SKIN BARRIER ALTERATIONS IN THE DEVELOPMENT OF IMMUNE-MEDIATED SKIN DISEASES

In this research project we investigated the characteristics and the connections between the immune and the physico-chemical skin barriers in health and in immune-mediated skin diseases, like atopic dermatitis and psoriasis. The alterations of the physico-chemical and immune barriers and their strong, but yet uncovered interdependences play major role in the initiation and maintenance of these skin diseases and dermatologists should pay more attention to define, measure and restore these defects.

Healthy skin has topographically different immune and physico-chemical barrier characteristics – the skin immune system is not unified

Until today the immune barrier of the healthy skin is considered to be unified on the whole body surface – however, recent indirect findings have challenged this dogma, as the microbial and the chemical milieu (e.g. sebum, sweat, pH) also exhibit remarkable differences on topographically distinct skin areas. First our aim was to compare the immune milieu of healthy sebaceous gland rich (SGR) and sebaceous gland poor (SGP) skin areas. For this purpose, immunohistochemical, immunocytochemical and quantitative real-time PCR analyses of thymic stromal lymphopoietin (TSLP) and other cytokines, immune cell markers and transcription factors were carried out in samples from SGP and SGR skin and also from papulopustular rosacea (PPR) samples, which is an inflammatory skin disease exclusively of SGP skin. TSLP mRNA and protein production was also studied in cultured keratinocytes. In SGR skin, higher TSLP expression, dendritic cell (DC) appearance without prominent activation and T cell presence with IL-17/IL-10 cytokine milieu were detected compared to SGP skin. Linoleic acid, a major sebum component, was found to induce TSLP expression dose-dependently in keratinocytes. In PPR, significantly decreased TSLP level and influx of inflammatory DCs and T cells with IL-17/IFN-γ cytokine milieu were observed. First in the literature we could detect that healthy SGR skin is characterized by a distinct, non-inflammatory immune surveillance, which was basically changed during inflammation. (Dajnoki, Z. et al. J Invest Dermatol 137 (5), 1114-1125., 2017).

As a next step we performed whole transcriptomic and subsequent pathway analyses to deeply analyse differences between SGR and SGP regions. We provided the first evidence that different skin regions exhibited characteristic innate and adaptive immune and barrier milieu as we could detect significantly increased chemokine (CCL2, 3, 19, 20, 23, 24) and

antimicrobial peptide (S100A7, A8, A9, lipocalin, β-defensin-2) expression, altered barrier (keratin 17, 79) functions and a non-inflammatory Th17/IL-17 dominance in SGR skin compared to SGP. Regarding pro-inflammatory molecules (IL-1α, IL-6, IL-8, IL-33, TNF-α), similarly low levels were detected in both regions. Our data may explain the well-known characteristic localization of some immune-mediated and autoimmune skin disorders, can establish the development of region specific barrier repair strategies and we also propose that the term „healthy skin control sample", widely used in experimental Dermatology, should only be accepted if researchers carefully specify the exact region of the healthy skin (along with the site of the diseased sample). (Béke, G. et al. Frontiers in Immunology, under review).

In order to study the photoprotective capacity of skin barrier in the future, a model system was worked out by transfecting keratinocytes with pseudouridine-modified mRNA (Ψ-mRNA) encoding CPD-photolyase. Major biological effects of UVB are attributed to cyclobutane pyrimidine dimers (CPDs), the most common photolesions formed on DNA. To investigate the contribution of CPDs to UVB-induced changes of gene expression, a photolyase mRNA-based experimental platform was developed and was able to demonstrate CPD-dependent and-independent events of UVB-induced cellular responses, and, as such, has the potential to identify novel molecular targets for treatment of UVB-mediated skin diseases. (Boros, G. et al. PLoS One 10 (6), e0131141., 2015.)

Altered adaptive and innate immune system as well as defective physico-chemical barrier are characteristics of atopic dermatitis (AD)

During the project we studied the number and function of regulatory T-cells (Tregs) in AD patients. Flow cytometry was utilised to determine the percentage of $CD4^+CD25^{bright}CD127^{-/low}FOXP3^+$ and skin-homing $CLA^+CD4^+CD25^{bright}FOXP3^+$ Tregs in healthy controls and AD patients. Although significantly increased percentages of Tregs were found in AD patients compared to healthy individuals, and significant correlation between the frequency of Tregs and disease severity was also detected, the suppressor activity of Tregs was altered in the presence of *Staphylococcus* enterotoxin B (SEB). In conclusion, the continuous presence of SEB on the skin of AD patients (which is a characteristic feature of AD skin) can trigger an acquired functional impairment of Tregs, and this can lead to increased function of effector T cells. The significant activation of Th2 type effector cells in the skin and also in the blood of AD patients

has already been detected by our group in a previous study. (Gáspár, K. et al. Acta Derm Venereol 95 (2), 151-155., 2015.)

Going further, the aim of our next study was to investigate the proportion of follicular helper T (TFH)-like cells in AD. The frequency of $CD4^+CXCR5^+ICOS^+PD\text{-}1^+$ TFH-like cells and their IL-21 cytokine production were determined by flow cytometry. Immunohistochemical analysis was also performed on skin biopsy specimens from AD patients for the detection of TFH markers. The percentages and absolute numbers of circulating TFH-like cells were significantly increased in children with AD compared to adult patients and healthy controls. IL-21 cytokine production of TFH-like cells was also elevated and showed a strong positive correlation with paediatric patients' SCORAD index. The expression of TFH-specific markers showed only a non-specific scattered pattern in skin biopsy specimens. This was the first study that has shown that TFH-like cell number and function is elevated in the peripheral blood of children with AD compared to adults. (Szabó, K. et al. Immunol Lett 189 101-108., 2017.)

Regarding, that DC-s play crucial role in the pathogenesis of AD we aimed to characterise myeloid pre-DCs separated from the blood of AD patients, to examine their phenotypic features and their chemokine production. Peripheral blood myeloid pre-DCs were separated from the blood of AD patients and healthy controls using the $CD1c^+/BDCA1^+$ magnetic separation kit. The expression of cell surface markers was measured by flow cytometry, while chemokine production was monitored with a chemokine antibody array. Pre-DCs from AD patients expressed higher levels of FcεRI and less CD206-mannose receptors on their surface and not only produced the AD-related chemokines CCL17 and CCL18, but also those chemokines which are characteristic of maturing DCs (CCL3, CCL4, CCL5, CCL9). Our results indicated that circulating pre-DCs derived from AD patients were more activated than those derived from healthy donors and therefore may contribute to the pathogenesis of AD. (Kapitány, A. et al. Acta Derm Venereol 97 (3), 325-331., 2017.)

Inflammation can impair the physico-chemical skin barrier, but the opposite question whether barrier alterations can affect keratinocyte immune responses is poorly investigated. To study the above topic we investigated whether the presence of filaggrin mutations in keratinocytes (which cause a physico-chemical skin barrier defect) influences the skin inflammation of AD patients. Filaggrin, cytokines (TSLP, IL-33) and CCL27 chemokine, T cells and DCs were stained by immunohistochemistry in biopsies from lesional skin of severe AD patients with and without filaggrin mutation and from healthy skin. Our results were also confirmed by

quantitative PCR analyses. No significant differences were found between the two patient groups. The expression of AD-specific cytokines showed significant correlation with histological severity. Our findings suggested that the immune-mediated skin inflammation was similar in the two severe patient groups and immune activation was connected rather to the severity of the disease, than to the origin of barrier alterations, since beside the filaggrin mutation, other forms of barrier defects are well-known in AD. (Dajnoki, Z. et al. Acta Derm Venereol 96 (5), 645-650., 2016.)

Hyper-IgE syndrome (HIES) is a severe primary immunodeficiency, characterized by increased serum IgE levels as well as recurrent infections and AD-like skin lesions. Our aim was to investigate the physico-chemical skin barrier alterations and allergic sensitization in STAT3-HIES patients in order to explore whether skin barrier dysfunction can play a role in the development of AD-like skin lesions in these patients. In our experiments STAT3 and filaggrin mutation analyses were performed in STAT3-HIES and AD patients. Impaired Th17 cell numbers, but normal physico-chemical barrier functions, as well as serum and stratum corneum TSLP levels were found in STAT3-HIES, while these parameters were significantly altered in AD patients. Allergic sensitization was detected in nearly all AD patients, while no signs of sensitization occurred in STAT3-HIES. Our study demonstrated that the skin barrier functions of STAT3-HIES patients are not damaged and they differ significantly from the altered skin barrier functions of AD patients. Our study underlines the importance of skin barrier in the development of allergic sensitization. (Mócsai, G. et al. J Clin Immunol 35 (7), 681-688., 2015.)

We also determined serum levels of various endogen steroids in AD patients compared to healthy volunteers. Steroid levels were measured by HPLC-MS/MS analysis and displayed significantly decreased plasma levels of the vitamin D signalling dependent metabolite DHEAS and increased levels of the cortisol precursor cortisone. We concluded that altered steroid levels in the plasma of patients with AD indicated altered vitamin D signalling (based on reduced DHEA sulfonation) and increased feedback for anti-inflammatory signalling (increased levels of cortisone) present in patients with AD. (Mihály, J. et al. B J Dermatol 172 (1), 285-288., 2015.)

Skin immune alterations are accompanied by severe systemic inflammation and comorbidities in psoriatic patients – psoriasis is more than just "skin deep"

Our aim was to characterize the phenotypic features as well as the cytokine and chemokine production of $CD1c^+$ myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) in the blood samples of psoriatic patients. Blood DCs were isolated by using a magnetic separation kit, and their intracytoplasmic cytokine production and CD83/CD86 as a maturation/activation marker expression was investigated by 8-colour flow cytometry. The chemokine production of both DC populations was investigated by flow-cytometry and ELISA. According to our results psoriatic $CD1c^+$ mDCs were in a premature state since their CD83/CD86 maturation/activation marker expression, IL-12 cytokine, CXCL9 and CCL20 chemokine production was significantly higher compared to control cells. On the other hand, blood pDCs neither produced any of the investigated cytokines and chemokines nor expressed CD83/CD86 maturation/activation markers. Our results indicate that in psoriasis not only skin but also blood mDCs perform Th1 polarizing and Th1/Th17 recruiting capacity, while pDCs function only in the skin milieu. (Khasawneh, A. et al. Immunol Lett 189 109-113., 2017.)

Limited data are available on the connection between the severity of skin inflammation, vitamin D3 status and bone mineral density (BMD) of patients with psoriasis or with psoriatic arthritis. Our study intended to explore possible correlations between vitamin D status and BMD, as well as the features of psoriasis and psoriatic arthritis. The proportion of patients with a low BMD value did not exceed that seen in the general population. We found an inverse correlation between the serum level of vitamin 25(OH)D3 and body mass index, as well as between the former and the severity of skin involvement. Furthermore, the activity of psoriatic arthritis was significantly higher in patients with inadequate vitamin D3 status. In patients with psoriatic arthritis, BMD significantly exceeded the values measured in patients suffering from psoriatic skin lesions only. Our findings suggest the importance of evaluating the vitamin D3 status and screening for comorbid conditions in patients with psoriasis or psoriatic arthritis. (Kincse, G. et al. J Dermatol 42 (7), 679-684., 2015.)

Going further, we explored other comorbidities of severe psoriatic patients and investigated whether there are any signs of subclinical cardiovascular disease (echocardiographic abnormalities) in these patients without clinically overt heart disease. As a second objective the influence of long term treatment with TNF-α inhibitors on the ventricular functions of

psoriatic patients was also investigated. Patients with severe psoriasis exhibited signs of subclinical cardiovascular disease significantly more frequently compared to controls and prolonged anti-TNF-α therapy had a beneficial effect on these signs. (Herédi, E. et al. J Eur Acad Dermatol Venereol 30 (9), 1531-1536., 2016.)

We also elaborated our patient registers containing clinical and laboratory data of AD and psoriatic patients. During this data collection we also registered data on their diminished quality of life and two publications summarised our observations. (Herédi, E. et al. Eur J Health Econ 15 (Suppl. 1), S111-S119., 2014.; Rencz, F. et al. J Eur Acad Dermatol Venereol 29 (7), 1398-1405., 2015.)

New diagnostic test in Autoimmune Chronic Urticaria

The correct diagnosis of autoimmune urticaria (AIU) is still difficult, since the "gold standard" tests are expensive and not available for clinical use. We aimed to create a practical diagnostic test with the combination of easily available anamnestic data, skin test and immune investigations in these patients. Logistic regression model was used to decide which combination of these parameters could increase the best sensitivity and specificity. We could develop a convenient and inexpensive combined test, which could nearly reach the sensitivity and specificity of the expensive and difficult gold standard test and can be recommended for diagnosing AIU in outpatient clinical settings. (Hajdu, K. et al. B J Dermatol 177 (3), 864-866., 2017.)