Investigation of the role of skin barrier alteration as an adjuvant for the initiation of region-specific inflammatory skin diseases

The major aim of the project was to characterize how alteration of the topographically different skin immune and permeability barrier composition and function correspond to the pathogenesis of region-specific immune-mediated skin diseases.

1. Investigations on the permeability and immune barrier differences of healthy skin regions:

The chemical milieu, microbiota composition, and immune activity show prominent differences in distinct healthy skin areas. The objective of the current study was to compare the major permeability barrier components (stratum corneum and tight junction (TJ)), investigate the distribution of (corneo)desmosomes and TJs, and measure barrier function in healthy sebaceous gland-rich (SGR), apocrine gland-rich (AGR), and gland-poor (GP) skin regions. Molecules involved in cornified envelope (CE) formation, desquamation, and (corneo)desmosome and TJ organization were investigated at the mRNA and protein levels using qRT-PCR and immunohistochemistry. The distribution of junction structures was visualized using confocal microscopy. Transepidermal water loss (TEWL) functional measurements were also performed. CE intracellular structural components were similarly expressed in gland-rich (SGR and AGR) and GP areas. In contrast, significantly lower extracellular protein levels of (corneo)desmosomes (DSG1 and CDSN) and TJs (OCLN and CLDN1) were detected in SGR/AGR areas compared to GP areas. In parallel, kallikrein proteases were significantly higher in gland-rich regions. Moreover, gland-rich areas were characterized by prominently disorganized junction structures ((corneo)desmosomes and TJs) and significantly higher TEWL levels compared to GP skin, which exhibited a regular distribution of junction structures. According to our findings, the permeability barrier of our skin is not uniform. Gland-rich areas are characterized by weaker permeability barrier features compared with GP regions. These findings have important clinical relevance and may explain the preferred localization of acantholytic skin diseases on gland-rich skin regions (e.g., Pemphigus foliaceus, Darier's disease, and Hailey-Hailey disease).

Accepted publication: Regional Differences in the Permeability Barrier of the Skin-Implications in Acantholytic Skin Diseases; A Kapitány, B Medgyesi, A Jenei, O Somogyi, L Szabó, K Gáspár, G Méhes, Z Hendrik, K Dócs, P Szücs, Z Dajnoki, A Szegedi*; Int J Mol Sci. 2021 Sep 27;22(19):10428. doi: 10.3390/ijms221910428. *These authors contributed equally this work.*

Epidermal keratinocytes (KCs) play a role in innate immune responses and can directly activate DCs and lymphocytes. KCs influence immune activity through the production of "epimmunome" molecules. According to recent data, epimmunome mediators include the following cytokines: IL-1α, IL-1β, IL-6, IL-8, IL-18, IL-24, IL-25, IL-33, IL-23, IL-17C, IL-36RA, IL-38, AMPs: S100A7, S100A8, S100A9, LCN2, cathelicidin (LL-37), and hBD2, and chemokines. Therefore, we aimed to determine these epimmunome molecules in topographically distinct areas of healthy skin, namely in the sebaceous (sebaceous gland-rich [SGR]), dry (gland-poor [GP]) and moist (axillary, apocrine gland-rich [AGR]) areas. Protein expression of the molecules was assessed by immunohistochemical staining and gene expression levels were compared by RT-qPCR. A literature search was also performed for functional characterization. According to our results, the three regions showed characteristically different cytokine patterns. GP was featured by an IL-25/IL-33/IL-36RA/IL-38/IL-18 cytokine milieu, SGR was characterized by IL-23/IL-17C/IL-18, and AGR skin exhibited a mixed IL-25/IL-33/IL-23/IL-18 profile. Literature analyses revealed different homeostatic and pro-inflammatory roles (Th2-related in GP, Th17-related in SGR, and mixed Th2/Th17 in AGR). During inflammation of epidermal challenge-driven diseases, the expression of those mediators (IL-33 in atopic dermatitis (AD), IL-23 in papulopustular rosacea (PPR), which were already expressed at higher levels under homeostatic

conditions, were significantly elevated, in contrast to non-epidermal-driven skin inflammation (psoriasis). These data indicate that healthy skin regions are equipped with different KC-derived cytokine profiles, which may determine their capability of mediator production also during inflammation.

Accepted publication: Cytokine profile of the epidermis is region specific and may determine the characteristics of inflammation; L Szabó, Z Dajnoki*, O Somogyi, K Gáspár, Z Hendrik, IL Szabó, AG Szöllősi, T Dinya, D Törőcsik, A Kapitány, A Szegedi; Exp Dermatol. 2023 Apr 23. doi: 10.1111/exd.14820. Online ahead of print. doi: 10.1111/exd.14820 *These authors contributed equally this work.*

2. Investigation on rosacea, a disease specifically localized to SGR:

Rosacea is a common chronic immune-mediated inflammatory skin disease of unknown cause mainly affecting SGR skin regions, particularly the central face, nose, chin, and forehead. It is characterized by severe skin dryness, elevated pH, transepidermal water loss, and decreased hydration levels. Until now, there has been no thorough molecular analysis of permeability barrier alterations in the skin of patients with rosacea. Thus, we sought to investigate the barrier alterations in papulopustular rosacea (PPR) samples compared with healthy SGR skin, using RNA sequencing analysis (each n=8). By applying a statistical cut-off level as a minimum of 1.5-fold alteration a total of 5,136 genes were found to be significantly differentially expressed between PPR and SGR. Then, significantly differentially expressed genes were subjected to pathway analyses by Cytoscape software ClueGO application which revealed 15 significantly enriched pathways related to skin barrier formation. RT-PCR and immunohistochemistry were used to validate the results of the pathway analyses. The results showed significant alterations regarding barrier components in PPR samples compared with SGR skin, including the cornified envelope and intercellular lipid lamellae formation, desmosome and tight junction organizations, barrier alarmins, and antimicrobial peptides. In summary, besides the well-known dysregulation of immunological, vascular, and neurological functions, we demonstrated prominent permeability barrier alterations in PPR at the molecular level. Our findings highlight the importance of barrier repair therapies for rosacea and we suggest that skin barrier restoring therapies should be incorporated into clinical guidelines for rosacea management, similar to that of AD. However, to determine if PPR barrier damage is the initiator of the disease or a consequence of manifested inflammation, the exact time of barrier disruption should be studied and a detailed analysis of the perilesional and/or nonlesional skin of patients with PPR should be performed in the future. Moreover, analogous experiments are needed in other subtypes of rosacea. Moreover, in this study, we highlighted that the barrier damage in PPR seemed to be unexpectedly similar to that of AD which finding needs further in-depth investigation in the second and third year of the current project.

Accepted publication: Rosacea Is Characterized by a Profoundly Diminished Skin Barrier; B Medgyesi, Z Dajnoki*, G Béke, K Gáspár, IL Szabó, EA Janka, S Póliska, Z Hendrik, G Méhes, D Törőcsik, T Bíró, A Kapitány, A Szegedi, J Invest Dermatol. (2020) 140, 1938-1950; doi:10.1016/j.jid.2020.02.025 *These authors contributed equally to this work*

3. Investigations on atopic dermatitis (AD), a disease specifically localized to SGP:

In AD, one of the most characteristic symptoms is chronic pruritus (or itch). However, its exact molecular mechanism is poorly understood and, consequently, available therapies are relatively ineffective. In order to clarify itch-related intercellular dialog murine models of acute and chronic itch, and samples from human AD and psoriasis were assessed. According to our findings, TRPV3 may have

a crucial role in mediating itch throughout the following mechanism of action: IL-31 induces the production and secretion of brain-derived natriuretic peptide (BNP) by sensory neurons. Then, BNP binds to NPR1 on keratinocytes and upregulate TRPV3. This TRPV3 activity leads to enhanced serpin E1 secretion which can activate sensory fibers and promotes pruritus in human skin. Our findings highlighted TRPV3 as a possible therapeutic target in disorders with chronic itch.

Accepted publication: Novel insights into the TRPV3-mediated itch in atopic dermatitis Ciara Larkin, Weiwei Chen, Imre Lőrinc Szabó, Chunxu Shan, Zsolt Dajnoki, Andrea Szegedi, Timo Buhl, Yuanyuan Fan, Sandra O'Neill, Dermot Walls, Wenke Cheng, Song Xiao, Jiafu Wang, Jianghui Meng J Allergy Clin Immunol. 2020 Oct 6;S0091-6749(20)31394-4. doi:10.1016/j.jaci.2020.09.028.

In another study, we aimed to investigate permeability barrier, cutaneous and blood immune responses following allergen immunotherapy (AIT) in AD. AIT is considered a curative treatment in some atopic diseases, but in AD contradictory clinical results exist and the action of AIT has not been elucidated. In the literature, there is no evidence for parallel investigations of permeability barrier, cutaneous and blood immune responses after AIT in AD. Mild-to-moderate AD patients (n= 14) with concomitant allergic rhinitis to house dust mites were involved. All patients received topical treatment, while eight patients were randomly selected for adjuvant AIT as well. At baseline and after 6 months, clinical, barrier and immunological investigations were performed. In the adjuvant AIT group, clinical parameters and barrier functions improved significantly. Post-AIT APT became negative in all patients in the AIT group but remained positive in the non-AIT group. Cutaneous DC and T cell counts decreased significantly after allergen challenge in the AIT group. According to our findings, AIT is a beneficial adjuvant treatment for sensitized AD patients. AIT improves not only clinical symptoms, but also permeability barrier functions. The effect of AIT on sensitization should be detected by APT, not by SPT.

Accepted publication: Improvement of clinical and immunological parameters after allergen-specific immunotherapy in atopic dermatitis K Hajdu, A Kapitány, Z Dajnoki, L Soltész, S Baráth, Z Hendrik, I Veres, A Szegedi, K Gáspár, J Eur Acad Dermatol Venereol. 2021 Jun;35(6):1357-1361. doi: 10.1111/jdv.17018.

In our third study we focused on Antimicrobial peptides (AMPs) which molecules are key factors in the pathogenesis of several immune-mediated skin diseases, including Th1/Th17-driven psoriasis (PsV) and rosacea. However, there is a lack of comprehensive studies of the expression of all 5 major antimicrobial peptide functional groups in atopic dermatitis. The aim of this study was to investigate the major antimicrobial peptide functional groups in lesional atopic dermatitis, non-lesional atopic dermatitis, and healthy control samples, using real-time quantitative PCR and immunohistochemistry. Lesional psoriatic skin was also examined as a diseased control. No differences in mRNA levels were detected between non-lesional atopic dermatitis and healthy control skin, and, at the protein level, the only change was the significantly decreased LL-37 in non-lesional atopic dermatitis. In lesional atopic dermatitis, several antimicrobial peptides were significantly altered at the mRNA level, while, at the protein level, all antimicrobial peptides were significantly upregulated or unchanged, except for LL-37, which decreased, compared with healthy controls. Antimicrobial peptides were similarly elevated in lesional atopic dermatitis and lesional psoriatic skin, with somewhat higher expression in lesional psoriatic skin, except for LL-37. In conclusion, LL-37 was the only antimicrobial peptide that was impaired in both non-lesional and lesional atopic dermatitis, highlighting its potential pathogenetic or exacerbating role in the initial stages of the disease.

Accepted publication: Antimicrobial Peptide Loss, Except for LL-37, is not Characteristic of Atopic Dermatitis; L Szabó†, A Kapitány†, O Somogyi, I Alhafez, K Gáspár, R Palatka, L Soltész, D Törőcsik, Z Hendrik, Z Dajnoki# and A Szegedi# #,†These authors contributed equally this work

4. Investigation on hidradenitis suppurativa, a disease specifically localized to AGR skin region:

Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease of the AGR skin region. The initial steps of HS development are not fully understood, despite intense investigations into immune barrier alterations in lesional HS skin. In our study, we aimed to systematically investigate the inflammatory molecules involved in three stages of HS pathogenesis, including healthy AGR, non-lesional HS, and lesional HS skin, with the parallel application of multiple mRNA and protein-based methods. Immune cell counts, Th1/Th17-related molecules, keratinocyte-related sensors, mediators, and pro-inflammatory molecules were investigated in the three groups by RNASeq, RT-qPCR, immunohistochemistry, and immunofluorescence. Epidermal changes were already detectable in non-lesional HS skin; the epidermal occurrence of antimicrobial peptides (AMPs), IL-1β, TNF-α, and IL-23 was highly upregulated compared with healthy AGR skin. In the lesional HS epidermis, TNF-α and IL-1β expression remained at high levels while AMPs and IL-23 increased even more compared with non-lesional skin. In the dermis of non-lesional HS skin, signs of inflammation were barely detectable (vs. AGR), while in the lesional dermis, the number of inflammatory cells and Th1/Th17-related mediators were significantly elevated. Our findings that non-lesional HS epidermal keratinocytes produce not only AMPs and IL-1β but also high levels of TNF-α and IL-23 confirm the driver role of keratinocytes in HS pathogenesis and highlight the possible role of keratinocytes in the transformation of non-inflammatory Th17 cells (of healthy AGR skin) into inflammatory cells (of HS) via the production of these mediators. The fact that epidermal TNF-α and IL-23 appear in non-lesional HS proves these cytokines as excellent therapeutic targets.

Accepted publication: Primary alterations during the development of hidradenitis suppurativa; Z Dajnoki, O Somogyi, B Medgyesi, A Jenei, L Szabó, K Gáspár, T Dinya, EA Janka, Z Hendrik, P Gergely, D Imre, S Póliska, D Törőcsik, C C Zouboulis, E P Prens, A Kapitány, A Szegedi; J. Eur. Acad. Dermatol. Venereol. 36 (3), 462-471, 2022. doi: 10.1111/jdv.17779.

According to the current literature, keratinocytes seem to be the crucial drivers of the initial pathogenic steps. However, the possible role of permeability barrier alteration in activating keratinocytes during HS development has not been clarified. We compared the major permeability barrier elements of non-lesional HS (HS-NL; n = 10) and lesional HS (HS-L; n = 10) skin with healthy AGR regions (n = 10) via RT-qPCR and immunohistochemistry. Stratum corneum components related to cornified envelope formation, corneocyte desquamation and (corneo)desmosome organization were analyzed along with tight junction molecules and barrier alarmins. The permeability barrier function was also investigated with transepidermal water loss (TEWL) measurements (n = 16). Junction structures were also visualized using confocal microscopy. At the gene level, none of the investigated molecules were significantly altered in HS-NL skin, while 11 molecules changed significantly in HS-L skin versus control. At the protein level, the investigated molecules were similarly expressed in HS-NL and AGR skin. In HS-L skin, only slight changes were detected; however, differences did not show a unidirectional alteration, as KRT1 and KLK5 were detected in decreased levels, and KLK7, KRT6 and DSG1 in increased levels. No significant differences in TEWL or the expression of junction structures were assessed. Our findings suggest that the permeability barrier is not significantly damaged in HS skin and permeability barrier alterations are not the driver factors of keratinocyte activation in this disease.

Accepted publication: New Data on the Features of Skin Barrier in Hidradenitis Suppurativa; Skin barrier alterations are not a characteristic feature of hidradenitis suppurativa; O Somogyi, Z Dajnoki*, L Szabó, K Gáspár, Z Hendrik, CC Zouboulis, K Dócs, P Szücs, K Dull, D Töröcsik, A Kapitány, A Szegedi; Biomedicines 2023 Jan 4;11(1):127. doi: 10.3390/biomedicines11010127. *These authors contributed equally this work*

To summarize our research findings, our skin has remarkable permeability and immunological barrier differences even under homeostatic conditions. These differences not only explain the localization of certain region-specific skin disorders but also have significant preventive/therapeutic potential, as the characteristics of the region determine the type of inflammation that develops there. This may in the future provide an opportunity to develop targeted topical (even preventive) therapies in region-specific inflammatory dermatoses such as rosacea, hidradenitis suppurativa, and atopic dermatitis.