SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Investigation of the skin immune system in health and inflammation

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The Examination took place at meeting room of Bldg. C, Department of Internal Medicine, Faculty of Medicine, University of Debrecen
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The online PhD Defense will be held at 2 p.m., 19th of October, 2020.

Live online access will be provided. If you wish to take part in the discussion, please send an e-mail to buzas@med.unideb.hu not later than 12 p.m. on the day before the discussion (18th of October, 2020). After the deadline, for technical reasons, it is no longer possible to join in to the defense.

INTRODUCTION

The skin is one of the largest organs in the human body and it is exposed to continuous physical, chemical and microbiological environmental stimuli. It performs a number of tasks to maintain its own and the body's homeostasis. Besides its static physicochemical barrier function – also known as permeability barrier (physical isolation from the environment, inhibition of penetration of pathogens, providing adequate hydration and pH, thermoregulation, and light protection) – the skin participates in maintaining a number of dynamic processes (tissue regeneration, transport processes, exocrine and neuroendocrine functions, synthesis of certain vitamins and hormones). The different types of cells that make up the skin, as well as the soluble mediator molecules they produce, form the skin's own immune system, which actively protects the deeper tissues of the human body (immunological barrier). The immunological barrier function of the skin is mediated by classical immune cells [dendritic cells (DCs), T cells and innate immune cells such as macrophages (MPs), granulocytes, etc.] and organ-specific cells such as keratinocytes (KCs), fibroblasts, glandular epithelial cells, etc. Together, they form a complex, well-coordinated system, thereby contributing to the maintenance of immunohomeostasis.

In my PhD work, among the several functions of the skin listed above, I investigated in details the cutaneous immunological processes in healthy and pathological conditions.

OBJECTIVES

Question No. 1: Is the immune system of the skin homogeneous in topographically different skin areas?

Research in the recent years has shown that the microbiota exhibits characteristic diversity in topographically distinct skin areas, i.e. it is not uniform across individual skin regions. For example, in oily (sebaceous gland-rich skin, e.g. face) skin areas, *Propionibacteria* and *Staphylococci* species; in wet (apocrine gland-rich skin, e.g. groin) areas, *Corynebacteria* and *Staphylococci* species are present, whereas in dry (gland-poor skin, e.g. legs) are characterized by the presence of certain species of *Actinobacteria*, *Proteobacteria* and *Bacteroides*. However, studies to date have not been conducted as to whether regional differences in the skin shown by

the microbiota and its environment are followed by the immunotopographic variability of the skin.

Therefore, several years ago, our group started an extensive series of studies aimed at assessing the Skin Immune System (SIS) in various skin regions under healthy conditions and in certain skin diseases (e.g. atopic dermatitis [AD], rosacea, hidradenitis suppurativa). As part of these experiments, in my PhD work, I performed a comparative analysis of the immune milieu of healthy skin regions that are poor (sebaceous gland poor, SGP) and rich in sebaceous glands (sebaceous gland rich, SGR).

Question No. 2: What are the characteristics of circulating DCs in AD?

In addition to T cells, DCs are considered to be the major participants in the pathogenesis of AD since they contribute to the development of AD-specific inflammation due to their T cell polarizing ability and their direct production of cytokines and chemokines. It has recently been suggested that peripheral blood, so-called pre-DCs, may be the precursors of not only healthy dermal DCs but also of inflammatory DCs of the skin. However, little information is available on whether DCs in the blood are involved in shaping the disease-specific cytokine-chemokine profile in AD patients.

In our previous study, we showed that there is a difference between the T-cell polarizing cytokine production in AD and healthy control blood pre-DCs. Continuing these series of experiments, in the second part of my PhD work, we performed a detailed phenotypic characterization of the cells, with particular emphasis on their activation and maturation phases as well as their chemokine production. In addition, we also examined whether pre-DCs in AD blood differ from de novo myeloid pre-DCs in healthy individuals and in those stimulated with *Staphylococcus* enterotoxin B (SEB) and TSLP (thereby imitating the AD microenvironment).

MATERIALS AND METHODS

Collection of samples and sample preparation

Skin Biopsies

Skin punch biopsies (0.5-1 cm^2) were taken from normal skin of 20 healthy individuals (10 from SGP and 10 from SGR skin sites) undergoing plastic surgery after obtaining written, informed consent, according to the Declaration of Helsinki principles. The study was approved by the local Ethics Committee of University of Debrecen, Hungary. All biopsies were cut into 2 pieces. For immunohistochemistry (IHC), half of the samples were embedded in paraffin after formalin fixation whereas the remaining parts were placed in RNAlater and stored at -70°C until RNA isolation. After Hematoxylin-Eosin staining, samples were sorted according to the number of sebaceous glands and were defined as SGP skin when containing $n \leq 1$ sebaceous gland and as SGR skin when containing $n \geq 3$ sebaceous glands in the field of view on 10x magnification in the microscope.

Blood samples

Peripheral blood was obtained from patients with chronic (severe) AD (n=12, 8 males, 4 females, age: 25.5±7.5 years); in addition, buffy coat (n=10) was used for the healthy controls. All patients fulfilled the diagnostic criteria established by Hanifin and Rajka. Patients who took part in this study had severe skin symptoms and had not been treated with oral glucocorticosteroids or other systemic immunomodulatory agents for at least 4 weeks or with antihistamines or topical corticosteroids for at least 5 days prior to blood sampling. Disease activities were determined by the severity scoring of atopic dermatitis (SCORAD index: 49.3±11.1); moreover, total serum IgE levels (2.598±1.402 kU/L) were also determined. In all patients, Hyper IgE Syndrome (HIES) was excluded according to the HIES clinical scoring system. Informed consent was obtained from all participants according to the Declaration of Helsinki principles. The local Ethics Committee of the University of Debrecen, Hungary, approved the study.

RNA isolation, Reverse Transcription and QRT-PCR

After removal of subcutaneous adipose tissue, all samples were homogenized with Tissue Lyser and total RNA was isolated from the human skin tissues. The concentrations and purities of the RNA samples were measured by means of a NanoDrop spectrophotometer, and its quality was checked using an Agilent 2100 Bioanalyzer. In the reverse transcription, 1µg of total RNA were

reverse transcribed into complementary DNA (cDNA) using the High Capacity cDNA Archive Kit. Previously samples were treated with DNase I enzyme. QRT-PCR measurements were carried out in triplicate using pre-designed FAM-MGB assays as well as TaqMan® Gene Expression Master Mix. All reactions were performed with a LightCycler® 480 System. Relative mRNA levels of target genes were calculated using the comparative ΔΔCT methods normalized to the expression of PPIA mRNA.

RNA sequencing and analysis

cDNA library for RNASeq was generated from 1μg total RNA using TruSeq RNA Sample Preparation Kit according to the manufacturer's protocol. Fragment size distribution and molarity of libraries were checked on an Agilent Bioanalyzer DNA1000 chip. Concentrations of RNASeq libraries were set to 10nM and 5 libraries were pooled together before sequencing. A single read 50bp sequencing run was performed on an Illumina HiScan SQ instrument and 16-18 million reads per sample were obtained. The CASAVA software was used for pass filtering and demultiplexing process. Sequenced reads were aligned to Human Genome v19 using TopHat and Cufflinks algorithms and bam files were generated. The StrandNGS software was used for further statistical analysis. Bam files were imported and normalized using DESeq algorithm. To identify statistically significant gene expression patterns between the different conditions, the non-parametric Wilcoxon Mann-Whitney test was used. Library preparations, sequencing and data analysis were performed at the Genomic Medicine and Bioinformatics Core Facility of University of Debrecen. RNASeq data have been deposited to Sequence Read Archive (SRA) database (https://www.ncbi.nlm.nih.gov/sra) under accession number SRP126212.

Pathway analyses

To map associated genes to their respective pathways, a complex interactive pathway analysis was performed using the default analysis parameters of the Ingenuity Pathway Analysis (IPA) software web-based application. Our input gene list contained those genes which showed significantly different expressions between the SGR and SGP groups. The goal of the analysis was to predict overrepresented pathways (significantly enriched terms), gene networks and upstream regulators (transcription factors, cytokines, chemokines) thereby helping to characterize the functional and molecular differences between the two types of skin regions. The gene list was imported directly from StrandNGS software into the IPA to perform IPA Core Analysis.

A focused enrichment analysis was also performed on immune system-related genes revealed by IPA and also those molecules which have been detected to be significantly differentially expressed by QRT-PCR or immunohistochemistry in our present and previous study. For this analysis, the ClueGo (v. 2.3.5) and CluePedia (v. 1.3.5) applications of the Cytoscape (www.cytoscape.org) software (v. 3.5.1) were employed which use Gene Ontology (GO) Biological Process (BP), GO Immune System Process (ISP), GO Molecular Function (MF), Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome Pathways databases. In our strict analysis, only statistically significantly ($p < 0.05$) enriched pathways were visualized with an additional criterion that enriched terms should have contained at least 9 genes from our input gene list.

Immunohistochemistry (IHC) and routine staining

For the IHC experiments, paraffin-embedded sections from skin tissues were deparaffinized. Heat-induced antigen retrieval was performed and sections were pre-processed with H_2O_2 for 10 minutes. Sections were stained with primary anti-human antibodies. Subsequently, anti-mouse/rabbit HRP-conjugated secondary antibodies were employed. Staining was detected with the Vector® VIP and ImmPACT™ NovaRED™ Kit. The detection of the given protein was carried out on all sections in parallel at the same time to enable us to evaluate comparable protein levels. Positive, Ig and isotype controls were also used to normalize staining against all proteins. Skin specimens were also stained with HE. Visual scoring of sebaceous glands' count was performed by a professional pathologist.

Whole-slide imaging

The slides were digitalized using a Pannoramic SCAN digital slide scanner with a Zeiss plan-apochromatic objective and Hitachi 3CCD progressive scan color camera. Immunostainings were analyzed with the Pannoramic Viewer software 1.15.2, using the HistoQuant and NuclearQuant applications. Regions of interest (ROIs) (n=20/slide) were selected and then the Field Area [FA (mm^2)] and the Mask Area [MA (mm^2)] were measured by the software. The FA shows the whole area of the ROI and the MA represents the positive area. The MA/FA values were counted for all ROIs. The expression of each protein was determined by 2 independent observers by using Pannoramic Viewer software.

Cell isolation and culturing

Peripheral Blood Mononuclear Cells (PBMCs) were obtained by Ficoll-Paque Plus gradient centrifugation from the peripheral blood of both AD patients and healthy controls. $CD1c^+$ myeloid DCs were isolated from PBMCs using the $CD1c^+$ ($BDCA1^+$) Dendritic Cell Isolation Kit according to the instructions of the manufacturer. Purified DCs were cultured in well-defined medium for 48 hours, in the presence (stimulated) or in the absence (unstimulated) of 30ng/ml TSLP and 100ng/ml SEB. DCs were cultured in 96-well plates and supernatants were collected for chemokine examinations.

Phenotypic characterization of DCs

After incubation, cells were harvested, resuspended in FACS buffer (PBS containing 1% bovine serum albumin) and aliquotted into four wells of a 96-well plate (one for the negative control, one for the isotype control, and two for the staining). Cells were stained with the following dye-labeled cell surface marker antibodies: APC-Cy7 anti-human CD1c and PerCP-Cy5.5 anti-human CD11c to identify $CD1c^+$/$CD11c^+$ cells; APC anti-human CD1a and APC anti-human CD207 to exclude LCs; and PE anti-human FcεRI, APC-Cy7 anti-human CD206, PerCP-Cy5.5 anti-human CD83 and PE anti-human CD86 to examine their characteristics, maturation and activation state. PE mouse IgG2b, κ isotype control, APC mouse IgG1, κ isotype control, PerCP/Cy5.5 mouse IgG1, κ isotype control, APC/Cy7 mouse IgG1, and κ isotype control were used as isotype controls.

Flow cytometry

Flow cytometry measurements were carried out on fixed cells in 96-well plates on a BD FACS Array instrument. Forward (FSC) and side light scattering (SSC) was used to detect cellular objects and to exclude debris and clustered cells. The given fluorescent signals were detected at the appropriate wavelength after corresponding excitation and filtration. Data was exported and evaluated using FCS Express 4 Flow Cytometry software.

Chemokine Array

Chemokine production was measured using the Proteome Profiler Human Chemokine Array Kit from the cell culture supernatant of the previously isolated unstimulated cells. The assay was carried out according to the instructions of the manufacturer.

ELISArray and conventional ELISAs

The production of CCL3, CCL4, CCL5, CXCL10, CCL17 and CCL22 chemokines were detected simultaneously from DC supernatants of 5 atopic and 5 control samples using the Human Common Chemokines Multi-Analyte ELISArray kit according to the manufacturer's guideline. The exact amount of the AD related CCL17, CCL18 and CCL22 chemokines was also analyzed using the CCL17/TARC, CCL18/PARC and CCL22/MDC Quantikine ELISA Kits, both according to the manufacturer's instructions. In the case of the latter, conventional ELISAs the supernatants of both unstimulated and stimulated cells were investigated.

Statistical analyses

For experiments on skin biopsies

Statistical analyses were performed using the GraphPad Prism software version 6. Statistical comparisons of two groups were done using the unpaired t-test. Differences between the groups were demonstrated using mean ± standard error of the mean. P-values <0.05 were considered statistically significant ($*p < 0.05$; $**p < 0.01$; $***p < 0.001$).

For experiments on blood samples

The SPSS ver. 18.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. To assess the distribution of the data, the Kolmogorov–Smirnov test was used. Because of their normal distribution, we determined mean with corresponding 95% confidence interval or mean ± standard deviation (SD) values and used the independent t-test for statistical comparison of the experimental data. To analyze the homogeneity of variance of these data, F test was used. Differences were considered to be significant when $p<0.05$.

RESULTS

I. Immunotopographical characterization of different normal skin regions

I.1. RNA Sequencing and IPA analyses reveal prominent differences between SGR and SGP skin regions

I.1.1. RNA Sequencing

In order to explore the in-depth differences between SGR and SGP skin, RNASeq analysis was performed on whole skin biopsy lysates of 6 SGR and 7 SGP patients. The StrandNGS software was then applied to create the heatmap and the principal component analysis (PCA) of the RNASeq data. Based on the heatmap and PCA generated by the software, the gene expression patterns of the two regions clearly distinguished from each other. It is also important to emphasize that the heatmap and the PCA also indicated that the gene expression profiles of the samples belonging to the given (SGP or SGR) group were similar irrespective of the origin of the specimen in a certain skin region.

The Mann-Whitney non-parametric statistical test ($p < 0.05$) was then performed to determine differential gene expression profiles of SGR and SGP samples. With this analysis, 1083 genes were found to be significantly differentially expressed in SGR compared with SGP skin; out of these, 672 genes showed higher, whereas 411 genes exhibited lower expressions in the SGR tissues

I.1.2. Ingenuity Pathway Analysis

Using the Ingenuity Pathway Analysis (IPA) software, the above 1083 genes were then subjected to two different types of functional, standard, non-restricted pathway analyses. First, we performed a non-restricted ***Canonical Pathway Analysis***, which revealed 40 significantly enriched terms (i.e. differentially appearing terms in the SGR samples when compared to the SGP regions). Of these 40 canonical pathways, the first 14 in the significance ranking list were all related to lipid metabolism (such as LXR/RXR Activation, FXR/RXR Activation, Stearate Biosynthesis I, etc.). This was not surprising at all since the *per definition* anatomical differences (presence or lack of sebaceous glands) of the two skin regions predisposed these results. The first (i.e. the most significant) pathway which explicitly related to the skin immune system was the IL-17 related one. Besides IL-17 signaling, the only pathway which could be partially connected to skin immune functions was “LPS/IL-1 mediated inhibition of RXR function”.

As a next step, a ***Regulatory IPA Analysis*** was applied. This revealed 8 signaling networks in which both upstream regulators and downstream cellular responses were identified in relation to certain gene panels. Three of these networks were linked to immune signaling processes and pathways, which also contained IL-17 related molecules, like CCL2, S100A8 and S100A9; all the other 5 pathways were somehow related to lipid metabolism.

I.1.3. Further analysis and validation strategies

Both the 2 pathway analyses presented above and our previous results highlighted that marked differences indeed exist in the expressions of innate and adaptive immune and also permeability barrier molecules between SGP and SGR region. These results encouraged us to validate the expression patterns identified by RNASeq by another method (i.e. by QRT-PCR), and by selecting multiple genes, on an extended number of samples (SGP: n=10, SGR: n=10). Since we were interested in defining whether the results (tendency and level of changes in the expressions of selected genes between the two regions) of the two mRNA based methods (RNASeq and QRT-PCR) were similar, mRNA expression levels of genes detected by QRT-PCR were compared to that of our previous RNASeq data set. Finally, to verify the differential expressions in the two skin regions also at the protein level, certain molecules were subjected to IHC and image analyses.

I.2. Prominent differences can be identified in the innate immune responses of the SGR and SGP skin regions

I.2.1. Expressions of antimicrobial peptides are significantly higher in SGR skin

First, we measured the expressions of antimicrobial peptides (AMPs) which exert antimicrobial activity and function as alarmin molecules. By employing QRT-PCR, gene expression levels of S100A7 (psoriasin), S100A8, S100A9, human β-defensin-2 [hBD-2 (DEFB4B)] and lipocalin (LCN2) were high and significantly increased in SGR skin, whereas these molecules only weakly expressed in SGP skin. Using RNASeq, expressions of all AMPs were elevated in SGR skin; the increases of S100A8 and S100A9 were found to be significant. Expressions of CAMP were very low both in SGP and SGR samples with a slight tendency of increase in SGR skin.

In the cases of S100A8 and LCN2, immunostaining was also performed and revealed significantly higher protein levels in SGR samples for both AMPs. LCN2 could not be detected

in SGP samples; however, in SGR skin, the apical layer of the epidermis and sebocytes showed slight positivity and its strongest expression was found in follicular KCs. Immunostaining of S100A8 also revealed prominent differences. This protein was detected at low levels in SGP skin; however, S100A8 was present at high levels in the upper layers of epidermal KCs, in follicular KCs and in sebocytes of SGR skin.

I.2.2. Expressions of chemokines are significantly higher in SGR skin

Then, the levels of certain chemokines (CCL2, CCL3, CCL19, CCL20, CCL23 and CCL24), produced by innate immune cells (KCs, DCs, macrophages), was determined. In SGP skin, by using QRT-PCR, expressions of CCL2, CCL19 and CCL20 were well detectable but levels of CCL3, CCL23 and CCL24 were very low. Of great importance, QRT-PCR revealed significantly higher levels of all investigated chemokines in SGR skin. Likewise, prominently – and in the cases of CCL2, CCL3, CCL19 and CCL23, significantly – higher expressions of these molecules in SGR skin was also verified by RNASeq. We also investigated the expressions of CCL2 and CCL20 at the protein level by IHC. CCL2 was highly expressed in the sebaceous glands in SGR skin. Although the epidermal CCL2 positivity was weak in both skin types, image analysis revealed a significantly higher expression in the SGR region.

I.2.3. No significantly different expression patterns between SGR and SGP skin regions are detected regarding innate immune system receptors and pro-inflammatory cytokines

RNASeq analysis revealed similar gene expression levels of the investigated receptors (TLR2, TLR3, TLR4 and NLRP3) in the two sample groups. The only exception was TLR3 which showed significantly increased expression in SGR skin. QRT-PCR measurements also provided similar data; i.e. there was no significant difference between the two skin areas in the expressions of the receptors.

In the next step, mRNA transcript levels of pro-inflammatory cytokines [namely IL-1α, IL-1β, IL-6, IL-8, IL-33 and tumor necrosis factor alpha (TNF-α)] were compared. According to RNASeq, the two skin regions were characterized by similar levels of expressions. Likewise, QRT-PCR revealed no significant differences between their mRNA levels, except for the significantly higher expression of IL-1β in SGR samples.

I.3. Expressions of barrier molecules are different in SGR and SGP skin regions

We were also interested in uncovering the potential differences in expressions of key molecules involved in the formation and maintenance of the epidermal barrier. By QRT-PCR, expressions of loricrin (LOR), late cornified envelope 1F (LCE1F), claudin 16 (CLDN16) and filaggrin (FLG) showed decreased expression in the SGR skin, whilst KRT17 and KRT79 expressed at higher levels in the SGR samples (in the cases of KRT17 and KRT79, the difference was statistically significant between the two skin areas). It should be emphasized that the different patterns were also confirmed by RNASeq. KRT17, LOR and FLG were also investigated at the protein level by IHC. Regarding FLG and LOR, no significant differences were identified between SGP and SGR regions, although the expression of LOR showed a tendency of decrease in SGR skin. Both proteins could be detected continuously with strong positivity in the granular layers of the epidermis and under the stratum corneum. KRT17 was present at significantly higher levels in SGR skin compared to SGP. In SGP skin, KRT17-specific immunoreactivity was present in the upper layers of the epidermis; in SGR skin samples, its expression was detected in the whole epidermis with the strong positivity in the upper layers.

I.4. The SGR skin region is characterized by a T_h17/IL-17 dominance

I.4.1. Expressions of T_h1, T_h2 and T_h22-related molecules are very low in the two skin regions

As a next step, we compared different T-cell subsets in SGR and SGP skin samples by investigating the expression of their signature and maturation cytokines, as well as their transcription factors. Gene expressions of molecules characteristic to T_h1 (IL-12B, TBX21, IFN-γ, TNF-α), T_h2 (IL-13, GATA3) and T_h22 (AHR, IL-22) cells were not different in the two skin regions. By QRT-PCR analysis, IL-12B and IL-22 were undetectable in either area, whereas expression of aryl hydrocarbon receptor (AHR) was significantly higher in SGR skin. Immunostaining of AHR also showed significantly higher protein levels in SGR samples. Namely, AHR was mainly expressed by KCs in their nucleus, but cytoplasmic staining in the epidermis was also detectable; a few cells in the dermis were also found positive for AHR.

I.4.2. T_h17-related molecules exhibit higher expressions in SGR skin

Although RNASeq data alone did not reveal significant differences in the expressions of T_h17-related molecules [IL-1β, IL-6, RORC, IL-23A, IL-17A, CCL20, IL-10 and transforming growth factor beta (TGF-β)], the in-depth bioinformatics pathway analyses (IPA), as shown above, have indeed identified the T_h17 pathway as a significantly enriched term. Therefore, we further assessed the expressions of these T_h17-cell coupled molecules by QRT-PCR. In perfect

agreement with our previous findings, a significantly higher expression of IL-17A was detected in SGR skin (in the cases of RORC and IL-10, the difference was not significant). In addition, markedly and significantly ($p<0.05$) elevated levels of IL-1β, IL-23A and CCL20 as well as a tendency of higher expression for IL-6 were detected in SGR compared to SGP skin.

I.5. A focused pathway analysis revealed the central role of IL-17 pathway in SGR skin

As a final step, we performed another in-depth bioinformatics pathway analysis with those immune system-related molecules which had exhibited significantly different expression either at the gene (by RNA Seq or QRT-qPCR) or at the protein level (by IHC) in our present and previous studies. By using a focused pathway analysis, the result of IPA could be confirmed; indeed, the IL-17 signaling pathway was found to be one of the most significantly enriched terms. Besides this pathway, multiple genes exhibiting roles in cytokine activity, cytokine-cytokine receptor activity, positive regulation of response to external stimulus, leukocyte chemotaxis, etc. were also identified among the significantly enriched pathways. Taken together, these data suggest that characteristic differences in activities/levels of the skin immune system, which is the first line defense of the human body, could be identified between SGR and SGP skin regions.

II. Phenotypic and functional characterization of dendritic cells isolated from peripheral blood of patients with AD

II.1. Blood pre-DCs from AD patients express significantly more FcεRI and show tendentious alterations of their maturation markers

In our experiments, we used peripheral blood samples from 12 patients with chronic (severe) AD and 10 healthy controls. After PBMCs isolation, CD1c+ DCs were separated and then subjected to analysis. To determine the phenotypes of the $CD1c^+$ pre-DCs separated from the blood and to exclude the non-relevant FACS-detected events, $CD1c^+$ /$CD11c^+$ pre-DCs were gated. More than 90% of the gated cells were positive for both the CD1c and CD11c markers; however, these cells did not express either CD207-et (Langerin, a LC marker) or CD1a.

When assessing the presence of the FcεRI, a significantly higher expression of this receptor was detected on the surface of AD pre-DCs compared to healthy pre-DCs; the difference was significant in the case of stimulated cells with SEB/TSLP. Examination of the surface markers reflecting the activation and maturation state of the cells showed the altered presence of these molecules on DCs derived from AD patients. The CD206 mannose receptor, which is known to be expressed on less matured cells, appeared on the surface of stimulated or unstimulated AD pre-DCs less frequently than on the surface of healthy pre-DCs. The CD83 and CD86 markers exhibited an opposite expression pattern as they were expressed more frequently on AD patient-derived cells (either stimulated or unstimulated) than the control cells. Although none of the 3 examined surface markers showed significantly altered expression on AD DCs, their consistent and unidirectional changes suggest that most of the AD DCs are in a pre-matured state.

II.2. *Blood pre-DCs from AD patients are able to produce both AD-specific and maturation-related chemokines*

II.2.1. Determination of chemokine production by Proteome Profiler Array

The chemokine production potential of the AD DCs was measured with a screening method which can simultaneously detect the release of 31 different human chemokines. These experiments showed that chemokines released from AD pre-DCs could be classified into two groups. The first group included those chemokines that were detected in the supernatants of both the patient-derived and the control pre-DCs in a similar amount (CXCL4, CXCL7, CXCL8 and CXCL16). The second group consist of those chemokines that were produced at significantly higher amounts or exclusively by the AD pre-DCs (CCL3, CCL4, CCL5, CCL17,

CCL18, CCL19, CCL20, CCL22, CXCL1, CXCL5 and CXCL10). In the cases of CCL5, CCL18, CCL19, CCL20 and CXCL5, the differences were not significant ($p>0.05$), whereas in the cases of CCL3, CCL4, CCL5 and CXCL10, the differences were statistically significant ($p<0.05$). It should be highlighted that, from this second group, CCL17, CCL18 and CCL22 are known as AD-related chemokines whereas CCL3, CCL4, CCL5 and CXCL10 can be defined as chemokines of maturing DCs.

II.2.2. Determination of increased chemokine production by 2 different ELISAs

The production of the most important chemokines from the second group was confirmed by a Multi-Analyte ELISArray from the supernatant of unstimulated cells. Similar to that observed during our studies using the Proteome Profiler Chemokine Array, CCL3 and CCL4 were detected at significantly higher amounts in the samples of AD pre-DCs compared to the supernatant of control pre-DCs. In the cases of CCL17, CCL22 and CCL5, the differences between the two groups did not reach significance, which could be due to either the different sensitivity levels of the two methods or the high SD values of the samples. In AD samples, CXCL10 was detected at a low level but it could not be detected in the supernatant of control cells at all.

In case of those cytokines (CCL17, CCL18 and CCL22) which play pivotal roles in the immunopathology of AD, more precise and reliable conventional ELISA investigations were also carried out on supernatants of unstimulated and stimulated pre-DCs. Unstimulated AD DCs produced both CCL17 and CCL22 in higher amounts than the control cells. The differences between the two groups became statistically significant after AD specific stimulation. We also found that CCL18 production was significantly higher in the supernatants of both unstimulated and stimulated AD DCs compared to healthy control cells. All these data suggest that $CD1c^+$ DCs isolated from the peripheral blood of AD patients show signs of early maturation and that they increase the production and release of multiple chemokines, some of which are characteristics for the disease.

DISCUSSION

Immunotopographical differences in human skin

Since previous data challenged the unified nature of skin immune system, in the first part of our study, we performed extended and comparative analyses of innate and adaptive immune responses, and also of barrier functions of SGR and SGP healthy skin regions. Considering that two healthy skin regions were investigated, we surprisingly found that the RNASeq analyses identified significantly differentially expressed genes between SGR and SGP samples in relatively high numbers. It was also a prominent result that the IPA canonical pathway analysis highlighted the importance of IL-17 signaling in the SGR skin region. These findings encouraged us to study in details the innate immune and barrier milieu influenced by IL-17-influenced in the mentioned two healthy skin regions; indeed, we analyzed characteristics and markers of five different molecular groups which play an important role in cutaneous functions. Although our reported data were mostly collected during transcriptomics analyses and, to lesser extent, from immunolabeling (hence future, detailed proteomics and functional studies are demanded and warranted), it can be postulated that there are indeed marked immunotopographical and barrier differences between the SGP and SGR regions of the human skin.

During the assessment of expressions of AMPs, we identified remarkable differences between SGR and SGP skin samples. AMPs are prominent effector mediators of the innate immune system with far more functions than their antimicrobial activity. Indeed, they play regulatory roles in angiogenesis, wound healing, cell proliferation, and differentiation; moreover, they exert immune-modulatory actions like stimulation of cytokine and chemokine production. Previous studies showed that hBD-1, hBD-2, hBD-3 and human CAMP were detectable at low levels in the differentiated epidermal layers of healthy skin, whereas RNase7 was highly expressed by healthy KCs. It was also shown that S100A7 is one of the most important AMPs in normal skin. It must be noted that in these previous investigations, the origin of healthy skin samples has not been specified. Up to now, only one workgroup examined the regional presence of some AMPs (S100A7, hBD-3 and RNase7) in distinct healthy skin areas. In perfect agreement with our current results, they found that all these proteins were expressed in higher amounts in the forehead (characteristic SGR regions) compared to lower leg (characteristic SGP region) shown by IHC. Besides, S100A7, hBD-2 and CAMP, S100A8, S100A9 and LCN2 were found to be undetectable or were not investigated previously in healthy skin. In our present

study, low levels of these AMPs (S100A7, A8, A9, CAMP, hBD-2, LCN2) were detected in SGP skin. Importantly, expressions of all of them, except CAMP, were found to be significantly higher in SGR skin. Furthermore, S100A8 and LCN2 proteins were found by IHC in both regions; these proteins were present in significantly higher amounts in SGR skin samples.

Our knowledge about the investigated chemokines, mainly derived from innate immune cells, is surprisingly quite incomplete with respect to the healthy skin. Our results showed that, in SGP samples, CCL3, CCL23 and CCL24 were hardly measurable whereas CCL2, CCL19 and CCL20 expressions were found in higher levels. In contrast, all six investigated chemokines were highly expressed in SGR samples, and their expressions were significantly higher compared to SGP samples. Previous studies, without indicating the investigated region, showed that a low expression of CCL20 in healthy skin whereas others were unable to visualize either CCL20 (or CCL2) by immunostaining. We found that albeit the sebaceous glands exhibited a prominent CCL2 staining, thereby resulting in a significantly higher CCL2 protein expression in SGR skin, the immunoreactivity of both CCL2 and CCL20 was very low or absent in the epidermis. These chemokines mainly target T cells and also affect DCs and macrophages. Our data correlate well with our previous observation that T cells and DCs are present at significantly higher numbers in SGR skin.

In the case of the most important KC sensors (TLR2, TLR3, TLR4, NLRP3) and pro-inflammatory cytokines (IL-1α, IL-6, IL-8, IL-33, TNF-α) produced mainly by innate immune cells, no significant differences were found between the two skin regions. These results were not surprising at all since healthy (non-inflammatory) skin samples were compared. The only exception was the significantly higher level of IL-1β in SGR skin; this molecule probably plays a role in establishing the later discussed T_h17/IL-17 cytokine milieu of this region.

Expressions of the late-terminal epidermal differentiation markers (LOR, LCE1F, FLG) as well as of the tight junction molecule CLDN16 were mostly lower in SGR than in SGP skin (albeit the difference was statistically insignificant). This may suggest that the epidermal barrier could be somewhat weaker in the SGR regions. This is supported by previous reports showing that the degree of transepidermal water loss, the increase of which correlates well with impaired barrier functions, is higher in characteristic SGR regions (different facial sites) vs. characteristic SGP regions (forearm, arm). In contrast, we found that mRNA levels of KRT17 and KRT79 were significantly higher in the SGR skin. Moreover, KRT17, which is usually expressed in

basal cells of epithelia (such as in SGP skin), is overexpressed in all layers of the epidermis in the SGR regions. Of further importance, previous studies have found that certain cytokines (IL-17, IL-22) of the T_h17/T_h22 pathways could upregulate the epidermal expression of KRT17 at gene and protein level and downregulate the expression of LOR at the mRNA level. Therefore, these alterations in barrier molecules of KCs may be the result of the later discussed T_h17/IL-17 cytokine milieu of SGR skin regions.

Of greatest importance, expressions of components of T_h17 signaling (T_h17 maturation cytokines and a T_h17 effector chemokine) were markedly and, in multiple cases, significantly (Il-17, IL-1β, IL-23A, CCL20) higher in SGR regions compared to SGP skin. These results correlated well with our previous data when we were able to detect significantly higher mRNA and protein expression of IL-17A by QRT-PCR and IHC. In this study we could reconfirm the presence of T_h17 cells in SGR skin. Moreover, not only the presence but also the central importance of IL-17 in SGR skin was observed as the above detailed differences in the expression of AMPs, chemokines and barrier molecules between SGR and SGP can be well explained by the effects of IL-17. It was previously shown that IL-17 can upregulate the cutaneous expressions of IL-1β, hBD-2, CAMP, S100A7, S100A8, S100A9, LCN2, CCL2, CCL20 and KRT17 at the mRNA and protein levels whereas it can downregulate LOR in KCs. Our focused pathway analysis – aimed to categorize (significantly) differentially expressed, immune system-related molecules into functional groups – also confirmed the key role of these molecules in the maintenance of the region-specific immune milieu of SGR skin. However, it should be emphasized that the detected effects of IL-17 in SGR skin appears to be a homeostatic phenomenon, and not an inflammatory response, since the expression of neutrophil chemoattractants (such as CXCL1, CXCL3, CXCL5, CXCL6, CXCL8, as determined by RNASeq analysis in this study), the production of multiple pro-inflammatory molecules, and the degree of neutrophil infiltration were not significant in SGR skin. Interestingly, among the pro-inflammatory molecules, IL-1β was the only one which exhibited higher expression in SGR skin. We contemplate that the higher IL-1β level can promote T_h17 cell development and can contribute to the IL-17 milieu, as detailed previously.

Along these lines, we think that the notable differences in the cutaneous immune and barrier parameters between SGR and SGP regions may be connected to the distinctions in the composition of microbiota and skin surface micromilieu between the two regions, since it is well-known that both the sebum and the skin microbiota can influence the immune functions

of cells in their microenvironment. It is also important to keep in mind that differences in the composition of the sebum and microbiota seen between the two regions develop during puberty. Researchers established a microbiota change on mouse skin and observed that the induction of IL-17A is a relatively conserved response of the skin, and that these T cell responses were able to promote skin innate responses (production of S100A8, A9). Since a similar, but physiological microbiota shift develops on the surface of human skin during puberty, we hypothesize that the T_h17/ IL-17 immune milieu in the SGR regions could be the remnant of this skin immune system adaptation during puberty in SGR skin and may also be associated with the development of acne in adolescents.

The skin immune system plays a crucial role in the pathogenesis of immune mediated inflammatory and autoimmune skin disorders. Some of these diseases favorably localize to specific skin areas; e.g. acne, rosacea and cutaneous lupus appear mostly on the face, scalp and chest, which are SGR areas. Since, until now, the composition and activation of the skin immune system was considered unique on the whole body, other causes were investigated in the background of the region specific localization of these diseases (sebum, microbiota, endocrine alterations, sunlight). Our present data allow one to consider this question from a new aspect and raise the possibility that region specific characteristics of skin immune system can have important contribution to the development of the region specific immune mediated skin diseases. The non-inflammatory T_h17/IL-17 guided immune and barrier milieu of SGR skin probably predispose this area for the development of inflammatory T_h17 type immune-mediated skin diseases, after disruption of steady-state condition (due to e.g. changes in sebum, microbiota, sun exposure and/or endocrine status). Recent data from the literature support this hypothesis, since in acne, rosacea and all forms of cutaneous lupus (DLE, SCLE, SLE) one of the major skin infiltrating lymphocyte subsets is the inflammatory type T_h17 cell population. Our recent data also raise the possibility that a disrupted tolerance and a switch from non-inflammatory $T_h17(\beta)$ cells to inflammatory $T_h17(23)$ cells may have special role in the development of SGR localized inflammatory skin diseases. According to our results, in SGR skin during steady-state, a homeostatic, tolerogenic TSLP epidermal expression can be detected whereas a significant loss of TSLP, together with the prominent influx of inflammatory DCs and inflammatory $T_h17(23)$/Th1 cells with IL-17/IFN-γ cytokine milieu, are observed during the development of rosacea.

The role of circulating DCs in AD

In addition to our studies on the healthy skin immune system, we also investigated an inflammatory skin disease, namely AD, which is associated the pathological changes of the immune system. Up to date, the phenotypic characteristics and chemokine production of the $CD1c^+$ blood pre-DCs of AD patients were poorly investigated. These pre-DCs are thought to be the precursors of skin dermal DCs, and they may also serve as the precursors of skin inflammatory DCs. Investigating blood pre-DCs can provide information on when or where the skin mDCs acquire their AD-specific characteristics, i.e. whether they exhibit disease-specific characteristics exclusively in the skin microenvironment or even in the blood. These questions can be best answered by assessing DCs directly separated from peripheral blood, instead of applying DCs that have been differentiated *in vitro* from monocytes. Although this method results in fewer cells from the same amount of blood, their characteristics more resemble the physiological conditions.

The expression of FcεRI on DCs in AD skin was published almost 20 years ago. The surface expression of this molecule shows high levels both on LCs and IDEC cells and is most probably involved in the regulation of inflammatory processes. Our own results confirmed a significantly elevated level of FcεRI on blood pre-DCs. The higher number of FcεRI receptors on the surface of blood $CD1c^+$ precursor cells may be the result of elevated serum IgE levels in AD patients. Blood DCs can use this receptor for IgE-mediated allergen presentation. Moreover, the presence of specific IgE and the receptor on their surface increase the efficiency of antigen uptake and presentation after arriving to the skin.

Based on the expression of the CD206 mannose receptor, immature and mature DCs can be distinguished, since most of the mature DCs do not express this protein on their surface in high amounts. We detected this marker in both the diseased and the control groups, but fewer AD pre-DCs expressed them; this suggests a pre-mature state of DCs in AD. With respect to the expressions of the CD83 and CD86 maturation and activation markers on the surface of blood DCs, the literature is very controversial. Expression levels of these markers were higher on the pre-DCs of AD patients, albeit neither CD206 nor CD83/CD86 showed significantly altered expression (even after stimulation) on DCs from AD patients. The consequent and unidirectional changes in their expressions suggest that AD pre-DCs are in a pre-matured state. The lack of significance in our present results and the inconsistency in the literature data related to these maturation markers can be explained by a recent observation that probably not the

entire blood $CD1c^+$ population, but rather a subpopulation, is in a pre-matured state. In that study a larger population of more active and larger DCs with decondensed nuclei and with low DAPI intensity was identified in the blood of AD patients. The AD characteristic cytokine production was assigned to these cells.

In our study, we investigated the chemokine production of AD pre-DCs, since DCs are important sources of chemokines, and there is no sufficient information on the chemokine production of these cells in the blood of AD patients. A Proteome Profiler Chemokine Array was used to identify the most important chemokines produced by AD pre-DCs *ex vivo*, followed by a confirmatory ELISArray method. Furthermore, we were also interested in the stimulatory effect of tissue specific microenvironment; therefore, we compared the chemokine producing capacity of unstimulated cells and DCs stimulated by TSLP and SEB by the more accurate conventional sandwich ELISA technique.

According to our results with Proteome Profiler Array the chemokines were divided into two groups: 1) chemokines produced by AD and control pre-DCs in similar amounts; 2) chemokines produced in a higher amount by AD pre-DCs. In the case of first group, we found that both AD and healthy pre-DCs produced CXCL4, CXCL7, CXCL8 and CXCL16 in similarly high amounts suggesting that their synthesis is a characteristic of blood pre-DCs but independent from the presence of AD.

The members of the second group (CCL3, CCL4, CCL5, CCL17, CCL18 and CCL22, except for CXCL10) were found to be released in higher amounts by blood pre-DCs of AD patients (detected by both Proteome Profiler Array and ELISAs). Among them CCL17, CCL18 and CCL22 are considered to be AD-related chemokines that are also produced by skin DCs of AD patients, and have an important role in regulating the T_h2 immune response and the trafficking of memory T cells. Gene expressions of the inflammation-related CXCL10, CCL3, CCL4 and CCL5 were detected not only in the skin of AD patients, but also in the skin of patients with other inflammatory skin diseases, indicating that the elevated level of these chemokines is a general characteristic of chronic inflammation, rather than an AD-specific factor.

The chemokine production pattern of blood DCs in AD patients can also give some information on their maturation, since DCs at different maturation stages produce different combinations of chemokines. Pre-mature DCs that show early signs of maturation start to produce CCL2, CCL3,

CCL4, CCL5, CXCL8 and CXCL10 in high amounts. In our study, the production of CCL3, CCL4, CCL5 and CXCL10 inflammatory chemokines of AD pre-DCs further confirmed their pre-mature status.

Taken together, the characterization of the cell surface markers and the chemokine production of blood pre-DCs in AD patients indicate that these cells are in a pre-mature stage, and are able to produce AD-specific chemokines even in the peripheral blood. Considering this AD-specific chemokine production and our earlier findings on their capacity to also release AD-specific cytokines, we suggest that the development of AD DCs are markedly influenced by the microenvironment in the blood, and not just by the skin milieu. It is also very likely that the chemokine and cytokine profiles of these DCs become even more intense and disease-specific following tissue-specific stimulation. The bloodstream of AD patients contains unique sets of cytokines (IL-4, IL-10, IL-13, CCL17 and TSLP) which can allow these cells to reach the early stage of maturation. Novel biological therapies (*e.g.* Dupilimab or anti-TSLP mAB) used against these molecules can modify the effect of the cytokines both in the skin and in the blood of AD patients, thus influencing the maturation and the function of the precursor DCs.

SUMMARY

In the first part of our study we performed whole transcriptomic and subsequent pathway analyses to assess the potential immunotopographical differences between SGR and SGP regions. We found that different skin regions exhibit a 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 T_h17/ 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 characteristic topographical localization of some immune-mediated and autoimmune skin disorders. In addition, 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).

In the second part of our work we aimed at investigating the phenotypic features and chemokine production of myeloid pre-dendritic cells of patients with AD ex vivo and after stimulation with *Staphylococcus* enterotoxin B (SEB) and thymic stromal lymphopoietin, representing an AD-like microenvironment. The expression of cell surface markers was measured by flow cytometry, while chemokine production was monitored with chemokine antibody array and confirmed by ELISAs. AD pre-dendritic cells expressed higher levels of FcεRI and the maturation and activation markers tended to be altered. They produced both AD (CCL17/18/22) and maturation-related (CCL3/4/5) chemokines at higher level than controls. The production of CCL3/4 and CCL18 were significantly higher even without AD-specific stimulation, while the production of CCL17 and CCL22 were significantly higher only after stimulation. These results indicate that circulating AD pre-DCs are premature and bear atopic characteristics even without tissue-specific stimulation, suggesting that their development is not only influenced by the skin microenvironment, but even earlier by the local milieu in the blood.

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List of publications related to the dissertation

1. **Béke, G.**, Dajnoki, Z., Kapitány, A., Gáspár, K., Medgyesi, B., Póliska, S., Hendrik, Z., Péter, Z., Töröcsik, D., Bíró, T., Szegedi, A.: Immunotopographical Differences of Human Skin. Front. Immunol. 9, 1-15, 2018. IF: 4.716
2. Kapitány, A., **Béke, G.**, Nagy, G., Doan-Xuan, Q. M., Bacsó, Z., Gáspár, K., Boros, G., Dajnoki, Z., Bíró, T., Rajnavölgyi, É., Szegedi, A.: CD1c+ Blood Dendritic Cells in Atopic Dermatitis are Premature and Can Produce Disease-specific Chemokines. Acta Derm.-Venereol. 97 (3), 325-331, 2017. DOI: http://dx.doi.org/10.2340/00015555-2540 IF: 3.127

List of other publications

3. Medgyesi, B., Dajnoki, Z., **Béke, G.**, Gáspár, K., Szabó, I. L., Janka, E., Póliska, S., Hendrik, Z., Méhes, G., Töröcsik, D., Bíró, T., Kapitány, A., Szegedi, A.: Rosacea is Characterized by a Profoundly Diminished Skin Barrier. J. Invest. Dermatol. [Epub ahead of print], 2020. IF: 6.29 (2018)
4. Szabó, I. L., Lisztes, E., **Béke, G.**, Tóth, K. F., Paus, R., Oláh, A., Bíró, T.: The phytocannabinoid (-)-cannabidiol (CBD) operates as a complex, differential modulator of human hair growth: anti-inflammatory submicromolar versus hair growth inhibitory micromolar effects. J. Invest. Dermatol. 140, 484-488, 2020. DOI: http://dx.doi.org/10.1016/j.jid.2019.07.690 IF: 6.29 (2018)

5. Jenei, A., Dajnoki, Z., Medgyesi, B., Gáspár, K., **Béke, G.**, Kinyó, Á., Méhes, G., Hendrik, Z., Dinya, T., Töröcsik, D., Zouboulis, C. C., Prens, E. P., Bíró, T., Szegedi, A., Kapitány, A.: Apocrine Gland-Rich Skin Has a Non-Inflammatory IL-17-Related Immune Milieu, that Turns to Inflammatory IL-17-Mediated Disease in Hidradenitis Suppurativa.
J. Invest. Dermatol. 139 (4), 964-968, 2019.
DOI: http://dx.doi.org/10.1016/j.jid.2018.10.020
IF: 6.29 (2018)

6. Szöllősi, A. G., Vasas, N., Angyal, Á., Kistamás, K., Nánási, P. P., Mihály, J., **Béke, G.**, Lisztes, E., Szegedi, A., Kawada, N., Yanagida, T., Mori, T., Kemény, L., Bíró, T.: Activation of Transient Receptor Potential Vanilloid 3 Regulates Inflammatory Actions of Human Epidermal Keratinocytes.
J. Invest. Dermatol. 138 (2), 365-374, 2018.
DOI: http://dx.doi.org/10.1016/j.jid.2017.07.852.
IF: 6.29

7. Bohn, T., Planchon, S., Leclercq, C. C., Renaut, J., Mihály, J., **Béke, G.**, Rühl, R.: Proteomic responses of carotenoid and retinol administration to Mongolian gerbils.
Food Funct. 9 (7), 3835-3844, 2018.
DOI: http://dx.doi.org/10.1039/C8FO00278A
IF: 3.241

8. Dajnoki, Z., Kapitány, A., **Béke, G.**, Szegedi, A.: A thymic stromal lymphopoietin szerepe a bőrben és egyéb barriereken.
Bőrgyógyász. Venerol. Szle. 93 (3), 119-125, 2017.
DOI: http://dx.doi.org/10.7188/bvsz.2017.93.3.6

9. Khasawneh, A., Baráth, S., Medgyesi, B., **Béke, G.**, Dajnoki, Z., Gáspár, K., Jenei, A., Pogácsás, L., Pázmándi, K. L., Gaál, J., Bácsi, A., Szegedi, A., Kapitány, A.: Myeloid but not plasmacytoid blood DCs possess Th1 polarizing and Th1/Th17 recruiting capacity in psoriasis.
Immunol. Lett. 189, 109-113, 2017.
DOI: http://dx.doi.org/10.1016/j.imlet.2017.04.005
IF: 2.436

10. Landrier, J. F., Kasiri, E., Karkeni, E., Mihály, J., **Béke, G.**, Weiss, K., Lucas, R., Aydemir, G., Salles, J., Walrand, S., de Lera, Á. R., Rühl, R.: Reduced adiponectin expression after high-fat diet is associated with selective up-regulation of ALDH1A1 and further retinoic acid receptor signaling in adipose tissue.
Faseb J. 31 (1), 203-211, 2017.
IF: 5.595

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11. Dajnoki, Z., **Béke, G.**, Kapitány, A., Mócsai, G., Gáspár, K., Rühl, R., Hendrik, Z., Juhász, I., Zouboulis, C. C., Bácsi, A., Bíró, T., Töröcsik, D., Szegedi, A.: Sebaceous gland rich skin is characterized by TSLP expression and distinct immune surveillance which is disturbed in rosacea.
J. Invest. Dermatol. 137 (5), 1114-1125, 2017.
DOI: http://dx.doi.org/10.1016/j.jid.2016.12.025
IF: 6.448

12. Dajnoki, Z., **Béke, G.**, Mócsai, G., Kapitány, A., Gáspár, K., Steuer-Hajdu, K., Emri, G., Nagy, B., Kovács, I., Beke, L., Dezső, B., Szegedi, A.: Immune-mediated Skin Inflammation is Similar in Severe Atopic Dermatitis Patients With or Without Filaggrin Mutation.
Acta Derm.-Venereol. 96 (5), 645-650, 2016.
DOI: http://dx.doi.org/10.2340/00015555-2272
IF: 3.653

13. **Béke, G.**, Kapitány, A., Dajnoki, Z., Steuer-Hajdu, K., Gáspár, K., Bíró, T., Szegedi, A.: A bőr immunrendszerének felépítése és működése.
Immunol. Szle. 7 (2), 4-11, 2015.

14. Aydemir, G., Kasiri, Y., Birta, E., **Béke, G.**, Garcia, A. L., Bartók, E. M., Rühl, R.: Lycopene-derived bioactive retinoic acid receptors/retinoid-X receptors-activating metabolites may be relevant for lycopene's anti-cancer potential.
Mol. Nutr. Food Res. 57 (5), 739-747, 2013.
DOI: http://dx.doi.org/10.1002/mnfr.201200548
IF: 4.909

15. Harangi, J., **Béke, G.**, Harangi, M., Mótyán, J. A.: The digestable parent cyclodextrin.
J. Incl. Phenom. Macrocycl. Chem. 73 (1-4), 335-339, 2011.
DOI: http://dx.doi.org/10.1007/s10847-011-0061-0
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