Dis 2018, 77(5): 636-643.

[14] Bijlsma JW, Welsing PM, Woodworth TG, et al: Early rheumatoid arthritis treated with tocilizumab, methotrexate, or their combination (U-Act-Early): a multicentre, randomised, double-blind, double-dummy, strategy trial. Lancet 2016, 388(10042): 343-355.

[15] Teitsma XM, Jacobs JWG, Welsing PMJ, et al: Patient-reported outcomes in newly diagnosed early rheumatoid arthritis patients treated to target with a tocilizumab- or methotrexate-based strategy. Rheumatology (Oxford) 2017, 56(12): 2179-2189.

[16] Teitsma XM, Marijnissen AK, Bijlsma JW, et al: Tocilizumab as monotherapy or combination therapy for treating active rheumatoid arthritis: a meta-analysis of efficacy and safety reported in randomized controlled trials. Arthritis Res Ther 2016, 18(1): 211.

[17] Teitsma XM, Jacobs JWG, Welsing PMJ, et al: Radiographic joint damage in early rheumatoid arthritis patients: comparing tocilizumab- and methotrexate-based treat-to-target strategies. Rheumatology (Oxford) 2018, 57(2): 309-317.

[18] Teitsma XM, Jacobs JWG, Concepcion AN, et al: Explorative analyses of protein biomarkers in patients with early rheumatoid arthritis achieving sustained drug-free remission after treatment with tocilizumab- or methotrexate-based strategies: from transcriptomics to proteomics. Clin Exp Rheumatol 2018.

[19] Ishiguro N, Atsumi T, Harigai M, et al: Effectiveness and safety of tocilizumab in achieving clinical and functional remission, and sustaining efficacy in biologics-naive patients with rheumatoid arthritis: The FIRST Bio study. Mod Rheumatol 2017, 27(2): 217-226.

[20] Choy E, Caporali R, Xavier R, et al: Subcutaneous tocilizumab in rheumatoid arthritis: findings from the common-framework phase 4 study programme TOZURA conducted in 22 countries. Rheumatology (Oxford) 2018, 57(3): 499-507.

[21] Bird P, Peterfy C, Countryman P, et al: AC-CUTE: An Open-Label Study to Evaluate Progression of Structural Joint Damage and Inflammation in Subjects with Moderate to Severe Rheumatoid Arthritis. Int J Rheumatol 2018, 2018: 8721753.

[22] Ebina K, Hashimoto M, Yamamoto W, et al: Drug retention and discontinuation reasons between seven biologics in patients with rheumatoid arthritis -The ANSWER cohort study. PLoS One 2018, 13(3): e0194130.

[23] Kremer JM, Rigby W, Singer NG, et al: Sustained Response Following Discontinuation of Methotrexate in Patients With Rheumatoid Arthritis Treated With Subcutaneous Tocilizumab: Results From a Randomized, Controlled Trial. Arthritis Rheumatol 2018, 70(8): 1200-1208.

[24] Strand V, Michalska M, Birchwood C, et al: Impact of tocilizumab monotherapy on patient-reported outcomes in patients with rheumatoid arthritis from two randomised controlled trials. RMD open 2017, 3(2): e000496.

[25] Strand V, Michalska M, Birchwood C, et al: Impact of tocilizumab administered intravenously or subcutaneously on patient-reported quality-of-life outcomes in patients with rheumatoid arthritis. RMD open 2018, 4(1): e000602.

[26] Tanaka Y, Kameda H, Saito K, et al: Effect of subcutaneous tocilizumab treatment on work/housework status in biologic-naive rheumatoid arthritis patients using inverse probability of treatment weighting: FIRST ACT-SC study. Arthritis Res Ther 2018, 20(1): 151.

[27] Kerekes G, Nurmohamed MT, Gonzalez-Gay MA, et al: Rheumatoid arthritis and metabolic syndrome. Nat Rev Rheumatol 2014, 10(11): 691-696.

[28] Fioravanti A, Tenti S, Bacarelli MR, et al: Tocilizumab modulates serum levels of adiponectin and chemerin in patients with rheumatoid arthritis: potential cardiovascular protective role of IL-6 inhibition. Clin Exp Rheumatol 2018.

[29] Szekanecz Z, Koch AE: Update on synovitis. Curr Rheumatol Rep 2001, 3(1): 53-63.
[30.] Gudmann NS, Junker P, Juhl P, et al: Type IV collagen metabolism is associated with disease activity, radiographic progression and response to tocilizumab in rheumatoid arthritis. Clin Exp Rheumatol 2018.
[31] Fleischer S, Ries S, Shen P, et al: Anti-interleukin-6 signalling therapy rebalances the disrupted cytokine production of B cells from patients with active rheumatoid arthritis. Eur J Immunol 2018, 48(1): 194-203.
[32] Szekanecz Z, Koch AE: Mechanisms of Disease: angiogenesis in inflammatory diseases. Nat Clin Pract Rheumatol 2007, 3(11): 635-643.
[33] Maracle CX, Tas SW: Inhibitors of angiogenesis: ready for prime time? Best Pract Res Clin Rheumatol 2014, 28(4): 637-649.
[34] Hirohata S, Abe A, Murasawa A, et al: Differential effects of IL-6 blockade tocilizumab and TNF inhibitors on angiogenesis in synovial tissues from patients with rheumatoid arthritis. Mod Rheumatol 2017, 27(5): 766-772.
[35] Low JM, Moore TL: A role for the complement system in rheumatoid arthritis. Curr Pharm Des 2005, 11(5): 655-670.
[36] Romano C, Del Mastro A, Sellitto A, et al: Tocilizumab reduces complement C3 and C4 serum levels in rheumatoid arthritis patients. Clin Rheumatol 2018, 37(6): 1695-1700.
[37] Bharucha KN, Brunner HI, Calvo Penades I, et al: Growth During Tocilizumab Therapy for Polyarticular-course Juvenile Idiopathic Arthritis: 2-year Data from a Phase III Clinical Trial. J Rheumatol 2018, 45(8): 1173-1179.
[38] Bielak M, Husmann E, Weyandt N, et al: IL-6 blockade in systemic juvenile idiopathic arthritis – achievement of inactive disease and remission (data from the German AID-registry). Pediatr Rheumatol Online J 2018, 16(1): 22.
[39] Kearsley-Fleet L, Beresford MW, Davies R, et al, Biologics for Children with Rheumatic Diseases S: Short-term outcomes in patients with systemic juvenile idiopathic arthritis treated with either tocilizumab or anakinra. Rheumatology (Oxford) 2018.
[40] Yasuoka R, Iwata N, Abe N, et al: Risk factors for hypersensitivity reactions to tocilizumab introduction in systemic juvenile idiopathic arthritis. Mod Rheumatol 2018: 1-4.
[41] Schirmer M, Muratore F, Salvarani C: Tocilizumab for the treatment of giant cell arteritis. Expert Rev Clin Immunol 2018, 14(5): 339-349.
[42] Samson M, Devilliers H, Ly KH, et al: Tocilizumab as an add-on therapy to glucocorticoids during the first 3months of treatment of Giant cell arteritis: A prospective study. Eur J Intern Med 2018.
[43] Reichenbach S, Adler S, Bonel H, et al: Magnetic resonance angiography in giant cell arteritis: results of a randomized controlled trial of tocilizumab in giant cell arteritis. Rheumatology (Oxford) 2018, 57(6): 982-986.
[44] Gloor AD, Yerly D, Adler S, et al: Immuno-monitoring reveals an extended subclinical disease activity in tocilizumab-treated giant cell arteritis. Rheumatology (Oxford) 2018.
[45] Cappelli LC, Palmer JL, Kremer J, Bingham CO, 3rd: Tocilizumab treatment leads to improvement in disease activity regardless of CCP status in rheumatoid arthritis. Semin Arthritis Rheum 2017, 47(2): 165-169.
[46] Diaz-Torne C, Ortiz MDA, Moya P, et al: The combination of IL-6 and its soluble receptor is associated with the response of rheumatoid arthritis patients to tocilizumab. Semin Arthritis Rheum 2018, 47(6): 757-764.
[47] Szekanecz Z: Cardiac effects of antirheumatic drugs. In: Atzeni F, Doria A, Nurmohamed M, Pauletto P (eds): The Heart in Systemic Autoimmune Diseases. 2nd edition. Elsevier, Amsterdam, 2017: 489-529.
[48] Provan S, Angel K, Semb AG, Atar D, Kvien TK: NT-proBNP predicts mortality in patients with rheumatoid arthritis: results from 10-year follow-up of the EURIDISS study. Ann Rheum Dis 2010, 69(11): 1946-1950.
[49] Yokoe I, Kobayashi H, Kobayashi Y, et al: Impact of tocilizumab on N-terminal pro-brain natriuretic peptide levels in patients with active rheumatoid arthritis without cardiac symptoms. Scand J Rheumatol 2018, 47(5): 364-370.
[50] Bay-Jensen AC, Platt A, Siebuhr AS, et al: Early changes in blood-based joint tissue destruction biomarkers are predictive of response to tocilizumab in the LITHE study. Arthritis Res Ther 2016, 18: 13.
[51] Aarden L, Ruuls SR, Wolbink G: Immunogenicity of anti-tumor necrosis factor antibodies-toward improved methods of anti-antibody measurement. Curr Opin Immunol 2008, 20(4): 431-435.
[52] Benucci M, Meacci F, Grossi V, et al: Correlations between immunogenicity, drug levels, and disease activity in an Italian cohort of rheumatoid arthritis patients treated with tocilizumab. Biologics 2016, 10: 53-58.
[53.] Burmester GR, Choy E, Kivitz A, et al: Low immunogenicity of tocilizumab in patients with rheumatoid arthritis. Ann Rheum Dis 2017, 76(6): 1078-1085.
[54] Agca R, Heslinga SC, Rollefstad S, et al: EULAR recommendations for cardiovascular disease risk management in patients with rheumatoid arthritis and other forms of inflammatory joint disorders: 2015/2016 update. Ann Rheum Dis 2017, 76(1): 17-28.
[55.] Choy E, Ganeshalingam K, Semb AG, et al: Cardiovascular risk in rheumatoid arthritis: recent advances in the understanding of the pivotal role of inflammation, risk predictors and the impact of treatment. Rheumatology (Oxford) 2014, 53(12): 2143-2154.
[56] Szekanecz Z, Kerekes G, Der H, et al: Accelerated atherosclerosis in rheumatoid arthritis. Ann N Y Acad Sci 2007, 1108: 349-358.
[57] Szekanecz Z: Pro-inflammatory cytokines in atherosclerosis. Isr Med Assoc J 2008, 10(7): 529-530.
[58] Giles J, Sattar N, Gabriel SE, et al: Comparative Cardiovascular Safety of Tocilizumab Vs Etanercept in Rheumatoid Arthritis: Results of a Randomized, Parallel-Group, Multicenter, Noninferiority, Phase 4 Clinical Trial [abstract]. Arthritis Rheumatol 2016, 68 (Suppl 10).
[59] Kim SC, Solomon DH, Rogers JR, et al: Cardiovascular Safety of Tocilizumab Versus Tumor Necrosis Factor Inhibitors in Patients With Rheumatoid Arthritis: A Multi-Database Cohort Study. Arthritis Rheumatol 2017, 69(6): 1154-1164.
[60] Generali E, Carrara G, Selmi C, et al: Comparison of the risks of hospitalisation for cardiovascular events in patients with rheumatoid arthritis treated with tocilizumab and etanercept. Clin Exp Rheumatol 2018, 36(2): 310-313.

[61] van den Oever IA, Sattar N, Nurmohamed MT: Thromboembolic and cardiovascular risk in rheumatoid arthritis: role of the haemostatic system. Ann Rheum Dis 2014, 73(6): 954-957.
[62] Ruiz-Limon P, Ortega R, Arias de la Rosa I, et al: Tocilizumab improves the proatherothrombotic profile of rheumatoid arthritis patients modulating endothelial dysfunction, NETosis, and inflammation. Transl Res 2017, 183: 87-103.
[63] Otsuka Y, Kiyohara C, Kashiwado Y, et al: Effects of tumor necrosis factor inhibitors and tocilizumab on the glycosylated hemoglobin levels in patients with rheumatoid arthritis; an observational study. PLoS One 2018, 13(4): e0196368.
[64] Robertson J, Porter D, Sattar N, et al: Interleukin-6 blockade raises LDL via reduced catabolism rather than via increased synthesis: a cytokine-specific mechanism for cholesterol changes in rheumatoid arthritis. Ann Rheum Dis 2017, 76(11): 1949-1952.
[65] Srirangan S, Choy EH: The role of interleukin 6 in the pathophysiology of rheumatoid arthritis. Ther Adv Musculoskelet Dis 2010, 2(5): 247-256.
[66] Szentpetery A, Horvath A, Gulyas K, et al: Effects of targeted therapies on the bone in arthritides. Autoimmun Rev 2017, 16(3): 313-320.
[67] Abu-Shakra M, Zisman D, Balbir-Gurman A, et al: Effect of Tocilizumab on Fatigue and Bone Mineral Density in Patients with Rheumatoid Arthritis. Isr Med Assoc J 2018, 20(4): 239-244.
[68] Chen YM, Chen HH, Huang WN, et al: Tocilizumab potentially prevents bone loss in patients with anticitrullinated protein antibody-positive rheumatoid arthritis. PLoS One 2017, 12(11): e0188454.
[69] Genovese MC, Kremer JM, van Vollenhoven RF: Transaminase Levels and Hepatic Events During Tocilizumab Treatment: Pooled Analysis of Long-Term Clinical Trial Safety Data in Rheumatoid Arthritis. Arthritis Rheumatol 2017, 69(9): 1751-1761.
[70] Morel J, Constantin A, Baron G, et al: Risk factors of serious infections in patients with rheumatoid arthritis treated with tocilizumab in the French Registry REGATE. Rheumatology (Oxford) 2017, 56(10): 1746-1754.
[71] Ahn SS, Jung SM, Song JJ, et al: Safety of Tocilizumab in Rheumatoid Arthritis Patients with Resolved Hepatitis B Virus Infection: Data from Real-World Experience. Yonsei Med J 2018, 59(3): 452-456.
[72] Bongartz T, Sutton AJ, Sweeting MJ, et al: Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. JAMA 2006, 295(19): 2275-2285.
[73] Wadstrom H, Frisell T, Askling J: Anti-Rheumatic Therapy in Sweden Study G: Malignant Neoplasms in Patients With Rheumatoid Arthritis Treated With Tumor Necrosis Factor Inhibitors, Tocilizumab, Abatacept, or Rituximab in Clinical Practice: A Nationwide Cohort Study From Sweden. JAMA Intern Med 2017, 177(11): 1605-1612.
[74] Mercer LK, Askling J, Raaschou P, et al: Risk of invasive melanoma in patients with rheumatoid arthritis treated with biologics: results from a collaborative project of 11 European biologic registers. Ann Rheum Dis 2017, 76(2): 386-391.

(Dr Szekanecz Zoltán, Debreceni Egyetem Általános Orvostudományi Kar Reumatológiai Tanszék, 4032 Debrecen, Nagyerdei krt. 98., e-mail: szekanecz.zoltan@med.unideb.hu)

Immunológiai szótár

GALT (gut associated lymphoid tissue; bélrendszerrel asszociált lymphoid szövet) Lymphoid szövet, amely szoros kapcsolatban áll a tápcsatornával, emberben pl. a mandulák, a Peyer-plakkok és a vakbél, nyúlban a sacculus rotundus, tyúkban a bursa Fabricii stb.

gamma-globulin (γ-globulin) A szérum globulinfrakciója, amelynek az anódmobilitása neutrális pH-n, elektroforézis során a legkisebb. Főleg immunglobulinok tartoznak ide.

γδT-sejtek γδ típusú T-sejt receptorral (TCR) rendelkező T-lymphocyták. Az egyedfejlődés során korán, a thymus fejlődése alatt jelennek meg. A perifériás nyirokszervekben az érett lymphocyták kb. 5%-át teszik ki. Bizonyos fajokban a γδ T-sejtek a domináns T-lymphocyták az epithelialis felszíneken, pl. a bőrben, az emésztő- és a nemi szervekben. Ezeknek a γδ T-sejteknek nagy része thymuson kívüli eredetű. A γδ T-sejtek többségén sem CD4-, sem CD8-molekulák nem találhatók, specificitásuk és funkciójuk ismeretlen. A γδ T-sejtek sohasem expresszálnak αβ TCR-t, és a T-sejtek egy külön vonalát képezik.

γ-lánc-betegség Ritka paraproteinaemia emberben, lymphomával kapcsolatos, amelyben abnormális monoklonális γ-láncok (pl. IgG-nehéz-láncok) találhatók a plazmában és a vizeletben, míg a könnyű láncok hiányzanak. A γ-láncokon deléció van, amely miatt a variábilis régió nagy része és a C_H1-régió egésze hiányzik, habár az N-terminális aminosavak egy része még megtalálható. Bizonyos esetekben a kapocsrégió is hiányozhat.

γ_m Az IgG γ-láncának (nehéz lánc) membránhoz kötött formája.

γ_s Az IgG γ-láncának (nehéz lánc) a szekretált formája.

Immunstimuláció. Mennyire hatásosak az „immunerősítők”?

Gergely Péter

Semmelweis Egyetem, Laboratóriumi Medicina Intézet, Budapest

Számtalan, jórészt gyógyhatásúnak vagy táplálékkiegészítőnek árusított immunerősítő készítmény van forgalomban: növényi kivonatok (pl. Echinacea), gombaeredetű immunstimulánsok (pl. béta-glükánok), baktériumeredetűek (pl. Broncho-Vaxom), szintetikus vegyületek (pl. inoziplex), nyomelemek (pl. cinksók), valamint többféle vitamin (pl. C-vitamin). A klinikai vizsgálatok eredményeit alapul véve, valamennyi készítmény rendelkezik valamilyen hatással a felső légúti gyulladásos betegségek megelőzésében, esetleg a hurutos tünetek javításában, bár ezek a hatások elég szerények. A legtöbb készítmény biztonságosan szedhető. A kérdés csak az, hogy érdemes-e tartósan, megelőző célzattal ilyen szereket szedni. Lehet-e ellenálló képességünket fokozni? A civilizált életmód valóban rontja ellenálló képességünket. Ezért egészséges életmóddal, pl. mozgással, egészséges táplálkozással, testsúlykontrollal, a dohányzás abbahagyásával stb. sokat javíthatunk rajta. Az egészséges életmódot azonban sokkal nehezebb betartani, mint naponta két tablettát bevenni. Ez az emberi tulajdonság élteti a „immunerősítők” (és egyéb csodaszerek) piacát.

Kulcsszavak: immunstimuláció, Echinacea, Broncho-Vaxom, Inosiplex, cink, probiotikum

IMMUNOSTIMULANTS. HOW EFFECTIVE ARE THEY?

There are a lot of products available on the market, promoted as immunostimulants of herbal (e.g., Echinacea), fungal (e.g., beta-glucans), bacterial (e.g., Broncho-Vaxom), or synthetic (e.g., inosipex) origin or trace-elements, such as zinc and various vitamins, such as vitamin C, named as food additives or nutraceuticals. Based on clinical studies, all products have some efficacy in prevention of common cold, or in reducing its symptoms, though these effects are usually minor. The majority of products are safe. The question arises whether do people require such products for a long time? Is it possible to increase our resistance to infections? It is well known that urban lifestyle decreases our resistance to infections. Therefore a healthy lifestyle, e.g., exercise, healthy food, weight control, stopping of smoking, etc., is advisable for all people. However, following of healthy lifestyle is more stressful compared to take one or two tablets a day. The market of these products, and other panaceas, is based on this human behaviour.

Keywords: immunostimulants, Echinacea, Broncho-Vaxom, Inosiplex, zinc, probiotics

Mindenki szeretne egészséges lenni vagy annak maradni, elkerülni a betegségeket. Egészségünk egyrészt rajtunk is múlik. De mennyit teszünk meg érte? A piac felismeri ezt az igényt és elég agresszíven megpróbálja kielégíteni.

Egészséges emberek számára hihetetlenül sok készítmény – részben táplálékkiegészítő, részben gyógyhatású készítmény – van forgalomban, amelyek alapvetően a légúti fertőzések megelőzésére szolgálnak – mármint a promóciók szerint. Látván-hallván ezt a zavarba ejtő bőséget, két kérdés merül fel bennünk:

- Kell-e egészséges embernek erősítenie az immunrendszerét (helyesebben fogalmazva: a ellenálló képességét)?
- Lehet-e erősíteni egyáltalán?

Mielőtt azonban a kérdésekre megkísérelnénk választ adni, tekintsük át ezeket a készítményeket.

Immunstimuláció, immunmoduláció

Ezt a két fogalmat gyakran használjuk szinonimaként. Valójában pedig az immunmoduláció használata a helyesebb, hiszen figyelembe veszi immunrendszerünk sokrétűségét, a pozitív és negatív hatások összefüggéseit. Igazi immunstimulációról csak a vakcináció esetében beszélhetünk, hiszen ott mind a szándék, mind az eredmény bizonyos irányú „stimuláció”, vagyis egy (esetleg több) konkrét kórokozóval szembeni védettség elérése.

Elsősorban a daganatos betegségek gyógyításában számtalan olyan készítményt próbáltak ki, melyek – legalábbis fel-

tételezetten – az immunrendszer befolyásolásán keresztül gátolták a daganatok növekedését. Ezen anyagok egy része in vitro vagy állatkísérletekben valóban immunstimuláns hatású, mások azonban daganatellenes hatásukat sokkal komplexebb módon váltják ki. Ezek egy részét szokás immunmoduláns vagy biológiai választ módosító (biological response modifier, BRM) anyagoknak is nevezni, beleértve elsősorban a citokineket, interferonokat, illetve hasonló készítményeket (pl. rintatolimod, linomid, bestatin stb.) is.

Ugyancsak ebbe a kategóriába sorolhatók az adjuvánsok. Adjuvánsoknak nevezzük az immunreakciókat fokozó anyagokat. Az aktív immunizációra szolgáló antigének, kivált a tisztított vagy szintetikusan előállított peptidek, igen kevéssé immunogének, csak adjuvánsokkal együtt adva hoznak létre kellő immunválaszt. Az adjuvánsok általában a monocyta-macrophag rendszert aktiválják.

A mindennapi gyakorlatban használt, immunstimulánsnak tartott készítmények csoportjait az 1. táblázat mutatja be.

1. táblázat. **A mindennapi gyakorlatban használt, immunstimulánsnak tartott készítmények csoportjai**

1. Növényi eredetű immunstimulánsok
   - Echinacea purpurea és más Echinacea fajok
   - flavonoidok
   - egyéb növényei anyagok
2. Gombaeredetű immunstimulánsok
   - Béta-glükánok, pl. lentinan
3. Baktériumeredetű immunstimulánsok
   - OM-85 (Broncho-Vaxom), Ribomunyl stb.
4. Szintetikus vegyületek
   - levamizol
   - inoziplex
   - pidotimod
5. Nyomelemek
   - cink
   - szelén
   - réz stb.
6. Vitaminok
   - D-vitamin
   - C-vitamin
   - A-vitamin és β-karotin
   - E-vitamin

Mit mondanak a klinikai vizsgálatok?

Bár valamennyi fentebb felsorolt (és fel nem sorolt) készítményt preklinikai (in vitro és in vivo) kísérletekben vizsgálták, ezeket az – egyébként ígéretes – eredményeket csak akkor terjeszthetjük ki emberekre, ha a készítmények hatásosságát megfelelő klinikai vizsgálatokban is igazolták. Ilyen vizsgálatok a kettős vak, randomizált (placebo-) kontrollos vizsgálatok (RCT-k). Ha sok ilyen vizsgálat történt, azokat metaanalízisek elemzik, ezeknek az elemzéseknek a legnagyobb a bizonyító (evidencia) értéke.

Bár többféle lehetőség is van, leggyakrabban a vírusos felső légúti hurut („megfázás, „common cold") kezelésében (a tünetek javulásában, a betegségtartam rövidítésében), illetve a betegség megelőzésében vizsgálták ezeket a készítményeket.

Növényi eredetű anyagok

a) Echinacea (kasvirág) különféle kivonatai: Habár gyakran Echinacea purpurea néven aposztrofálják, valójában az Echinacea génusz 9 fajából és azok különféle részéből készült kivonat van forgalomban. Ezek fő hatónyagai polifenolok, poliszacharidok és alkamidok. Ezek immunológiai, valamint egyéb élettani hatásait preklinikai vizsgálatokból elég jól ismerjük.
   Egy Cochrane metaanalízis 24 RCT-t vizsgált, melyekben felső légúti vírusinfekciókban adták [1]. A vizsgálatok zöme a betegség első tüneteitől kezdve adta a szert, és a tünetek javulását mérte. Valamelyes hatás mutatkozott a placebóhoz viszonyítva, de ez klinikailag kevéssé jelentős. A vizsgálatok másik része preventív célzattal adta, így nem volt szignifikáns változás a megbetegedettek számában, viszont sokan abbahagyták a mellékhatások (gyomorpanaszok, arthralgia-myalgia, allergia) miatt.

b) Flavonoidok: A flavonoidok (rutin, kvercetin, polifenolok stb.) élettani – immunológiai, gyökfogó, érrendszerre gyakorolt stb. – hatásai jól ismertek, könyvtárnyi irodalom áll rendelkezésünkre. A növények, ill. növényi eredetű élelmiszerek mind tartalmaznak különféle flavonoidokat, tehát ezeket akarva-akaratlanul is bevisszük szervezetünkbe. Klinikai vizsgálat is nagyon sok van (a PubMed több mint 2000 klinikai vizsgálatot mutat a „flavonoids" kereső kifejezéssel). A sokféle adatból csak mutatóba néhány:
   - Zöld tea, katechinek és theanin. Egy RCT-ben egészségügyi dolgozókban szignifikánsan csökkentette (4,1% vs. 13,1%) a léguti infekciók előfordulását [2].
   - Polifenolok (pl. vörös szőlő, cékla). Egy RCT szerint a polifenolok szignifikánsan csökkentették a megfázásos tüneteket – szubjektív score alapján mérve [3].
   - Egy COCHRANE metaanalízis szerint a Pelargonium-kivonat csökkentette a megfázás tüneteit (elsősorban a bronchitises tüneteket), de a bizonyíték (evidencia) gyenge [4].
   - Kvercetin adása nem csökkentette a légúti infekciók előfordulását [5].
   - A troxerutin 11%-kal csökkentette a megfázásos tüneteket [6].

– Egy újabb metaanalízis 14 RCT-t analizált. A különféle flavonoidok adása (0,2–1,2 g napi adagban) 33%-kal csökkentette a légúti infekciók előfordulását és 40%-kal csökkentette a betegség tartamát [7].

Összességében tehát megállapítható, hogy a flavonoidok valamennyire hatásosak mind a légúti fertőzések megelőzésében, mind a tünetek javításában.

c) Egyéb növényi anyagok: Számtalan növényi eredetű „immunerősítő” kivonat/őrlemény stb. van forgalomban. Ezek egy része viszonylag jobban ismert, mint pl. a ginzeng (Panax quinquefolius és P. ginseng és más fajok), fokhagyma, mások kevésbé, mint pl. a goji, bodza, gyömbér, oregánó, mirha stb.
   – A ginzeng hatását vizsgálták (Seida és mtsai, 2011): nem befolyásolta a légúti infekciók előfordulását, de rövidítette a betegség lefolyását [8].
   – Egy metaanalízis szerint – egyetlen RCT alapján – a legalább 3 hónapon át tartó napi fokhagymakivonat (180 mg allicin tartalommal) csökkentette a légúti infekciók számát és rövidítette azok lefolyását, az evidencia azonban gyenge (a korábbi metaanalízisek adataiból nem volt hatékonyság leszűrhető) [9].
   – A kínai népi gyógyászat számos szert és azok keverékét alkalmazza a meghűléses panaszok kezelésére. Egy metaanalízis 17 klinikai vizsgálatot elemzett (3212 beteg), de a heterogenitás miatt nem lehetett megállapítani, hogy a kezelés hatásos volt-e [10].

Béta-glükánok

Egyik legtöbbet vizsgált ilyen készítmény a Lentinus edodes gombából készült lentinán, melynek tumorellenes/immunmoduláns hatásai jól ismertek. Egy metaanalízis szerint a klinikai vizsgálatokban kemoterápia mellett megnövelte a betegek túlélését és javult a terápiára adott válasz [11].

Krónikus légúti betegségben szenvedő gyermekekben az élesztőeredetű glükán (glucan #300) fokozta a mucosalis immunitást és javította az általános állapotot – sajnos ez utóbbi paraméter pontos definíciója hiányzik [12].

A glükánok légúti infekciót megelőző hatását nem vizsgálták.

Baktériumeredetű immunstimulánsok (kivonatok)

Többféle készítmény is van forgalomban, ezek légúti patogén baktériumok (pl. Haemophilus influenzae, Streptococcus pneumoniae, Klebsiella pneumoniae stb.) különféle módon nyert lizátumai vagy kivonatai. Immunológiai hatásaik részben általánosak, adjuváns típusúak, részben antigénspecifikusak.

a) Egy metaanalízis (15 RTC, 2557 beteg) különféle polivalens baktériumlizátumok hatékonyságát vizsgálta gyermekekben és felnőttekben [13]. Mindkét korcsoportban csökkentette a légúti infekciók számát. COPD-ben is hatásos volt, de a kis esetszám miatt az eredmény nem volt szignifikáns.
b) OM-85 (Broncho-Vaxom). Egy metaanalízis (9 RCT) szerint a kezelés szignifikánsan csökkentette gyermekekben a visszatérő légúti infekciók számát (32% vs. 58,2%) [14].
c) Ribomunyl (bakteriális riboszóma kivonat): Egy metaanalízis 9 RCT alapján szignifikáns csökkenést (2,39 vs. 3,35) észlelt a visszatérő fertőzések számában 6 hónap alatt [15].

Összességében tehát megállapítható, hogy a polivalens baktériumkivonatok hatásosak a légúti fertőzések megelőzésében.

Szintetikus vegyületek

A preklinikai vizsgálatok egyértelmű immunstimuláns aktivitást mutattak mindhárom készítménnyel.

a) Levamizol: A levamizol a visszatérő légúti infekciók számát szignifikánsan csökkentette [16]. Sajnos súlyos mellékhatások jelentkeztek (súlyos neutropenia, agranulocytosis), ezért ma már erre a célra nem alkalmazzák.
b) Inosiplex (inozin pranobex, Isoprinosine): Egykor számos vírisinfekcióban (vírushepatitisek, vírusencephalitisek stb.) adták, ezekben egyrészt nem volt elég hatásos, másrészt új, ma már hatékony gyógyszerek állnak rendelkezésünkre. Egy RCT nem találta hatásosnak gyermekek visszatérő légúti infekcióinak prevenciójában [17]. Egy újabb RCT, más végpontokat vizsgálva, azonban hatásosnak és biztonságosnak találta [18]. Az infekció első tüneteitől kezdődően adva gyorsabb javulást eredményezett, és az 50 év alatti korcsoportban a teljes remisszió is hamarabb következett be.
c) Pidotimod (Axil): A pidotimod egy karboxilsav-származék. Egészséges gyermekekben nem volt hatásos a légúti infekciók prevenciójában [19], visszatérő légúti infekciókban szenvedő gyermekekben viszont hatékonyan csökkentette az infekciók számát [20].

Nyomelemek

A nyomelemek élettani – ezen belül immunológiai – hatásai elég jól ismertek. A legtöbb adat a hiányukban jelentkező tünetekből, illetve az ilyen esetekben adott pótló kezelésből származik. Általános alultápláltság mellett (elsősorban a fejlődő országokban) nyomelemek hiánya is kialakulhat. A fokozott hajlam légúti, enteralis, malária stb. infekciókra és az

infekciók súlyosabb kimenetele a nyomelemek hiányának egyik következménye.

a) Cink (Zn): A cink immunológiai hatásai preklinikai vizsgálatokból jól ismertek.
   - Egy metaanalízis 13 RCT alapján azt a következtetést vonta le, hogy (1) a légúti infekció első 24 órájában adva megrövidíti a betegség lefolyását és csökkenti a tüneteket – a nagyfokú heterogenitás miatt azonban az eredményeket óvatosan kell kezelni, (2) 5 hónapon át adva gyermekekben pedig csökkenti a légúti infekciós esetek számát [21]. Ezt a Cochrane-analízist később visszavonták.
   - Egy nemrégi metaanalízis cink-glükonát szopogatására (75–100 mg – lassú és tökéletesebb felszívódás) a megfázásos tünetek csökkentek és 33%-kal csökkent átlagban a betegség tartama [22].
   - Cink adása fokozta a koleravakcina hatékonyságát [23].
   - Verruca vulgaris kezelésében önmagában is [24], ill. lokális kezeléssel kombinálva [25] hatásosnak bizonyult.
   - Vassal kombinálva 40%-kal csökkentette (vélhetően alultáplált) csecsemők légúti és emésztőrendszeri infekcióit [26].
   - Rézzel és szelénnel kombinálva csökkentette a nosocomialis pneumoniák előfordulását égés után (80% vs. 33%) [27].
   - Szelénnel és vitaminokkal (C-, E-vitaminnal és béta-karotinnal) kombinálva csökkentette idősotthonban lakók légúti infekcióinak számát, és javította a vakcinára adott immunválaszt, de nem befolyásolta az urogenitalis infekciókat [28].

b) Réz (Cu): A réz elsősorban a phagocyták működéséhez szükséges. Önmagában a réz adását légúti infekciókban nem vizsgálták. Más nyomelemekkel – és vitaminokkal – együtt adva csökkentette a nosocomialis infekciók előfordulását [27], illetve idősekben rövidítette a légúti infekciók lefolyását [29]. Ezen utóbbi eredményeket, más hasonló vizsgálatokéval együtt, azonban kétségbevonták [30] – ezért a közleményt később visszavonták.

c) Szelén (Se): Immunológiai, antioxidáns és daganatgátló hatása mellett hiánya az immunfunkciók romlásához vezet. Különféle gyulladásos betegségekben szérumszintjét csökkentnek találták. HIV-fertőzötteknek adva, a szövődmények száma csökkent. Szepszisben adva – egy metaanalízis szerint – a mortalitást csökkentette [31]. C-vitaminnal, cinkkel és telítetlen zsírsavakkal együtt adva csökkentette a légúti infekciók számát [32]. Légúti infekciókban egyetlen – kínai nyelvű – közlemény van [32], amelyben a szelénadás rövidítette az RSV-infekció lefolyását.

d) Többi nyomelem (molibdén, kobalt, vanádium stb.): Immunológiai hatásaik alig ismertek, hiányuk azonban csökkent immunfunkciókhoz is vezet(het). Ezekkel a nyomelemekkel – beleértve azok kombinált készítményeit – klinikai vizsgálatok nem történtek.

Probiotikumok

A probiotikumok szó görög eredetű, és jelentése „életért". Élő mikroorganizmusok (pl. Lactobacillus rhamnosus, L casei, Bifidobacterium lactis) orális bevitelét jelenti megfelelő mennyiségben. A probiotikumok elsősorban a veleszületett immunrendszert befolyásolják (pl. fagocitózist), javítják a bélfal barrierfunkcióit és a lokális (mucosalis) immunitást, de még számos más hatással is rendelkeznek.

Egy metaanalízis (12 RCT, 3720 egyén) szerint a probiotikumok adása – a placebóhoz viszonyítva – csökkentette a légúti infekciók előfordulását és rövidítette azok lefolyását [34].

Vitaminok

A vitaminok esetében is elsősorban a hiányuk okozta immunológiai rendellenességeket ismerjük.

a) C-vitamin: Egy metaanalízis szerint a napi >200 mg C-vitamin bevitele nem csökkentette a légúti infekciók előfordulását (megelőző kezelés során), de rövidítette a betegség időtartamát, kivált gyermekekben [35].

b) D-vitamin: Egy másodlagos analízis szerint a szérum 25(OH)D-vitamin-szintje negatív korrelációt mutat a heveny légúti infekciók számával [36]. Hasonlót találtak közösségben szerzett pneumoniában is [37].
   Egy vizsgálat (VIDARIS) szerint nagy adagú (100 000 E/nap) D3-vitamin adása nem csökkentette sem a légúti infekciók gyakoriságát, sem azok súlyosságát [38].
   Egy metaanalízis (11 RCT, 5660 egyén) szerint a napi D-vitamin-bevitel csökkenti a légúti infekciók gyakoriságát, de a vizsgálati eredmények heterogenitása miatt ezt óvatosan kell értékelni [39].
   A legújabb metaanalízis szerint (25 RCT, 11 321 egyén) a napi D-vitamin-pótlás csökkenti a heveny légúti infekciók előfordulását, legerősebb a hatás a D-vitamin-hiányos egyénekben [40].
   Mind ez idáig 5 metaanalízis született e témában, ebből 2 pozitív, 3 negatív eredménnyel zárult, azaz a D-vitaminnak vélhetően nincs megelőző hatása.

c) A-vitamin: A retinoidok immunológiai hatásai régóta ismertek. Különösen fontos az A-vitamin a mucosalis immunitás fenntartásában. Ugyancsak jól ismert, hogy A-vitamin-hiányban a hasmenéses, légúti infekciós betegségek gyakoribbak [41], az A-vitamin-hiány különösen komoly probléma a fejlődő országokban.
   A magasabb plazma-béta-karotin-koncentrációjú egyének között kevésbé gyakoriak a heveny légúti infekciók [42].
   Egy meta-analízis szerint az A-vitamin adása csak az

akut hasmenéses esetek számát csökkentette, a légúti infekciókét nem [43]. A szerzők szerint csak azoknak a gyermekeknek érdemes A-vitamin-pótlást adni, akik alultápláltak és A–vitamin-hiányban szenvednek. Egy újabb metaanalízis megerősíti, hogy A-vitamin-hiányos gyermekeknek (5 éves kor alatt) érdemes és kell A-vitamin-pótlást adni, mert ezzel mind a morbiditást, mind a mortalitást jelentősen csökkenteni lehet [44].

d) E-vitamin: Az Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study in Finland, 1985–1993 másodlagos analízise megvizsgálta, hogy a dohányos (≥20 cigaretta/nap) idős (50–69) férfiak pneumoniakockázata csökken-e E-vitamin adására [45]. Bár a kezelés hatására 73%-os csökkenést tapasztaltak, az adatok óvatosan kezelendők, ill. további vizsgálatokat javasol a szerző – elsősorban a jelentős heterogenitás (I^2-statisztikával mérve) miatt.

Összegezve, a vitaminok pótlása csak vitaminhiány esetén fejt ki meghűlés elleni védőhatást.

Összesítés

Habár mind szerkezetüket, mind hatásaikat tekintve a fenti készítmények igen sokfélék, készült egy metaanalízis, mely összesítve analizálja az „immunstimuláns” készítmények hatásosságát gyermekek visszatérő légúti fertőzésében [46]. Összesen 35 RCT adatait elemezték (4060 egyén), a kezelés átlagosan 41%-kal csökkentette a fertőzések számát – az evidencia mértékét (grade) mérsékeltnek minősítették.

Az immunstimuláció biztonságossága

A biológiai választ módosító szerek (biológiai terápia) kapcsán számos – korábban nem észlelt – nemkívánatos hatást észleltek:

- tumorlízis szindrómát,
- kapillárisszivárgás (capillary leak) szindrómát,
- citokinvihart – pl. a szuperagonista anti-CD28 antitest esetén („londoni katasztrófa”),
- autoimmun reakciókat.

Ugyancsak jól ismert az adjuvánsok okozta betegség, az ASIA-szindróma (Autoimmune/inflammatory Syndrome Induced by Adjuvants).

Ugyanakkor a fentebb tárgyalt „immunerősítők” esetében ezek a súlyos nemkívánatos hatások nem észlelhetők.

- Növényi eredetű anyagok esetén a gyomor-bél panaszok a leggyakoribbak, legkomolyabb probléma az allergia, amit Echinacea készítmények esetében néhány alkalommal, újabban más készítményeknél is észleltek.
- A baktériumkivonatok ritkán gyomor-bél panaszokat és nagyon ritkán allergiás tüneteket okozhatnak.
- A levamizolt ma már erre a célra nem alkalmazzuk; az inoziplex hosszabb időn át adva emeli a húgysavszintet, bár köszvényt kiváltó hatását nem észlelték – egyébként hosszú időn át, preventív célzattal, nem érdemes adni.
- Jól ismertek (habár elhanyagolhatók) a vitaminok túladagolásával kapcsolatos mellékhatások.
- A nyomelemek (Zn, Cu) csak a bevittnél sokszorta nagyobb adagokban okozhatnak toxikus tüneteket. A készítmények igen kis adagban tartalmazzák a nyomelemeket, toxikus mellékhatásokról nem számoltak be.

Összefoglaló megjegyzések és javaslatok

Láthatjuk, hogy valamennyi készítménycsoport rendelkezik valamilyen hatással a vírusok által okozott felső légúti gyulladásos betegségek megelőzésében, esetleg a hurutos tünetek javításában, bár ezek a hatások elég szerények. A legtöbb készítmény biztonságosan szedhető. A kérdés csak az, hogy egészséges egyénnek érdemes-e tartósan, megelőző célzattal ilyen szereket szedni. Lehet-e más módon ellenálló képességünket fokozni (szándékosan nem azt írom, hogy immunrendszerünket erősíteni)?

Az nem kétséges hogy a civilizált életmód valóban rontja ellenálló képességünket. Ennek egyrészt az ülő életmód, a kevés mozgás, a bezártság, a tömegközlekedés és még számtalan más – de jól ismert, és itt nem részletezett – oka van. Ezért egészséges életmóddal sokat javíthatunk rajta. Bizonyosan sokkal többet, mint különféle pasztillák, cseppek bevételével.

Javaslatok az egészséges életmódra:

- a dohányzás kerülése (abbahagyása),
- mértékletes alkoholfogyasztás (pontosabban a mértéktelen fogyasztás kerülése),
- rendszeres, aktív testmozgás,
- egészséges, változatos táplálkozás (esetleg probiotikumok fogyasztásával kiegészítve),
- testsúlykontroll,
- a stressz kerülése (ill. megfelelő kezelése, pl. agykontrollal, NEM gyógyszerekkel!),
- elegendő, pihentető alvás,
- az infekciók kerülése kivált „megfázásos” időszakban”.

Az egyetlen probléma ezekkel a jó tanácsokkal, hogy sokkal egyszerűbb reggel vagy este egy-két kapszulát vagy tablettát bevenni, mint a fentieket megfogadni. Még akkor is, ha tudjuk, hogy a fenti tanácsok sokkal többet használnak. Ez az emberi tulajdonság élteti az „immunerősítők” (és egyéb csodaszerek, ránctalanítók stb.) piacát.

IRODALOM

[1] Karsch-Völk M, Barrett B, Kiefer D, et al: Echinacea for preventing and treating the common cold. Cochrane Database Syst Rev 2014; (2): CD000530.

[2] Matsumoto K, Yamada H, Takuma N, et al: Effects of green tea catechins and theanine on preventing influenza infection among healthcare workers: a randomized controlled trial. BMC Complement Altern Med 2011; 11: 15.

[3] Schütz K, Sass M, de With A, et al: Immune-modulating efficacy of a polyphenol-rich beverage on symptoms associated with the common cold: a double-blind, randomised, placebo-controlled, multi-centric clinical study. Br J Nutr 2010; 104(8): 1156-64.

[4] Timmer A, Günther J, Motschall E, et al: Pelargonium sidoides extract for treating acute respiratory tract infections. Cochrane Database Syst Rev 2013; (10): CD006323.

[5] Heinz SA, Henson DA, Austin MD, et al: Quercetin supplementation and upper respiratory tract infection: A randomized community clinical trial. Pharmacol Res 2010; 62(3): 237-42.

[6] Turner RB, Fowler SL, Berg K: Treatment of the common cold with troxerutin. APMIS 2004; 112(9): 605-11.

[7] Somerville VS, Braakhuis AJ, Hopkins WG: Effect of Flavonoids on Upper Respiratory Tract Infections and Immune Function: A Systematic Review and Meta-Analysis. Adv Nutr 2016; 7(3): 488-97.

[8] Seida JK, Durec T, Kuhle S: North American (Panax quinquefolius) and Asian Ginseng (Panax ginseng) preparations for prevention of the common cold in healthy adults: a systematic review. Evid Based Complement Alternat Med 2011; 2011: 282151.

[9] Lissiman E, Bhasale AL, Cohen M: Garlic for the common cold. Cochrane Database Syst Rev 2014; (11): CD006206.

[10] Zhang X, Wu T, Zhang J, et al: Chinese medicinal herbs for the common cold. Cochrane Database of Systematic Reviews 2007; (1): CD004782.

[11] Wang H, Cai Y, Zheng Y, et al: Efficacy of biological response modifier lentinan with chemotherapy for advanced cancer: a meta-analysis. Cancer Med 2017; 6(10): 2222-2233.

[12] Vetvicka V, Richter J, Svozil V, et al: Placebo-driven clinical trials of yeast-derived β-(1-3) glucan in children with chronic respiratory problems. Ann Transl Med 2013; 1(3): 26.

[13] Cazzola M, Anapurapu S, Page CP: Polyvalent mechanical bacterial lysate for the prevention of recurrent respiratory infections: a meta-analysis. Pulm Pharmacol Ther 2012; 25(1): 62-8.

[14] Schaad UB: OM-85 BV, an immunostimulant in pediatric recurrent respiratory tract infections: a systematic review. World J Pediatr 2010; 6(1): 5-12.

[15] Boyle P, Bellanti JA, Robertson C: Meta-analysis of published clinical trials of a ribosomal vaccine (ribomunyl) in prevention of respiratory infections. BioDrugs 2000; 14(6): 389-408.

[16] Van Eygen M, Znamensky PY, Heck E, Raymaekers I: Levamisole in prevention of recurrent upper-respiratory-tract infections in children. Lancet 1976; 1(7956): 382-5.

[17] Litzman J, Lokaj J, Krejcí M, et al: Isoprinosine does not protect against frequent respiratory tract infections in childhood. Eur J Pediatr 1999; 158(1): 32-7.

[18] Beran J, Šalapová E, Špajdel M; Isoprinosine Study (EWO ISO-2014/1) Team: Inosine pranobex is safe and effective for the treatment of subjects with confirmed acute respiratory viral infections: analysis and subgroup analysis from a Phase 4, randomised, placebo-controlled, double-blind study. BMC Infect Dis 2016; 16(1): 648.

[19] Mameli C, Pasinato A, Picca M, et al:; AX-Working Group: Pidotimod for the prevention of acute respiratory infections in healthy children entering into daycare: A double blind randomized placebo-controlled study. Pharmacol Res 2015; 97: 79-83.

[20] Licari A, De Amici M, Nigrisoli S, et al: Pidotimod may prevent recurrent respiratory infections in children. Minerva Pediatr 2014; 66(5): 363-7.

[21] Singh M, Das RR: Zinc for the common cold. Cochrane Database Syst Rev 2011; (2): CD001364.

[22] Hemilä H: Zinc lozenges and the common cold: a meta-analysis comparing zinc acetate and zinc gluconate, and the role of zinc dosage. JRSM Open 2017; 8(5): 205427041769429

[23] Karlsen TH, Sommerfelt H, Klomstad S, et al: Intestinal and systemic immune responses to an oral cholera toxoid B subunit whole-cell vaccine administered during zinc supplementation. Infect Immun 2003; 71(7): 3909-13.

[24] Al-Gurairi FT, Al-Waiz M, Sharquie KE: Oral zinc sulphate in the treatment of recalcitrant viral warts: randomized placebo-controlled clinical trial. Br J Dermatol 2002; 146(3): 423-31.

[25] Akhavan S, Mohammadi SR, Modarres Gillani M, et al: Efficacy of combination therapy of oral zinc sulfate with imiquimod, podophyllin or cryotherapy in the treatment of vulvar warts. J Obstet Gynaecol Res 2014; 40(10): 2110-3.

[26] Baqui AH, Zaman K, Persson LA, et al: Simultaneous weekly supplementation of iron and zinc is associated with lower morbidity due to diarrhea and acute lower respiratory infection in Bangladeshi infants. J Nutr 2003; 133(12): 4150-7.

[27] Berger MM, Eggimann P, Heyland DK, et al: Reduction of nosocomial pneumonia after major burns by trace element supplementation: aggregation of two randomised trials. Crit Care 2006; 10(6): R153.

[28] Girodon F, Galan P, Monget AL, et al: Impact of trace elements and vitamin supplementation on immunity and infections in institutionalized elderly patients: a randomized controlled trial. MIN. VIT. AOX. geriatric network. Arch Intern Med 1999; 159(7): 748-54.

[29] Jain A: Influence of vitamins and trace-elements on the incidence of respiratory infection in the elderly. Nutr Res 2001; 22: 85-87.

[30] Sternberg S, Roberts S: Nutritional supplements and infection in the elderly: why do the findings conflict? Nutr J 2006; 5: 30.

[31] Alhazzani W, Jacobi J, Sindi A, et al: The effect of selenium therapy on mortality in patients with sepsis syndrome: a systematic review and meta-analysis of randomized controlled trials. Crit Care Med 2013; 41(6): 1555-64.

[32] Raposo SE, Fondell E, Ström P, et al: Intake of vitamin C, vitamin E, selenium, zinc and polyunsaturated fatty acids and upper respiratory tract infection-a prospective cohort study. Eur J Clin Nutr 2017; 71(4): 450-457.

[33] Liu X, Yin S, Li G: [Effects of selenium supplement on acute lower respiratory tract infection caused by respiratory syncytial

virus]. Zhonghua Yu Fang Yi Xue Za Zhi 1997; 31(6): 358-61. Chinese.
[34] Hao Q, Dong BR, Wu T: Probiotics for preventing acute upper respiratory tract infections. Cochrane Database Syst Rev 2015; (2): CD006895.
[35] Hemilä H, Chalker E: Vitamin C for preventing and treating the common cold. Cochrane Database Syst Rev 2013; (1): CD000980.
[36] Ginde AA, Mansbach JM, Camargo CA Jr: Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. Arch Intern Med 2009; 169(4): 384-90.
[37] Jovanovich AJ, Ginde AA, Holmen J, et al: Vitamin D level and risk of community-acquired pneumonia and sepsis. Nutrients. 2014; 6(6): 2196-205.
[38] Murdoch DR, Slow S, Chambers ST, et al: Effect of vitamin D3 supplementation on upper respiratory tract infections in healthy adults: the VIDARIS randomized controlled trial. JAMA 2012; 308(13): 1333-9.
[39] Bergman P, Lindh AU, Björkhem-Bergman L, Lindh JD: Vitamin D and Respiratory Tract Infections: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. PLoS One 2013; 8(6): e65835.
[40] Martineau AR, Jolliffe DA, Hooper RL, et al: Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. BMJ 2017; 356: i6583.
[41] Qi YJ, Niu QL, Zhu XL, et al: Relationship between deficiencies in vitamin A and E and occurrence of infectious diseases among children. Eur Rev Med Pharmacol Sci 2016; 20(23): 5009-5012.
[42] van der Horst-Graat JM, Kok FJ, Schouten EG: Plasma carotenoid concentrations in relation to acute respiratory infections in elderly people. Br J Nutr 2004; 92(1): 113-8.
[43] Chen H, Zhuo Q, Yuan W, et al: Vitamin A for preventing acute lower respiratory tract infections in children up to seven years of age. Cochrane Database Syst Rev 2008; (1): CD006090.
[44] Imdad A, Mayo-Wilson E, Herzer K, Bhutta ZA: Vitamin A supplementation for preventing morbidity and mortality in children from six months to five years of age. Cochrane Database Syst Rev 2017; 3: CD008524.
[45] Hemilä H: Vitamin E administration may decrease the incidence of pneumonia in elderly males. Clin Interv Aging. 2016; 11: 1379-1385. eCollection 2016.
[46] Del-Rio-Navarro BE, Espinosa-Rosales FJ, Flenady V, Sienra-Monge JJL: Immunostimulants for preventing respiratory tract infection in children. Cochrane Database of Systematic Reviews 2006; (4): CD004974.

(Dr. Gergely Péter, Semmelweis Egyetem, Laboratóriumi Medicina Intézet, 1089 Budapest, Nagyvárad tér 4., pgergely@alexmed.hu)

Immunológiai szótár

gátlási teszt (1) Vizsgálati módszer, amelyben valamely aktivitást, pl. vírusét, a titrálandó antitestet tartalmazó szérum hígítási sorával gátolnak. (2) Standard agglutinációs, precipitációs stb. teszt gátlása a megfelelő – a specificitás biztosítása érdekében tisztított – antigén adásával.

génknockout egér Olyan egér, amelyben egy kiválasztott gént inaktív mutánssal helyettesítenek, így az illető gén által kódolt protein hiányzik. A mutáns gént in vitro egy vektorral viszik be az embrionális őssejtbe, majd in vivo implantációt követően normál magzati fejlődés játszódik le. Az utódok közül a géndefektusra homozigóta egyedeket tenyésztik tovább. A génknockout egereket az immunológiában és egyéb területeken használják meghatározott fehérjék funkciójának tanulmányozására, a hiányzó működés segítségével. Elnevezésükre a hiányzó funkció jellemző, pl. „perforin-knockout egér”.

germinális centrum (csíraközpont) B-lymphocyták szférikus aggregációja, amely a lymphoid szöveteken belül az elsődleges follikulusokban, thymusdependens antigénekkel való stimuláció eredményeként alakul ki. A T-sejt-területen való antigénfelismerést követően a B-sejtek az elsődleges follikulusokba vándorolnak, ahol masszív proliferációt követően létrejön a germinális centrum, s ennek révén a kisebb, recirkuláló B-sejtek a köpenyzónába kerülnek (follicularis köpeny). Néhány nap elteltével a centrumra egy sötét zóna – amelyben néhány follicularis dendritikus sejt (FDC) található és centroblastokban gazdag –, valamint egy világos zóna jellemző, ez utóbbi antigént hordozó FDC-k hálózatát és centrocytákat tartalmaz, melyek az FDC-k felszínén az antigénnel kapcsolatba lépnek. A germinális centrum a B-sejtek izotípusváltásának és az affinitásmaturációnak a helye. Azok a sejtek, amelyek az antigénre nagy affinitással reagálnak, pozitív szelekción mennek keresztül és életben maradnak. A többi sejt apoptózissal elpusztul, s maradványaikat macrophagok távolítják el. A germinális centrumokban levő B-sejtek oligoklonálisak, amely egy bizonyos antigénre való válaszra enged következtetni. A szelekciót követően a sejtek plazmasejtekként elhagyhatják a germinális centrumot, a kérgi kötegekbe vagy a csontvelőbe vándorolhatnak, vagy memória B-sejtekként a marginális zónába kerülnek. Megjegyzendő, hogy a germinális centrumokban elegendő T-sejt van ahhoz, hogy a T- és a B-sejtek között interakció jöhessen létre (pl. a CD40L és a CD40 között).

Mozgásban

Mónok Gabriella

Gyerekeimmel együtt verselve

Sziszeg a fagy, a tél hava lejárt,
az új idő egy kicsit most megállt:
Nincs hó, nincs dér, csak játékos fény,
cikázik a szellő, s könnybe lábad a jég.
Gyerekek futnak, jár a hinta már,
a fűben hever ezernyi kis bogár.
Ugrál a tücsök, vékony hangon zenél,
rigópár tipeg a kertben, kukacot remél.

Gyerekeim némán ámulnak nagyon,
amint cica söpör végig a füves udvaron.
Kergetik egymást a szalmabálán már,
kétségbe ejtve egy ártatlan egérpárt.
Jól járt a kandúr, lesz megint bújócska,
egéranyó a családot ismét jól eldugta.
Ugrál a béka a hínár tetején,
halacskák úszkálnak a vízben még.

Ám a teknős a tóba egy nagyot csobbant,
És kutatva a vizet, egy halacskára pottyant.
Vízipók résen volt, elszaladt onnan,
Egy tavirózsa szirmán megpihenve gyorsan.
Őzike a tóhoz inni ballagott, de
Meglátta a békát és ugrott egy nagyot!
Megijedt ő nagyon, de mégsem lépte agyon,
Mert a béka már fürge volt nagyon!

Brummog a medve, dübörög a rét,
A nagyvad immár csatára kész!
Félhetnek a méhek, a méznek már lőttek,
Nem maradt semmi az okos méhésznek.
Vöröslik a fű, zörög a bozót,
Üdvözlik a ravaszt, a libát megfogót,
Lopakodik lassan a tyúkok óljához,
de kiabál a kakas a védő kutyához.

A Labrador lelkesen ugatja az eget,
majd gyors iramban a kiskertbe üget,
Megérzi a rókát a közelgő bokorban,
S nem téveszti össze az egyik rokonnal!
Elijeszti mára a négylábú ravaszt,
S megvédi a kertben élő picike kakast!
Zajos nap után már mindenki fáradt,
A gyerekek álmuk mélyén nevetnek párat!

2013. március 3.

NÉZZÉK,

ÚJRA AKTÍV LEHETEK, MINT AZELŐTT!*

A Cosentyx®▼ az első és egyetlen humán IL-17A gátló terápia spondylitis ankylopoetica kezelésére

* A 150 mg Cosentyx kezelésben részesült spondylitis ankylopoeticában szenvedő betegek 61%-a elérte az ASAS 20 választ a MEASURE 2 vizsgálatban a 16. héten. Ezekben a betegekben a betegségaktivitás jelentősen csökkent (BASDAI), a gerincmobilitás (BASMI) a kiinduláshoz képest jelentősen javult a 16. héten placebóhoz hasonlítva. Ezenkívül az egészségi állapottal összefüggő életminőségben (ASQoL), a fizikális funkciókban (BASFI) és a fáradtság (FACIT-F) tekintetében is jelentős javulás mutatkozott a placebóhoz képest. A tapasztalat javulások fennmaradtak az 52. hétig.

COSENTYX® 150 mg szekukinumab előretöltött injekciós tollban
Kiadhatóság: Korlátozott érvényű orvosi rendelvényhez kötött gyógyszer.
Osztályozási alkategória SZ. A forgalomba hozatali engedély jogosultjának helyi képviselője: Novartis Hungária Kft. (Pharma részleg), 1114 Budapest, Bartók Béla út 43-47. Tel: 06-1-457-6500, Fax: 06-1-457-6600.
A forgalomba hozatali engedély száma: **EU/1/14/980/005**

Bővebb információért olvassa el a gyógyszer alkalmazási előírását!

A hatályos „alkalmazási előírás" teljes szövegét megtalálja az Országos Gyógyszerészeti és Élelmezés-egészségügyi Intézet (www.ogyei.gov.hu/gyogyszeradatbazis/) vagy az Európai Gyógyszerügynökség (www.ema.europa.eu) honlapokon.

A gyógyszer társadalombiztosítási támogatásban nem részesül, társadalombiztosítási támogatással nem rendelhető, így közfinanszírozás alapjául szolgáló ár, támogatási összeg és térítési díj nem adható meg.

Az EMA forgalomba hozatali engedély kiadásának dátuma: 2015.01.15.

Társadalombiztosítási támogatási kérelem benyújtásának dátuma: 2016.12.10.

▼Ez a gyógyszer fokozott felügyelet alatt áll, mely lehetővé teszi az új gyógyszerbiztonsági információk gyors azonosítását. Az egészségügyi szakembereket arra kérjük, hogy jelentsenek bármilyen feltételezett mellékhatást.

NOVARTIS

Cosentyx® secukinumab

HU1803786657 Lezárás dátuma: 2018. március 5.

A teljes remisszió lehetősége akár MTX nélkül is[1,2]

MIKOR KEZDJÜK EL A RoACTEMRA KEZELÉST?

Közel 10 év bizonyított hatékonyság és tapasztalat a rheumatoid arthritisben[3,4,5]

RoACTEMRA®
tocilizumab

1. RoACTEMRA SmPC, www.ema.europa.eu
2. Gabay C, et al. Lancet 2013; 381: 1541–1550.
3. EMA. Doc Ref EMEA/CHMP/580914/2008. 2008. Available at http://www.ema.europa.eu/docs/en_GB/document_library/Summary_of_opinion_-_Initial_authorisation/human/000955/WC500059466.pdf Last accessed October 2017.
4. Jones G et al. J Rheumatol 2017; 44(2): 142–146.
5. Dougados M et al. Ann Rheum Dis 2013; 72(1): 43–50.

Dokumentum zárásának időpontja: 2018.09.25.

Bővebb információért olvassa el a gyógyszer alkalmazási előírását: www.ogyei.gov.hu/gyogyszeradatbazis. Az aktuális árak tekintetében kérjük, ellenőrizze a www.neak.gov.hu honlapon a Publikus gyógyszertörzs – lakossági tájékoztató alatt található információkat.

További információ:
Roche (Magyarország) Kft. 2040 Budaörs, Edison u. 1.
Tel: 06-23-446 800, Fax: 06-23-446 860
Email: hungary.medinfo@roche.com
Internet: www.roche.hu

HU/RA/0918/0026