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Received: 7 December 2022 | Revised: 16 March 2023 | Accepted: 11 April 2023

DOI: 10.1111/exd.14820

Experimental Dermatology WILEY

RESEARCH ARTICLE

Cytokine profile of the epidermis is region specific and may determine the characteristics of inflammation

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Funding information
EFOP-3.6.1-16-2016-00022; Hungarian Research Grants, Grant/Award Number: NKFIH K-128250, NKFIH K-142348 and NKFIH PD131689; János Bolyai Research Scholarship of the Hungarian Academy of Sciences; The New National Excellence Program, Grant/Award Number: ÚNKP21-5 and ÚNKP-22-3

Abstract

Recent data indicate that distinct skin areas show different microbial/chemical milieu. Keratinocytes (KC) respond to these stimuli by producing cytokine mediators. Therefore, we aimed to determine KC-derived cytokine expression in distinct healthy skin regions (gland-poor [GP], sebaceous gland-rich [SGR] and apocrine gland-rich [AGR]), and their changes in skin diseases of the given regions (atopic dermatitis [AD], papulopustular rosacea [PPR] and psoriasis). Cytokines were analysed at the mRNA and protein levels, and literature analysis was performed for functional categorization. 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 proinflammatory roles of these cytokine patterns (Th2 related in GP, Th17 related in SGR and mixed Th2/Th17 in AGR). In skin diseases which are primarily epidermal cytokine-driven (AD, PPR), the level of the regionally characteristic cytokines were further elevated, in contrast to the autoantigen-driven psoriasis, where the cytokine pattern was independent from the localization. Healthy skin regions are equipped with different KC-derived cytokine profiles, which may influence each region's capability of mediator production in certain types of dermatoses.

KEYWORDS
apocrine gland, atopic dermatitis, cytokines, epimmunome, psoriasis, rosacea, sebaceous gland

Abbreviations: AD, atopic dermatitis; AGR, apocrine gland-rich; AMPs, antimicrobial peptides; CCL, C-C motif chemokine ligand; CD, Crohn's disease; CXCL, C-X-C motif chemokine ligand; DC, dendritic cells; GP, gland-poor; hBD2, human beta defensin 2; IBD, inflammatory bowel disease; IF, immunofluorescence; IHC, quantitative immunohistochemistry; IL, interleukin; KC, keratinocytes; LCN2, lipocalin-2; PPR, papulopustular rosacea; PsV, psoriasis vulgaris; RA, receptor antagonist; RT-qPCR, quantitative real-time polymerase chain reaction; S100, S100 calcium binding protein; sfTSLP, short form thymic stromal lymphopoietin; SGR, sebaceous gland-rich; Th, T helper; Treg, regulatory T cell; UC, ulcerative colitis.

Lilla Szabó and Zsolt Dajnoki contributed equally to this work.

Anikó Kapitány and Andrea Szegedi contributed equally to this work.

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1 | INTRODUCTION

Our skin is a barrier organ that protects the body, while also sensing and transducing external influences from the outside world. The physiological properties and chemical milieu (e.g. pH, secretion of glands) of adult human skin are not unified. In parallel, the microbiota on the surface of healthy apocrine gland-rich (AGR, moist), sebaceous gland-rich (SGR, sebaceous) and gland-poor (GP, dry) skin areas also exhibit prominently distinct compositions.[1] The majority of our skin is GP skin (~75%, extremities, lower back, abdomen, etc.), while the remaining ~25% of our skin is covered by two smaller niches, SGR (scalp, face, chest and upper back) and AGR (axilla, inguinal and anogenital area) regions (Figure S1).[1] Interestingly, under steady state, these two smaller niches have different immune activity compared with GP region. Our research group earlier demonstrated that SGR and AGR regions are equipped with significantly more non-inflammatory T helper (Th)17 [Th17(β)] cells, higher numbers of homeostatic dermal dendritic cells (DCs), and higher amounts of epidermal chemokines and antimicrobial peptides (AMPs).[2–4]

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, which term was introduced by Swamy et al. in 2010.[5,6] 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-36 receptor antagonist (IL-36RA), IL-38, AMPs: short form thymic stromal lymphopoietin (sfTSLP), S100 Calcium Binding Protein (S100)A7, S100A8, S100A9, lipocalin-2 (LCN2), cathelicidin (LL-37), and human beta defensin 2 (hBD2), and chemokines.[6–8]

Production of epimmunome molecules is profoundly determined by the microbiota, which is continuously sensed by KCs.[9–11] Due to the variant microbiota composition of the skin,[1] KCs may produce distinct amounts and patterns of epimmunome mediators in different regions (GP, SGR and AGR). Our research group have already shown significant differences in the levels of AMPs and chemokines among skin regions.[2,3] However, regional differences in cytokine-type epimmunome mediators remain unexplored.

Epimmunome production seems to be an important initiator of certain immune-mediated skin diseases, especially where barrier damage or microbiota alteration are key pathogenic factors (so-called outer epidermal challenge induced outside-in skin disorders). One such disease is atopic dermatitis (AD) which is initiated by barrier dysfunction followed by high IL-25 and IL-33 production and typically appears on dry GP skin region.[12,13] Barrier damage and changes in the production of epimmunome molecules (loss of sfTSLP, high AMP level) are also suggested as drivers of papulopustular rosacea (PPR), which is localized to sebaceous SGR skin area.[4,14–17] On the other hand, psoriasis, initiated rather by autoantigen presentation instead of outer epidermal/barrier challenges from the outside world, can be considered an inside-out dermatosis.[18]

The objective of this study was (i) to determine the relative expression of cytokine-type epimmunome mediators in the three healthy skin regions, (ii) to calculate their absolute cytokine profiles, (iii) to better understand the function of the epimmunome profiles in the distinct regions by a literature search and (iv) to assess the most prominent epimmunome changes in outer epidermal challenge-driven outside-in and inside-out skin diseases (AD, PPR and psoriasis respectively). Our results show that healthy skin regions are equipped with characteristically different epimmunome supplies which seems to influence their capability of epimmunome production also in outside-in, outer epidermal challenge-driven inflammations. This phenomenon may explain the characteristic immune phenotype and localization of this type of skin diseases.

2 | MATERIALS AND METHODS

2.1 | Biopsies

Skin biopsies (0.5–1 cm^2) were taken from healthy SGR (mean age ± SD: 63.4 ± 15.43 years), AGR (mean age ± SD: 63.3 ± 10.48 years) and GP (mean age ± SD: 48.1 ± 15.94 years) skin sites of 10 healthy individuals undergoing plastic surgery (Table S1, Figure S1). Lesional PPR (mean age ± SD: 53.3 ± 14.70) and AD (mean age ± SD: 29 ± 8.25) samples were obtained from the affected skin area of 7 individuals. Lesional PsV (mean age ± SD: 68 ± 15.52) and Scalp Ps (mean age ± SD: 57 ± 18.12) samples were obtained from 6 individuals (Table S2). Each biopsy was divided in half; one part was stored in RNA later at −70°C until further use, while the other part was formalin-fixed and paraffin-embedded. Written, informed consent according to the Declaration of Helsinki principles was obtained by all individuals before participating in the study. The study was approved by the Regional Ethics Committee (certificate number: IV/2072-2/2020/EKU).

2.2 | RNA isolation and reverse transcription

Samples were homogenized in Tri reagent (Sigma-Aldrich, St. Louis, MO) with Tissue Lyser (Qiagen) using innuSPEED Lysis Tubes prefilled with metal beads (Analytik Jena). Total RNA was extracted from the biopsies. RNA concentration and purity were measured with NanoDrop spectrophotometer (Thermo Fisher Scientific, Bioscience, Waltham, MA) and RNA quality was checked using Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA was reverse transcribed into complementary DNA (cDNA) using the High Capacity cDNA Archive Kit (Invitrogen, Life Technologies, San Francisco, CA), according to the manufacturer's instructions.

2.3 | RT-qPCR

RT-PCR was carried out in triplicate using pre-designed MGB assays ordered from Applied Biosystems (Life Technologies). The following TaqMan Gene Expression assays were used: CXCL8 (Hs00174103_m1), IL1A (Hs00174092_m1), IL1B

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(Hs00174097_m1), IL6 (Hs0098569_m1), IL17C (Hs00171163_m1), IL18 (Hs01038788_m1), IL23 (Hs00900829_g1), IL24 (Hs01114074_m1), IL25 (Hs03044841_m1), IL33 (Hs00369411_m1), IL36A (Hs00205367_m1), IL36RN (Hs01104220_g1), IL37 (Hs00367201_m1), IL38 (Hs00544661_m1) and PPIA (Hs9999904_m1). All reactions were performed with an ABI PRISM 7000 Sequence Detection System. Relative mRNA levels were calculated using the $2^{-\Delta\Delta Ct}$ method normalized to the expression of PPIA mRNA.

2.4 | Immunohistochemistry

For immunohistochemistry analyses, paraffin-embedded sections from healthy controls were deparaffinized. Sections were preprocessed with 3% H_2O_2 for 15 min, then, heat-induced antigen retrieval was performed. After blocking with 1% bovine serum albumin (BSA) for 1 hour, sections were incubated with primary antibodies against human IL-1α (rabbit polyclonal IgG [GTX113088], GeneTex), human IL-1β (rabbit polyclonal IgG [ab9722], Abcam, Cambridge, United Kingdom), human IL-6 (mouse polyclonal IgG [SAB1400139], Sigma-Aldrich), human IL-8 (mouse monoclonal IgG [BMS136], eBioscience), human IL-17C (rabbit polyclonal IgG [PA5-34860], Invitrogen), human IL-18 (rabbit monoclonal IgG [ab24309], Abcam), human IL-23 (rabbit polyclonal IgG [PA5-20239], Invitrogen), human IL-24 (mouse monoclonal IgG [MA5-27140], Invitrogen), human IL-25 (mouse monoclonal IgG [MA1_41067], Invitrogen), human IL-33 (mouse monoclonal IgG [MA5-15772], Thermofisher), human IL-36RA (rabbit polyclonal IgG [PA5-72779], Invitrogen) and human IL-38 (mouse monoclonal IgG [14-7385-82], Invitrogen).

Subsequently, the following horseradish peroxidase–conjugated secondary antibody was employed: anti-mouse/rabbit (Daco). Before and after incubating with antibodies, washing of samples was performed for 5 min, 3 times in each step. Signals were detected with the Vector NovaRed Kit (Vector Laboratories, Burlingame, CA, USA). Sections were counterstained with methylene green, dehydrated and covered with glass coverslip. The detection of one 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.

The details of IHC Quantification and Scoring System can be found in the Supplementary material.

2.5 | Immunofluorescent staining and quantification

Immunofluorescent staining was performed similarly as previously described in the immunohistochemistry section till the time point of the application of secondary antibodies. After incubating with primary antibodies, Alexa FluorTM 555 goat anti-mouse IgG (H + L) and Alexa FluorTM 555 goat anti-rabbit IgG (H + L) secondary antibodies were applied (ThermoFisher Scientific). To evaluate immunofluorescence staining, at least 3 images were taken per section at 200× magnification. Fiji (ImageJ) was used to determine the total intensity value per epidermal area in 8-bit grayscale images. Because IL-33 localizes to the nucleus, positivity was measured only for the nuclear area for this cytokine.

Further details are available in the Supplementary material.

3 | RESULTS

3.1 | Distinct mRNA levels of epimmunome characterize different skin regions

First, we investigated cytokine-type epimmunome mediators known from the literature: *IL25*, *IL33*, *IL23A*, *IL17C*, *IL36RA*, *IL37*, *IL38*, *IL18 IL1B*, *IL24*, *IL6*, *IL1A*, *C-X-C Motif Chemokine Ligand [CXCL] 8* and *IL36A* by RT-qPCR.[6–8] Since regional expression of AMP- and chemokine-type epimmunome molecules have been studied in our previous studies, they were not part of the current research.[2,3] According to our findings, the mRNA levels of *IL25*, *IL36RA*, *IL37*, *IL38* and *IL18* were significantly higher in GP region compared with SGR area. On the other hand, *IL1B* was significantly upregulated in SGR skin (Figure 1, Table S3). When comparing AGR area with GP region, *IL36RA*, *IL38* and *IL18* mRNA levels were significantly lower in AGR skin (Figure 1, Table S3). When comparing AGR and SGR regions, *IL33* and *IL6* mRNA levels were significantly higher and *IL1B* mRNA level was significantly lower in AGR area compared with SGR skin (Figure 1, Table S3).

3.2 | Distinct epidermal protein levels of epimmunome characterize different skin regions

Next, we studied the epidermal protein expression of epimmunome mediators (IL-25, IL-33, IL-23, IL-17C, IL-36RA, IL-38, IL-18, IL-1α, IL-1β, IL-6, IL-8 and IL-24) in the three healthy skin areas using IHC. The IL-18, IL-1β, IL-23, IL-25, IL-33 and IL-17C were homogeneously distributed in the epidermis, IL-33 and IL-18 showed nuclear localization, while other cytokines could be observed in cytoplasm of keratinocytes. The highest staining intensity for IL-1α, IL-24, IL-36RA and IL-38 was detected in the stratum granulosum with decreasing levels towards the basal epidermal layer. Staining for IL-6 and IL8 was much weaker but exhibited a similar decreasing pattern towards basal KCs. The staining pattern was similar in the distinct regions in case of all molecules (Figures 2, S2, and Table S3).

When comparing SGR and GP skin areas, the protein levels of IL-25, IL-33, IL-36RA and IL-38 were significantly higher in GP region. In contrast, IL-17C and IL-23 protein levels were significantly higher in SGR samples compared with GP samples. Then, we compared AGR and GP regions and found significantly higher IL-17C protein expression in AGR area. When comparing AGR and SGR skin regions, IL-1β, IL-24, IL-25 and IL-33 protein levels were significantly higher in AGR region (Figures 2, S2, and Table S3).

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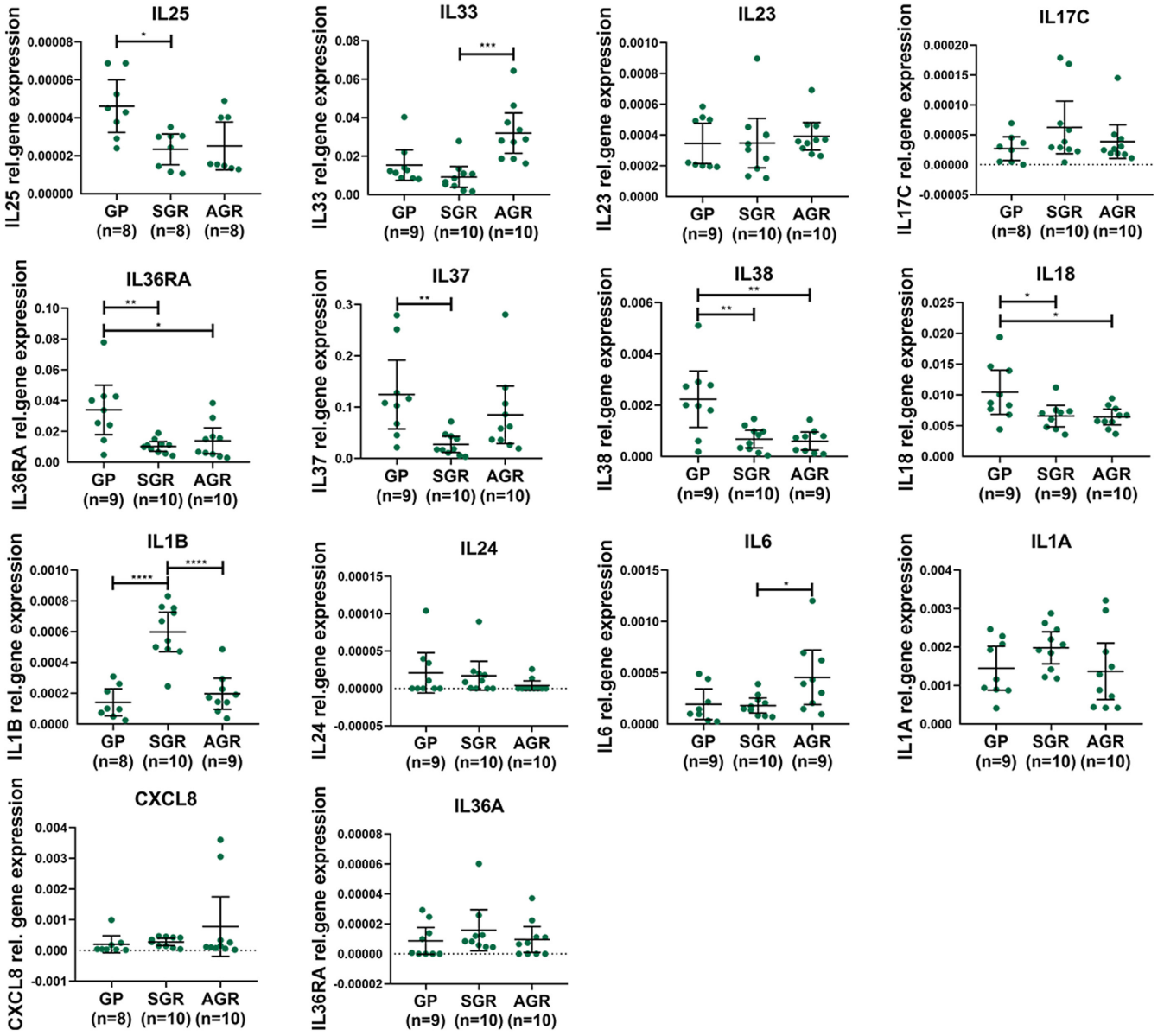

FIGURE 1 The mRNA expression levels of cytokine-type epimmunome mediators in healthy skin areas. The mRNA levels of IL25, IL33, IL23, IL17C, IL36RA, IL37, IL38, IL18, IL1A, IL1B, IL6, CXCL8, IL36A and IL24 were detected by RT-qPCR. The graphs show the median ± 95% confidence interval (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, as determined by one-way analysis of variance followed by Tukey's post hoc test in case of normal distribution or Kruskal–Wallis test followed by Dunn's post hoc test when data distribution was not normal). AGR, apocrine gland-rich; CXCL, C-X-C Motif chemokine ligand; GP, gland-poor; IL, interleukin; KC, keratinocyte; RA, receptor antagonist; SGR, sebaceous gland-rich; Th, T helper.

The incongruence between mRNA and protein analyses can be explained by the fact that there are several steps between transcription and protein synthesis (RNA processing, transcriptional and translational modifications) that can affect the amount of protein produced. However, in addition to these events, the discrepancy could be primarily due to that epidermal staining was quantified in our protein-based study, as the detection of epimmunome production by KCs was the focus of our current research, and cytokine production by glandular cells localized in the dermis have not been specifically measured (cytokine production by certain glands can significantly influence PCR findings, but this is beyond the scope of this study, see Figures S3 and S4).

To further confirm our epidermal protein findings, we performed additional immunofluorescence (IF) staining to detect and quantify molecules with significantly different epidermal protein expression (previously detected by IHC), as the intensity of fluorophores is linearly proportional to the protein content (Figure S5). Using this approach, we found that epidermal IL-17C, IL-23, IL-25, IL-33, IL-36RA and IL-38 protein levels were always consistent with measurements of IHC findings.

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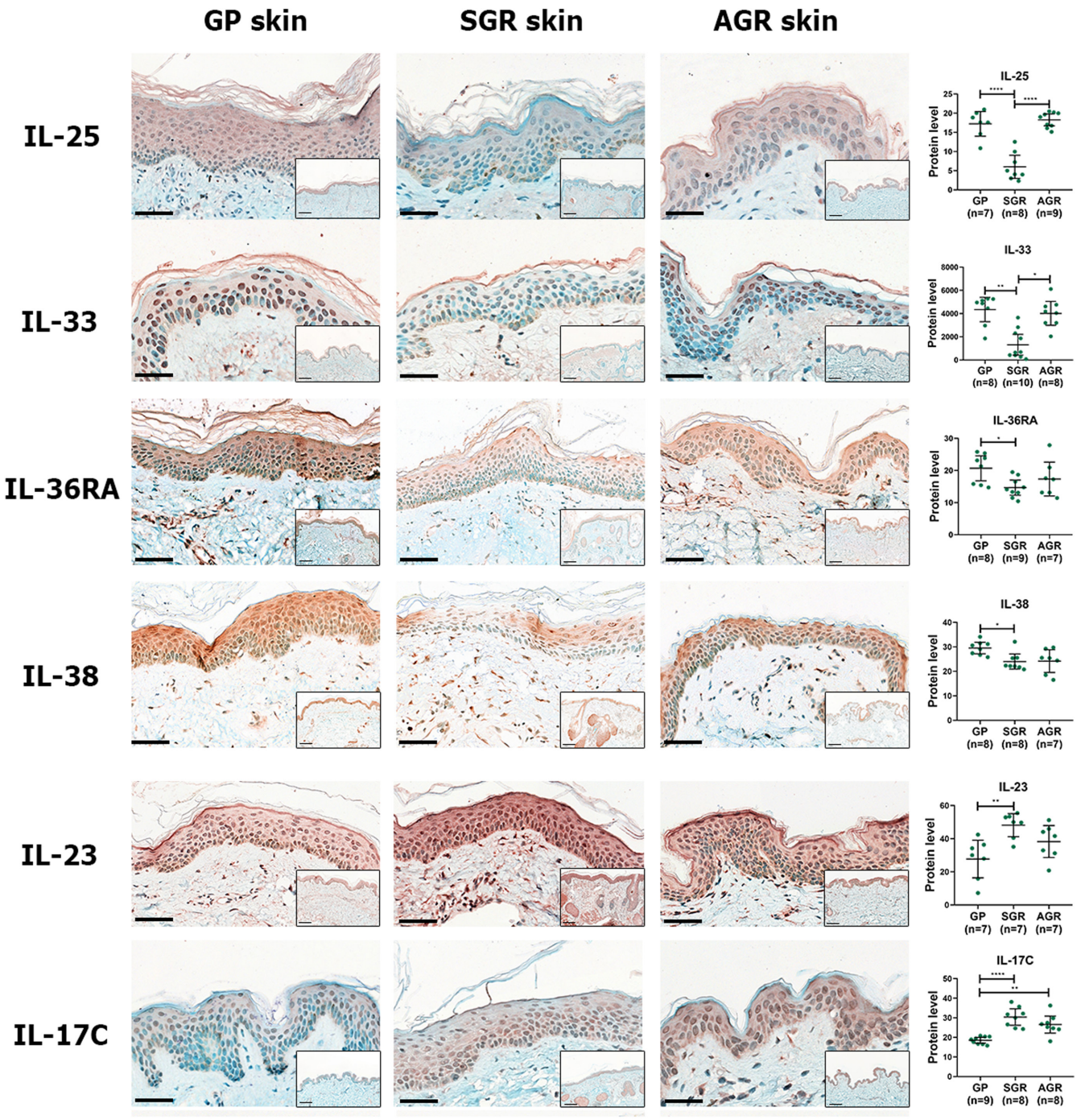

FIGURE 2 Prominent epimmunome-related differences characterize distinct skin areas at the protein level. Representative images for immunostaining and quantification of epidermal levels of IL-25, IL-33, IL-36RA, IL-38, IL-23 and IL-17C (scale bar = 50 μm). The low magnification images in the bottom right corners of the bigger images represent the typical tissue characteristics of each skin region (sebaceous glands in the SGR, apocrine glands in the AGR and the absence of glands in the GP region; scale bar = 200 μm). The graphs show the median ± 95% confidence interval of measured protein levels (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, as determined by one-way analysis of variance followed by Tukey's post hoc test in case of normal distribution or Kruskal–Wallis test followed by Dunn's post hoc test when data distribution was not normal). AGR, apocrine gland-rich; GP, gland-poor; IL, interleukin; RA, receptor antagonist; SGR, sebaceous gland-rich.

3.3 | Different skin regions bear unique homeostatic epimmunome profiles with distinct immune activity

Since we studied healthy regions, we could not apply control skin samples in a conventional way. Therefore, to define the unique epimmunome pattern for each region, a simplified, scoring system was developed. For each molecule, the median value of data representing each region was ranked based on three predefined ranges represented by (+), (++) or (+++) marks (Table 1). Those molecules with three marks (+++) were subsequently considered to be representative of a given region. According to our findings, SGR area can

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TABLE 1 Distinct epimmunome mediators characterize different skin regions.

	GP	SGR	AGR
IL-25	+++	+	+++
IL-33	+++	+	+++
IL-23	+	+++	+++
IL-17C	+	+++	++
IL-36RA	+++	+	++
IL-38	+++	+	++
IL-18	+++	+++	+++
IL-1α	+	++	++
IL-1β	+	+	+
IL-6	++	+	++
IL-8	++	+	++
IL-24	+	+	++

Note: For each molecule, the median value of the data representing each region was ranked based on three predefined ranges represented by (+), (++) or (+++) marks.

Abbreviations: AGR, apocrine gland-rich; AMP, antimicrobial peptide; CCL2, C-C Motif Chemokine Ligand 2; GP, gland-poor; IL, interleukin; SGR, sebaceous gland-rich; Th, T helper.

be characterized by a prominent presence of IL-23, IL-17C and IL-18. GP region exhibited high IL-25, IL-33, IL-36RA, IL-38 and IL-18 protein levels. AGR region was represented by the presence of IL-25, IL-33, IL-23 and IL-18, bearing the characteristics of SGR and GP areas.

Next, to find out the steady-state role of the epimmunome profiles of each region, we reviewed the literature concerning both the homeostatic and inflammatory functions of the identified molecules (Table 2). According to the literature, the epimmunome mediators of GP area induce regulatory T cells (Treg) under steady state, while in inflammation, they promote Th2 and/or inhibit Th17 responses. The mediators of SGR area maintain balanced skin-colonizing microbiota by sustaining a non-inflammatory Th17 immune milieu, while, during inflammation, they initiate inflammatory Th17 responses. AGR region has characteristics of both SGR and GP regions (Table 2).

3.4 | Regional epimmunome profiles reflect epidermal cytokine production in outside-in skin diseases

Our literature analysis indicated that the three regions are characterized by either Th2-related or Th17-related homeostatic epidermal epimmunome profiles. Therefore, next, we determined how these regional epimmunome profiles change under inflammatory conditions. The most important KC-produced Th2- and Th17-related cytokines (Th17: IL-23 and IL-17C; Th2: IL-33, IL-25, IL-36RA and IL-38) were investigated by immunofluorescent staining in AD and PPR, which can be considered epidermal challenge-driven, outside-in diseases of GP and SGR regions (Figure 3). Psoriatic lesions of the same GP and SGR regions were also immunostained as controls, to detect epimmunome-related changes in an inside-out dermatosis (Figure 3).

In AD, the epidermal fluorescence intensity of IL-33, IL-25, IL-36RA and IL-38 cytokines, which characterize GP region, was significantly increased, while IL-23 and IL-17C levels did not change. In contrast, in PPR, the intensity of IL-23 and IL-17C, cytokines of healthy SGR, became highly elevated, while the expression of IL-33, IL-25, IL-36RA and IL-38 did not increase. In both psoriatic groups, independent of the region (PsV obtained from GP regions and scalp psoriasis from SGR region), IL-23 and IL-17C was significantly upregulated, and IL-33 and IL-25 were unchanged, while IL-36RA and IL-38 were decreased (Figure 3).

These results suggest that, in outside-in, outer epidermal challenge initiated diseases, inflammatory epimmunome production closely resembles the homeostatic region-specific epimmunome pattern, although in an exaggerated amount. On the contrary, in skin inflammation of different origin (like psoriasis) the developing inflammatory epidermal cytokine milieu is independent from the homeostatic epimmunome milieu of a given skin region.

4 | DISCUSSION

In our study, we performed the comparative analysis of cytokine-type epimmunome mediators at the mRNA (by RT-qPCR) and protein levels (by IHC and IF) among the three healthy skin regions. We also defined the absolute homeostatic epimmunome profiles of these areas by using a simplified scoring system. According to our findings, distinct healthy skin regions are equipped with different homeostatic epithelial epimmunome profiles. Based on our epidermal protein data, GP skin is characterized by an IL-25/IL-33/IL-36RA/IL-38/IL-18 epidermal epimmunome milieu, SGR region exhibits an IL-23/IL-17C/IL-18 epimmunome pattern, and AGR region bears an IL-25/IL-33/IL-23/IL-18 milieu. Then, to better understand the role of the epimmunome profile of distinct healthy skin regions, we carried out a comprehensive literature search. We found that the characteristic epimmunome sets of the three skin regions differ in the mechanisms of maintaining homostasis, as mediators of GP seem to promote Tregs, cytokines of SGR areas induce non-inflammatory Th17 cells, while AGR area bears both characteristics (Table 2). On the contrary, these epimmunome sets are also different in their proinflammatory capabilities, as the region-specific epimmunome composition of SGR area seems to favour a type 3 (Th17-related), GP region promotes a type 2 (Th2-related) adaptive immune inflammation, while AGR area bears both characteristics.

In perfect agreement with these findings, Del Duca and colleagues demonstrated higher CXCL8, IL-1β, IL-6 and IL-23 mRNA levels in SGR area (back), while GP region (arm, outer thigh and abdomen) was characterized by higher IL-33 and IL-37 mRNA levels.[39] In another study, regulatory T cell (Treg) counts were found to differ among distinct skin sites, and according to the opinion of the authors, the distinct Treg counts can be responsible for the different

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TABLE 2 Roles of epimmunome molecules under homeostatic and inflammatory conditions.

Molecule	Cytokine family	Receptors	Alarmin/ Epimmunome	Cell sources	Role in homeostatic conditions	Role in inflammatory conditions	Refs.
Th2-related							
IL-25	IL-17	IL-17RA/IL-17RB	Epimmunome	KC, Th2, mast cell, EC, eosinophil, basophil	Tolerance through Tregs	Promotion of Th2 response, inhibition of Th1 and Th17 responses, induction of Th2 cytokine production	[7,19–22]
IL-33	IL-1	ST2/IL-1RAcP	Alarmin	KC, EC, fibroblast, endothelial cell	Tolerance through Tregs	Promotion of Th2 response, induction of Th2 cytokine production	[7,19,22–24]
Th17-related							
IL-23	IL-12	IL-23R	Epimmunome	KC, macrophage, activated DC	Regulation of microbial homeostasis	Promotion of Th17 response	[7,19,25]
IL-17C	IL-17	IL-17RA/IL-17RE	Epimmunome	KC, EC	Epithelial barrier reinforcement, maintenance of microbial homeostasis	Promotion of Th17 response	[7,19,26–28]
Anti-inflammatory cytokines							
IL-36RA	IL-1	IL-36R/IL-1RacP1	Epimmunome	KC, endothelial cells, T cell, macrophage, DC		Inhibition of Th17 cytokines and IL-36 cytokines	[7,19,29]
IL-37	IL-1	IL-18Rα/IL-1R8 and IL-18BP	Epimmunome	KC, myeloid DC, NK, B cell, monocytes	Tolerance through Tregs	Inhibition of Th1/Th2/Th17 responses, production of Th2 cytokines, inhibition of Th17 cytokines	[7,19,30,31]
IL-38	IL-1	IL-36R, IL-1R1, IL-1RAPL1	Epimmunome	KC, EC, B cells	Tolerance through Tregs	Inhibition of Th1 and Th17 responses and cytokines	[7,19,32,33]
Proinflammatory cytokines							
IL-18	IL-1	IL-18R (IL-18Rα/ IL-38Rβ)	Epimmunome	KC, EC, DC		Promotion of Th1 and Th2 responses *(cytokine milieu dependent)*	[7,19,34,35]
IL-1α IL-1β	IL-1	IL-1R1/ IL-1R2	Epimmunome Alarmin	KC, macrophage, monocyte, lymphocyte, neutrophil, fibroblast	Dampening the role of Tregs	Promotion of Th17 response	[7,19,36]
IL-6	IL-6	IL-6R	Epimmunome	KC, monocyte, macrophage, T cell, B cell, mast cell, endothelial cell	Inhibition of Treg differentiation	Promotion of Th17 response	[7,19,37]
IL-8	CXC	CXCR1, CXCR2	Epimmunome	KC, EC, macrophage, monocyte, lymphocyte, endothelial cell, fibroblast, neutrophil, smooth muscle cell		Attracts neutrophils, NK cells, T cells	[7,19]
IL-24	IL-20	IL-20R1/IL-20R2 and IL-22R1/IL-20R2	Epimmunome	KC, Th2, Th17, B cell, monocyte, macrophage, NK cell, mast cell	Maintenance of homeostasis	Promotion of Th2 cell responses, inhibition of Th1/Th17 responses, regulation of the pathogenic Th17 response	[7,19,38]

Note: Epimmunome: all molecules used by epithelial cells to instruct immune cells. Alarmin: epimmunome mediators that are mainly released as a result of degranulation, cell injury or death.
Abbreviations: DC, dendritic cells; EC, epithelial cell; IL, interleukin; KC, keratinocytes; NK, natural killer; Th, T helper; Treg, regulatory T cell.

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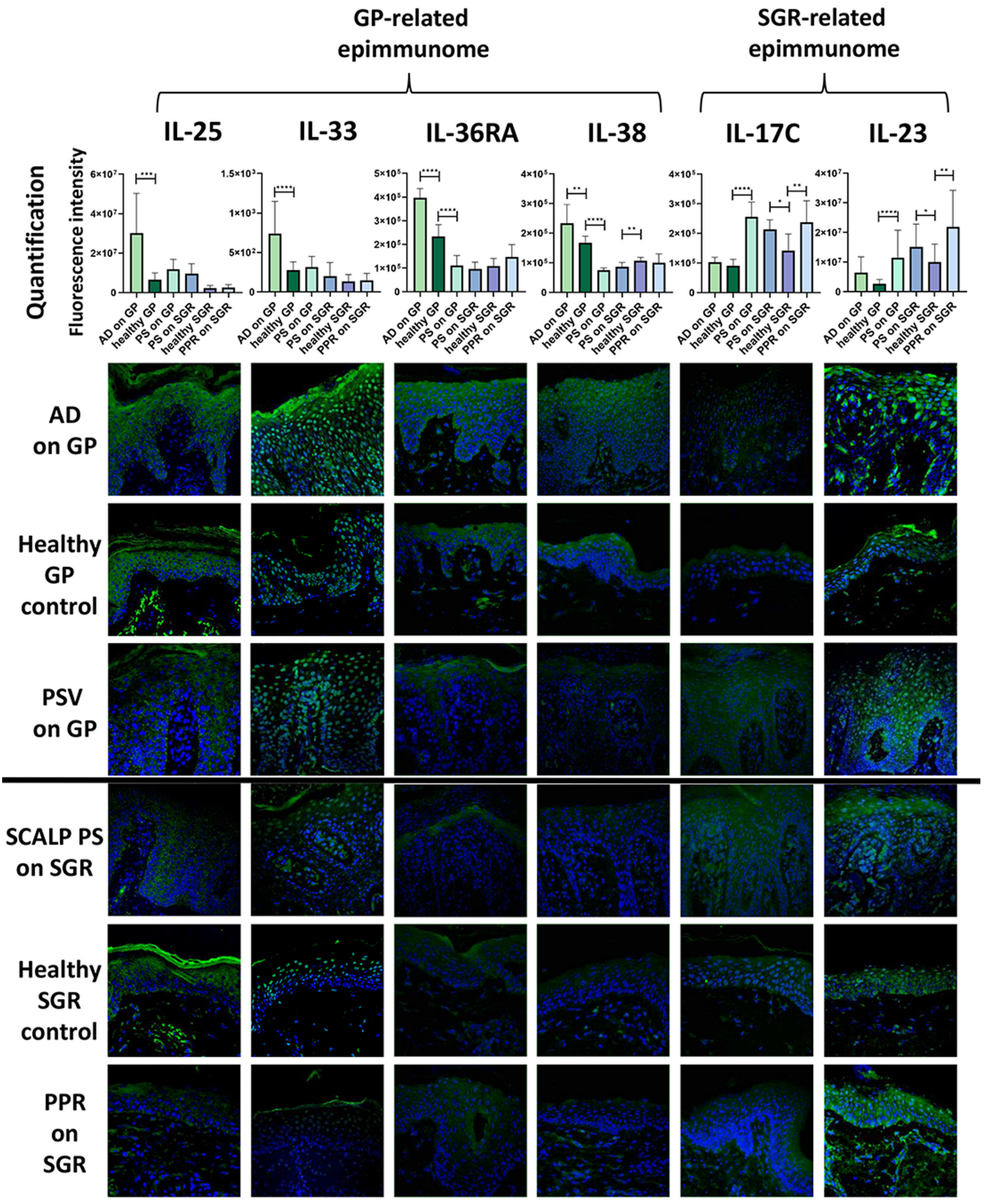

FIGURE 3 The homeostatic epimmunome patterns of the regions are reflected in the epidermal cytokine production in skin diseases. Immunofluorescent staining was performed in the GP ($n=7$) and SGR ($n=7$) areas and their respective model diseases, including the Th2-driven AD ($n=7$) of the GP skin area and the Th1/17-mediated PPR ($n=7$) of the SGR skin. In addition to healthy GP and SGR skin samples, psoriatic (scalp [$n=6$] and PsV [$n=6$]) samples were also used as additional control samples. Statistical analyses between the protein levels of diseased samples and their respective healthy controls were determined by unpaired *t* test in case of normal data distribution or Mann Whitney test in case data distribution was not normal. AD, atopic dermatitis; GP, gland-poor; KC, keratinocyte; PPR, papulopustular rosacea; PsV, psoriasis vulgaris; Ps, psoriasis; SGR, sebaceous gland-rich; Th, T helper.

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probability of cutaneous metastases on distinct regions.[40] These results are also in agreement with our previous investigations, as we revealed a prominent presence of non-inflammatory Th17(β) cells accompanied by a prominent presence of Th17-related AMPs (LCN2 and S100A8) and chemokines (CCL2) in healthy SGR and AGR regions.[2,4]

Several factors, such as microbiota, lipid composition, moisture and pH may be responsible for the epimmunome-related differences of these regions.[4] However, the microbiota diversity of healthy skin seems to be the crucial factor contributing to these differences, as the microbiota is in close and continuous contact with the KCs, and their regionally distinct compositions may train them differently.[9–11,41] In addition to the unique bacterial composition of SGR, AGR and GP regions, SGR and AGR skin areas also favour the colonization of different fungal communities.[1,42,43] Recent sequencing-based studies revealed that *Malassezia* is the dominant fungi on the human skin surface and its absolute abundance is extremely high in SGR region, with a prominent presence in AGR area as well.[42–44] More importantly, *Malassezia* species were found to trigger Th17 responses, which could play a role in the initiation of Th17-related epimmunome production in SGR and AGR epidermis.[45]

In addition to homeostatic conditions, we determined the changes in epimmunome production of GP and SGR regions in their characteristic inside-out skin diseases (AD and PPR, respectively). We detected the most prominent KC-derived epimmunome molecules including IL-17C, IL-23, IL-25, IL-33, IL-36RA and IL-38 in AD and PPR. Lesional skin samples from both SGR and GP regions of psoriatic patients were also assessed to determine epimmunome changes in an outside-in skin disease. According to our findings, when an outside-in, epidermal challenge-induced skin disease develops in GP region, IL-33, IL-25, IL-36RA and IL-38 (homeostatic cytokines of GP) levels rise significantly, while IL-23 and IL-17C (low amounts in GP under steady-state) levels do not change, resulting consequently a type 2 (Th2 mediated) inflammation, as in AD. In SGR region, when epidermal challenges from the outside world induce inflammation, IL-23 and IL-17C (homeostatic cytokines of SGR) levels increase prominently, while IL-33, IL-25, IL-36RA and IL-38 (low amounts in SGR under steady-state) are not induced, and this can promote a type 3 (Th17 mediated) inflammation, like in PPR. In agreement with our current findings, De Benedetto et al. showed that barrier damage-induced epidermal milieu can promote distinct types of adaptive immune activation in certain tissue environments, thereby inducing either type 2 or type 3 immune response.[46] In contrast, both GP (PsV) and SGR (scalp psoriasis) areas act similarly with highly upregulated IL-23 and IL-17 in psoriasis, which is initiated rather by auto-antigens presentation by DCs than by a challenge in the outer epidermal layers.[18] Based on these results, in epimmunome-driven skin diseases the expression of KC-derived epimmunome mediators, which were already present at higher levels under homeostatic conditions in the given skin region, are elevated significantly.

Importantly, the region-specific epimmunome production under both homeostatic and inflammatory conditions is not just a feature of skin.[47–49] In the gut, distinct levels of TSLP, IL-25 and IL-23 have been demonstrated in the small intestine and colon, which may influence the characteristic localization of certain inflammatory bowel diseases, such as Crohn's disease (Th1/17-driven) and ulcerative colitis (Th2-driven).[50–55]

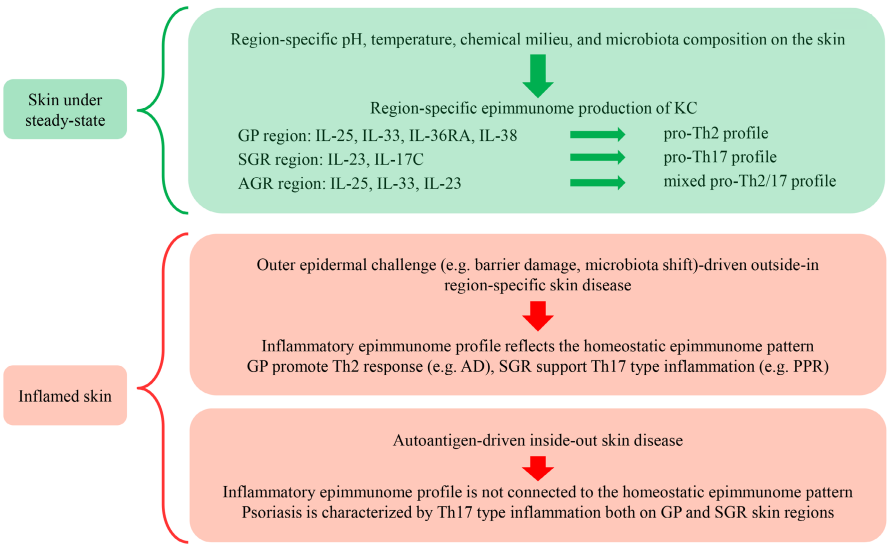

FIGURE 4 Cytokine profile of the epidermis is region-specific and may determine disease characteristics. Healthy skin regions are equipped with different epimmunome supplies, which may determine their capability of epimmunome production in outside-in skin diseases driven by epidermal challenges from the outside world, and can also influence disease immune phenotype and localization. On the contrary, in autoantigen-driven dermatosis, such as psoriasis, the developing inflammatory epidermal cytokine milieu is independent from the given homeostatic epimmunome milieu of a given skin region as regardless of the region where it develops, it is characterized by the same type of inflammatory response. AD, atopic dermatitis; AGR, apocrine gland-rich; GP, gland-poor; KC, keratinocyte; PPR, papulopustular rosacea; SGR, sebaceous gland-rich; Th, T helper.

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In summary, we propose that skin regions acquire a specific epimmunome production capability due to their unique immune training by their characteristic microbiota under steady-state conditions (Figure 4). This may influence the mediator production of KCs even in pathogenic circumstances and may determine the complex immune feature and location of inflammatory skin disorders, like AD (Th2 inflammation on GP) and PPR (Th17 inflammation on SGR), where an epidermal challenge from the outside initiates the disease through epimmunome production (Figure 4). In contrast, inflammation of inside-out skin disorders (e.g. psoriasis) is independent of the homeostatic epimmunome profile of the region (Figure 4). Based on our findings, we also propose that the specific differences in skin regions should be taken into account in barrier repair therapy of a given skin area.

AUTHOR CONTRIBUTIONS

A.Sz was involved in conceptualization and funding acquisition. A.K., L. Sz. and Zs. D. were involved in methodology. O.S., L. Sz., Zs. D., and IL.Sz. were involved in investigation. A. Sz., K.G., D.T., T.D., and IL.Sz. were involved in resources. L.Sz. and O.S. were involved in formal analysis. Z.H. and Zs.D. were involved in software. A.Sz., Zs.D., L.Sz., A.K., D.T. and AG.Sz. were involved in writing—original draft and writing—review and editing. Zs.D. was involved in visualization.

ACKNOWLEDGEMENTS

The publication was supported by Hungarian Research Grants (NKFIH K-142348, NKFIH K-128250, NKFIH PD-131689) and the EFOP-3.6.1-16-2016-00022 project. The project was co-financed by the European Union and the European Social Fund. This project was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences and the ÚNKP-21-5 New National Excellence Program of the Ministry for Innovation and Technology. This project was also supported by the ÚNKP-22-3 New National Excellence Program of the Ministry for Culture and Innovation from the source of the national research, development and innovation fund.

FUNDING INFORMATION

Hungarian Research Grants (NKFIH K-142348, NKFIH K-128250, NKFIH PD-131689), EFOP-3.6.1-16-2016-00022 project, János Bolyai Research Scholarship of the Hungarian Academy of Sciences, and the New National Excellence Program (ÚNKP-21-5 and ÚNKP-22-3).

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Schematic representation of the distribution of topographical different human skin regions.

Figure S2. Protein expression levels of epimmunome molecules in healthy skin areas. Representative images for immunostaining and quantification of epidermal levels of IL-1α, IL-1β, IL-6, IL-8 and IL-24 (scale bar = 50 μm). The low magnification images in the bottom right corner of bigger images represent the typical tissue characteristics of each skin region (sebaceous glands in the SGR, apocrine glands in the AGR and the absence of glands in the GP region; scale bar = 200 μm). The graphs show the median ± 95% confidence interval (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, as determined by one-way analysis of variance followed by Tukey's post hoc test in case of normal distribution or Kruskal–Wallis test followed by Dunn's post hoc test when data distribution was not normal). AGR, apocrine gland-rich; GP, gland-poor; IL, interleukin; SGR, sebaceous gland-rich.

Figure S3. Representative images for immunostaining of dermal staining patterns of IL-25, IL-33, IL-36RA, IL-38, IL-23 and IL-17C. Small images represent the glandular expression of the cytokines. AG, apocrine gland; AGR, AG-rich; GP, gland-poor; SG, sebaceous gland; SGR, SG-rich; SwG, sweat gland.

Figure S4. Representative images for immunostaining of dermal

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staining patterns of IL-18, IL-1α, IL-1β, IL-6, IL-8 and IL-24. Small images represent the glandular expression of the cytokines. AG, apocrine gland; AGR, AG-rich; GP, gland-poor; SG, sebaceous gland; SGR, SG-rich; SwG, sweat gland.

Figure S5. Immunofluorescence findings confirm the epimmunome-related differences of distinct skin areas. Representative images for immunofluorescence staining and quantification of epidermal levels of IL-25, IL-33, IL-36RA, IL-38, IL-23 and IL-17C. The graphs show the median ± 95% confidence interval of measured protein levels (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, as determined by one-way analysis of variance followed by Tukey's post hoc test in case of normal distribution or Kruskal–Wallis test followed by Dunn's post hoc test when data distribution was not normal). AGR, apocrine gland-rich; GP, gland-poor; IL, interleukin; RA, receptor antagonist; SGR, sebaceous gland-rich.

TABLE S1. Characteristics of skin samples from healthy individuals. AGR, apocrine gland-rich; F, female; GP, gland-poor; M, male; SD, standard deviation; SGR, sebaceous gland-rich.

TABLE S2. Characteristics of skin samples from patients. AD, atopic dermatitis; F, female; M, male; PPR, papulopustular rosacea; scalp Ps, scalp psoriasis; PsV, Psoriasis vulgaris; SD, standard deviation.

TABLE S3. The pairwise comparison of epimmunome mediator mRNA and protein levels from healthy skin samples. Statistical analyses between protein and mRNA levels were determined by one-way analysis of variance followed by Tukey's post hoc test in case of normal data distribution or Kruskal–Wallis test followed by Dunn's post hoc test when data distribution was not normal. The bold type indicates data with significant differences. AGR, apocrine gland-rich; FC, fold change; GP, gland-poor; IHC, immunohistochemistry; IL, interleukin; KC, keratinocyte; ND, not determined; ns, not significant; qRT-PCR, quantitative real-time PCR; RA, receptor antagonist; SGR, sebaceous gland-rich.

How to cite this article: Szabó L, Dajnoki Z, Somogyi O, et al. Cytokine profile of the epidermis is region specific and may determine the characteristics of inflammation. *Exp Dermatol*. 2023;00:1-12. doi:10.1111/exd.14820

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