REGION SPECIFIC DIFFERENCES IN KERATINOCYTES' FUNCTIONS AND THEIR ROLE IN SKIN DISEASES

According to our previous results we could state that our skin is not equal on the body. There are topographically different regions with different immune and permeability barrier characteristics. These regions overlap with the microbiota differences on the skin surface, and parallel with that, three main distinct skin areas seem to occur: sebaceous/apocrin gland poor (SGP/AGP), sebaceous gland rich (SGR) and apocrine gland rich (AGR) regions. In the present project, we aimed to further characterize the differences of the immune and permeability barriers on distinct skin regions (SGP/AGP, SGR, AGR) focusing primarily on the keratinocytes (KCs). We also aimed to study the role of these regional barrier differences in the development of region-specific, immune-mediated skin diseases, like rosacea, hidradenitis suppurativa, atopic dermatitis and acne, and compared it with a non-region specific disease (psoriasis).

1. Investigation of the homeostatic immune and permeability skin barriers on 3 body regions

a, Earlier, we characterized the immune and barrier milieu of healthy SGR skin, and now we proceeded to analyse AGR skin. We could unambiguously show that AGR skin possesses distinct immune and barrier milieu, compared to AGP areas. All the characteristic adaptive and innate immune features of AGR skin were also presented in HS in robust and widespread forms, accompanied by the occurrence of activation and inflammation markers.

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b, We compared the permeability barrier components and measured their function in SGR, AGR, and GP skin regions. According to our findings, the permeability barrier of our skin is not uniform, similarly to the immune and microbiota barriers. 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.

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c, We developed a novel approach for the examination of AMPs in the outermost layer of the epidermis, namely stratum corneum (SC). The SC sample collection by tape stripping was

coupled with detection by highly specific and sensitive parallel reaction monitoring (PRM)-based mass spectrometry. Significant topographical differences were described among GP, SGR and AGR healthy skin regions. We applied a minimally invasive, reproducible approach for sampling, which can be assessed for research and diagnostic purposes and for monitoring the effectiveness of therapies in skin diseases.

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d, We also investigated the „alarmin" type of cytokines produced by KCs of the 3 main skin regions. 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. During inflammation of epidermal challenge-driven diseases (rosacea and atopic dermatitis), the expression of those mediators (IL-33 in AD, IL-23 in PPR), which were already expressed at higher levels under homeostatic conditions, were significantly elevated, in contrast to non-epidermal-driven skin inflammation (psoriasis).

Accepted publication: Szabó L et al., Experimental Dermatology, 2023. Apr 23. doi: 10.1111/exd.14820.

e, In order to collect information on the characteristics of SGR skin, functional investigations on SZ95 sebocytes were also conducted. As EGF receptor (EGFR) is expressed throughout the proliferating and the lipid-producing layers of sebaceous glands (SGs) in healthy skin, we investigated the effect of EGF on SZ95 sebocytes and how it may alter the changes induced by palmitic acid (PA), a major sebum component with bioactive roles. Altogether, our results reveal that PA-induced lipid accumulation and inflammation can be modulated by EGF in sebocytes, which also highlights the need for system biological approaches to better understand sebaceous (immuno)biology.

Accepted publication: Törőcsik D et al., Frontiers Immunology, 2021.May 6;12:600017, doi:10.3389/fimmu.2021.600017

f, In another study we investigated and identified miR-146a to have the highest induction in the TLR1/2 and 4 activated SZ95 sebocytes and found that its increased levels led to the down-regulation of IL-8 secretion, decreased the chemoattractant potential and stimulated the proliferation of sebocytes. Our findings suggest, that miR-146a could be a potential player in acne pathogenesis by regulating inflammation, inducing proliferation and, through the indirect down-regulation of GNG7, promoting the lipid production of sebocytes.

Accepted publication: Dull K et al., Scientific Reports, 2021. 11(1):21510. doi:10.1038/s41598-021-00907-1

2. Investigation of immune-mediated diseases of the SGR skin region (acne, rosacea)

a, In light of our and others new immunological and dermatological data, we proposed a new concept for acne vulgaris pathogenesis, which might facilitate a better understanding of this disease leading to improved treatments. We proposed that acne vulgaris represented naturally developing, transient inflammatory interaction of adolescent facial skin with its new adolescence microbial/chemical milieu (*Cutibacterium acnes*, sebum), replacing a state of previous childhood skin homeostasis. This concept might explain why acne is characterized by strong regional and age specificity, prevalent occurrence and resolution.

Accepted publication: Szegedi A. et al.,Trends in Immunology, 2019. Oct; 40(10):873-876. doi: 10.1016/j.it.2019.08.006.

b, Until now, there has been no thorough molecular analysis of permeability barrier alterations in rosacea. Thus, we aimed to investigate the barrier alterations in rosacea samples compared with healthy SGR skin. The results showed significant alterations in barrier components in rosacea samples compared with SGR skin, including the cornified envelope and intercellular lipid lamellae formation, desmosome and tight junction organizations, barrier alarmins, and antimicrobial peptides. The cause and effect connection between the immune barrier activation and permeability barrier damage needs further evaluations in order to develop even disease preventive therapies.

Accepted publication: Medgyesi et al., Journal of Investigative Dermatology, 2020. 140: 1938-1950; doi:10.1016/j.jid.2020.02.025

3. Investigation of immune-mediated diseases of the AGR skin region (hidradenitis suppurativa - HS)

a, I was co-author of a review article summarizing the main knowledge about the pathogenesis, clinical features and therapy of hidradenitis suppurativa (HS). This anniversary article of 43 research-performing authors from all around the globe summarized the evidence of the intense HS clinical and experimental research during the last 15 years in all aspects of the disease and provides information of the developments to come in the near future.

Accepted publication: Zouboulis CC et al., Experimental Dermatology,. 2020. Dec;29(12):1154-1170. doi: 10.1111/exd.14214

b, We aimed to systematically investigate the inflammatory molecules involved in three clinical stages of HS development, including healthy AGR, non-lesional HS and lesional HS

skin. Our findings confirmed the driver role of keratinocytes in HS pathogenesis and highlighted 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 several mediators.

Accepted publication: Dajnoki zs et al., Journal of the European Academy of Dermatology and Venereology, 2022. Mar;36(3):462-471. doi:10.1111/jdv.17779

c, In another investigation a multicentre, cross-sectional cost-of-illness study was performed among 200 adult HS patients. We evaluated direct medical (physician consultations, inpatient admissions, medical, and surgeries), direct non-medical (transportation and caregiving), and indirect costs (productivity loss). This was the first study to assess both direct and indirect costs in HS patients. HS imposes a substantial burden on patients and society, predominantly arising from productivity loss and biological therapy. Resource utilization data and cost-of-illness estimates provide valuable inputs into cost-effectiveness analyses of health interventions in HS.

Accepted publication: Gáspár K et al., Expert Review of Pharmacoeconomics & Outcomes Research, 2022. Apr;22(3):399-408. doi:10.1080/14737167.2021.1895753

d, The possible role of permeability barrier alterations in activating keratinocytes during HS development have 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. No significant functional differences 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: Somogyi O et al., Biomedicines, 2023. 11: 127. doi:10.3390/biomedicines 11010127

e, In another investigation we performed culture-based analyses of skin bacteria in lesional moist, and unaffected dry and sebaceous skin regions of hidradenitis suppurativa patients. We enrolled 11 HS patients (mean age: 32.55 years; eight male/three female), and 14 age- and sex-matched healthy controls (mean age: 33.21 years; 11 male/three female) in this study. We took swabs of lesions, the dry region extensor elbow and the sebaceous retroauricular areas of study subjects. Genus-level composition of samples showed clear differences between HS lesions and the corresponding moist regions of healthy controls, but the non-lesional extensor elbow and retroauricular regions of HS patients were also different from the same regions of

controls. These findings suggest that skin microbial alterations in HS may have generic aspects that may not be restricted to lesions, nor apocrine gland-rich, moist areas.

Accepted publication: Antal D al., Journal of the European Academy of Dermatology and Venereology, 2022. Sep;36(9):e731-e733. doi: 10.1111/jdv.18254

f, In our next study we investigated the occurence of chronic inflammatory intestinal disorders (CIID) in HS. A high prevalence of CIID was detected in the examined HS population. The non-invasive fetal calprotectin test had high sensitivity and specificity for diagnosing CIID in HS patients. Concomitant CIID and HS may indicate the need for an early biological treatment start.

Accepted publication: Palatka R et al., Dermatology, 2023. Apr 5. doi: 10.1159/000530434

4. Investigation of immune-mediated disease of the GP skin region (atopic dermatitis)

a, The most prominent and debilitating symptom of atopic dermatitis is chronic pruritus (itch). We have aimed to address the gap in knowledge by specifically focusing on clinically relevant intercellular communication in human skin cells, murine models of acute and chronic itch, and samples from human atopic dermatitis and psoriasis. Our recent study provided a clear link between TRPV3 and dermatitis. In atopic dermatitis conditions, IL-31 induces BNP synthesis and release from sensory neurons. On the basis of these results, we believe that targeting TRPV3 signalling will prove beneficial for the treatment of chronic itch conditions.

Accepted publication: Larkin C et al., Journal of Allergy and Clinical Immunology, 2021. 147(3):1110-1114.e5. doi:10.1016/j.jaci.2020.09.028

b, In another study we investigated immune parameters in the blood and skin and detected clinical, and barrier changes after allergen specific immunotherapy (AIT) in atopic dermatitis (AD). AIT proved to be a beneficial adjuvant treatment for sensitized AD patients. AIT improved not only clinical symptoms, but also permeability barrier functions. The effect of AIT on sensitization should be detected by Atopy Patch test, not by Skin Prick Test.

Accepted publication: Hajdú K et al., Journal of the European Academy of Dermatology and Venereology, 2021. Jun;35(6):1357-1361. doi:10.1111/jdv.17018

c, We aimed to determine the relationship between plasma 25-hydroxy-vitamin D3 ($25(OH)D_3$) levels as well as the levels of the ligand of the vitamin D receptor (VDR) heterodimerization partner, the retinoid X receptor (RXR) and the active vitamin A5 derivate 9-*cis*-13,14-dihydroretinoic acid (9CDHRA) and AD severity. In consequence, the metabolic activation of vitamin D from $25(OH)D_3$ towards $1,25(OH)_2D_3$ as well as the co-liganding of

the RXR by 9CDHRA may be an important signaling mechanism, an important marker for AD development and severity as well as the basis for novel nutritional and pharmaceutical AD-treatment options. The novel RXR-ligand, besides the well-known VDR-ligand, positively correlates with atopic dermatitis severity markers.

Accepted publication: Lucas R et al., Dermatology, 2022. 238(6):1076-1083. doi: 10.1159/000524343

d, In another study TRPV3 (transient receptor potential vanilloid 3) expression was investigated in AD. We provided the first evidence that the expression of TRPV3 is markedly upregulated in non-lesional human AD epidermis, similar to lesional AD samples. Of further importance, by using the patch-clamp method on cultured healthy and non-lesional AD keratinocytes, we also show that this upregulation is functional as determined by the significantly augmented TRPV3-specific ion current (induced by agonists) on cultured non-lesional AD keratinocytes when compared to healthy ones.

Accepted publication: Vasas N et al., Experimental Dermatology, 2022. May;31(5):807-813. doi: 10.1111/exd.14530

5. Investigation of immune-mediated disease without region specificity (psoriasis)

a, In our preliminary studies we could detect differences in the PARP1 and TRPV1 expression between the 3 main skin regions. Therefore we take part in an experiment in which these molecules were studied in a psoriasis model. We showed that PARP1 expression is reduced in human psoriatic lesions compared with control skin samples. In imiquimod-treated HPV-KER keratinocytes, PARP inhibition recapitulated the in vivo findings, namely keratinocyte hyperproliferation; furthermore, the mRNA expression of psoriasis-associated cytokines (IL6, IL1β, IL8, IL17 and IL23A) was also induced. The inhibition of TRPV1 abrogated the effects of the combined imiquimod + PARP inhibitor treatment.

Accepted publication: Kiss B. et al., Experimental Dermatology, 2019. Nov 22. doi: 10.1111/exd.14061.

b, In the next study we wanted to prove, that regional immune differences of the skin have influence on the development of region specific skin diseases, but not on the development of non region specific ones, like psoriasis. We detected the immune milieu of psoriasis on different skin regions. Our findings supported that, in spite of the significant differences between healthy SGR and SGP skin immune milieu, psoriatic plaques developing on these distinct areas bear similar cellular, molecular, and barrier characteristics.

Accepted publication: Gáspár K et al., Acta Dermato Venereologica,. 2020. Jul 2;100(14):adv00203. doi:10.2340/00015555-3553.

c, To investigate the association of psoriasis with established obesity-related gene variants, we conducted a population-based case-control study including 3541 subjects (574 psoriasis cases and 2967 controls from the general Hungarian population). Our results suggested that in psoriatic patients, there are prominent differences in the causes of obesity that should be accounted for, including not only environmental factors but also patient characteristics, such as the time of disease onset as well as genetic factors.

Accepted publication: Szentkereszty-Kovács Z et al., Life (Basel), 2021. 11(10):1086. doi:10.3390/life11101086

In summary of our present project, we proved that not only the microbiota and chemical mantle of human skin show three different topographical regions, but parallel, the immune and permeability barrier of these areas are also distinct, which can make these skin regions prone to the development of "region-specific" inflammatory skin diseases, like HS on AGR, acne and rosacea on SGR and AD on GP areas.