SHORT COMMUNICATION

Comparison of Immune and Barrier Characteristics in Scalp and Skin Psoriasis

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Recent studies have found different microbiota and chemical milieus in different healthy skin areas. In addition, topographically distinct immune and barrier characteristics have been identified (1–3). Based on these results, the skin cannot be considered a unified organ: 3 different skin niches can be distinguished: sebaceous gland poor (SGP), sebaceous gland rich (SGR), and apocrine gland rich (AGR) skin regions (1, 3). Certain immune-mediated skin diseases localize primarily to one of these regions; for example, atopic dermatitis appears mostly in SGP regions, acne and rosacea appear in SGR regions, and hidradenitis suppurativa in AGR regions.

Other skin diseases can develop in any skin regions, like psoriasis, where lesions can manifest in either SGP regions (psoriasis vulgaris of SGP skin), or SGR regions (scalp psoriasis), and AGR regions (inverse psoriasis).

This study aimed to compare the immune and barrier features of psoriasis localized to SGP areas (psoriasis vulgaris) and psoriasis on SGR regions (scalp psoriasis), to determine if the immune milieu of healthy skin influences the immune characteristics and, consequently, the treatment of psoriasis on distinct skin regions.

METHODS AND RESULTS

Since psoriasis is a T helper (Th)1/Th17-mediated skin disorder (4), the current study investigated the Th1- and Th17-related immune and barrier alterations in lesional skin samples of patients with psoriasis vulgaris on SGP skin and scalp psoriasis (each $n=6$) (Table SI[1]) by immunohistochemistry and RT-qPCR (for details see Appendix S1[1]).

Immunostaining of $CD4^+$ T cells, $CD11c^+$ myeloid dendritic cells (mDCs) and $CD1a^+$ Langerhans cells (LCs) revealed no significant differences between psoriasis vulgaris on SGP skin and scalp psoriasis on SGR skin (Fig. S1[1]).

Gene expression levels for Th1- and Th17-related cytokines, as interferon (IFN)γ, IL-12, IL-17 and IL-23 were similar (Table SII[1]), and the protein levels of IFNγ, IL-17 and IL-23 were also similar between the 2 groups. No differences in the Th17-related chemokines, CCL2 and CCL20, were detected at the mRNA level (Table SII[1]). In contrast, the protein expression of CCL20 was significantly higher in scalp psoriasis compared with psoriasis vulgaris of SGP skin (Table SII[1], Fig. S2[1]).

Expression of the most common pro-inflammatory cytokines, IL-1β and tumour necrosis factor alpha (TNF-α), was investigated at the mRNA level, while immunostaining for TNF-α was also performed. Their expression at the mRNA level (Table SII[1]) and TNF-α protein levels were similar in the 2 psoriatic sample groups (Table SII[1], Fig. S2[1]).

To further investigate the Th17-related components of the innate immune response, this study assessed the gene expression levels of different AMPs (S100A7/8/9, human beta defensin (DEFB)4B, lipocalin (LCN)2), and protein levels for LCN2 and S100A8. There were no significant differences between the 2 psoriatic groups, at either gene or protein levels (Table SII[1], Fig. S3[1]), except for the gene expression of LCN2, which was significantly higher in scalp psoriasis (Table SII[1]).

Finally, the mRNA levels of key molecules involved in the formation and maintenance of the epidermal barrier (loricrin (LOR), filaggrin (FLG), keratin (KRT) 6, KRT17) were also examined by qPCR, while LOR, FLG, and KRT17 were evaluated, using immunohistochemistry. Expression of these molecules was similar in psoriasis samples from SGR and SGP skin, at both gene and protein levels (Table SII[1], Fig. S3[1]).

DISCUSSION

This study compared the immune characteristics of psoriasis vulgaris on SGP skin and scalp psoriasis on SGR skin to determine whether the inflammation developed in the 2 subtypes of psoriasis are influenced by the primarily distinct immune milieu of the different healthy skin areas. The results show that the mediators of both innate immune responses and Th1/Th17 type adaptive immune pathways were expressed similarly in scalp psoriasis and psoriasis vulgaris of SGP skin (**Fig. 1**). In addition, no significant differences could be detected in the expression of barrier molecules (Fig. 1). Significant differences were found only in *LCN2* mRNA and CCL20 protein expression, with higher levels in scalp psoriasis. Since these parameters have previously been shown to be elevated even in healthy SGR skin compared with SGP (3), these differences may reflect the original immune characteristics of SGR skin region rather than psoriasis-related features (Fig. 1).

The immune characteristics of psoriasis in different skin areas have been compared in a few publications (5–9), but only 2 publications have focused on comparing scalp psoriasis and psoriasis vulgaris (5, 6). The research focus and applied methods of these 2 studies were different from ours; furthermore, barrier components were not examined at the protein level (5, 6). Moreover, the conclusions of these 2 articles appear to be contradictory. In general, the results of the current study are in line with that of Ruano and colleagues (5), who, despite revealing some differences in the magnitude of dysregulation between the 2 forms of psoriasis by transcriptomic analyses, concluded

[1]https://www.medicaljournals.se/acta/content/abstract/10.2340/00015555-3553

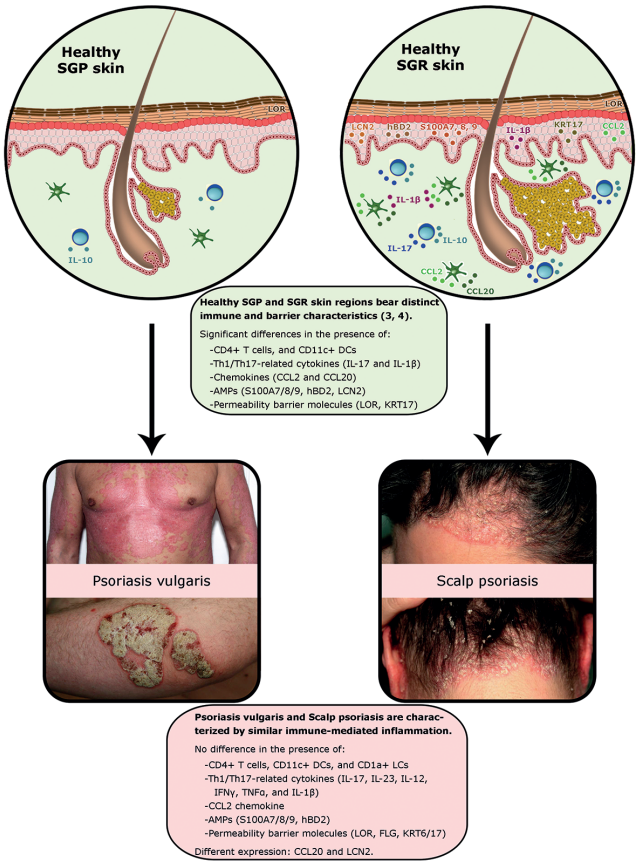

Fig. 1. Although the immune and barrier characteristics of healthy sebaceous gland poor (SGP) and sebaceous gland rich (SGR) skin are different, the psoriatic plaques developing on these distinct areas bear similar cellular, molecular and barrier characteristics. AMP: antimicrobial peptide; CCL: chemokine (C-C motif) ligand; hBD2: human beta defensin2; FLG: filaggrin; IFNG: interferon gamma; IL: interleukin; KR: keratin; LCN: lipocalin; LOR: loricrin; TNFA: tumor necrosis factor alpha.

that the immune mechanisms of scalp psoriasis are fundamentally similar to that of skin psoriasis (5). In another study, Ahn et al. concluded that distinct psoriasis subtypes display differences in IL-17, IFN-γ and IL-22 production (6). Although these results appear to be contrary to ours, these differences were significant only when palmoplantar psoriasis were compared with conventional plaque psoriasis (6). In another publication, chronic plaque psoriasis and inverse psoriasis, characteristic of AGR skin, were compared, and IL-17 was identified as their major shared pathway (9). These results support our findings showing that psoriasis localized to different skin areas share similar IL-17 related immune characteristics.

In clinical practice, the treatment of scalp psoriasis is considered more difficult than that of psoriasis vulgaris on SGP skin, since the high density of hair follicles and pilosebaceous units makes the application of local therapy and phototherapy technically complicated. Therefore, new formulations, such as foam or gel, were developed as new treatment modalities for this region (10). Clinical practice and studies show that biological treatments have the same efficacy for psoriasis vulgaris and scalp psoriasis (11). These findings are supported by the current study, showing that, in spite of the significant differences between healthy SGR and SGP skin immune milieu, psoriatic plaques developing on these distinct areas bear similar cellular, molecular, and barrier characteristics (Fig. 1). In summary, the results of this study suggest that, although the formulation of the local therapy needs to be different for psoriasis localized to the scalp vs SGP skin areas, there is no indication for the development of active ingredients with different mechanisms of action.

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The authors have no conflicts of interest to declare.

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Appendix S1

SUPPLEMENTARY MATERIALS AND METHODS

Skin biopsies

Written informed consent, according to the Declaration of Helsinki principles, was obtained from all patients involved in the study. The National Medical Research Council approved the study. Skin biopsies (8-mm punch biopsies) were collected from the skin lesions of 12 patients with psoriasis, including 6 samples from scalp psoriasis and 6 from psoriasis vulgaris of SGP skin (Table SI[1]). Part of each biopsy was paraffin-embedded and used for immunohistochemistry (IHC) and part of each biopsy was stored in RNAlater (Qiagen, Hilden, Germany) at –70°C until RNA isolation for RT-PCR.

Immunohistochemistry staining

For IHC analyses, freshly prepared paraffin-embedded sections from scalp psoriasis and from psoriasis vulgaris of SGP skin were used. After samples were deparaffinized and rehydrated, samples were incubated in 3% H_2O_2 for 15 min to eliminate endogenous peroxidase activity, followed by heat-induced antigen retrieval. After blocking in 1% bovine serum albumin (BSA) solution, sections were incubated overnight at 4°C with primary antibodies against human CD4 (rabbit monoclonal IgG [ab133616]: Abcam, Cambridge, UK), human CD11c (rabbit monoclonal IgG [ab52632]: Abcam), human CD83 (mouse monoclonal IgG [ab123494]: Abcam), human CD1a (mouse monoclonal IgG [AM33361PU-T]: Acris, Rockville, MD, USA), human IL-17 (rabbit polyclonal IgG [bs-2140R]: Bioss Antibodies, Woburn, MA, USA), human IL-23 (rabbit polyclonal [PA5-20239]: Thermo Fisher, Rockford, IL, USA), human IFN-gamma (rabbit polyclonal [NBP1-19761]: Novus Biologicals, Littleton, CO, USA), human TNF-alfa (mouse monoclonal IgG [SAB1404480-100UG]: Sigma-Aldrich, St. Louis, MO , USA), human CCL2/MCP1 (mouse monoclonal IgG1 [NBP2-22115]: Novus Biologicals), human CCL20/MIP-3-α (mouse monoclonal IgG [LS-B7409]: LifeSpan Biosciences, Seattle WA, USA), human lipocalin/NGAL (rabbit polyclonal IgG [PA5-32476]: Invitrogen, Life Technologies, San Francisco, CA, USA), human S100A8 (rabbit polyclonal IgG [HPA024372]: Sigma-Aldrich), human loricrin (rabbit monoclonal IgG [NBP1-33610]: Novus Biologicals), human filaggrin (mouse monoclonal IgG: [ab17808]: Abcam), and human KRT17 (rabbit polyclonal IgG [ab53707]: Abcam). After primary antibody incubation, anti-mouse/rabbit HRP-conjugated secondary antibodies (Dako, Agilent Technologies, Santa Clara, CA, USA) were applied. Washing steps before and after incubating with antibodies were performed with TBST for 5 min, 3 times per step. Vector® VIP and ImmPACT™ NovaRED™ Kits (VECTOR Laboratories, Burlingame, CA, USA) were used to detect signals. Sections were background stained with methylene green. The detection of each protein was carried out on all sections in parallel at the same time, so that we could evaluate comparable protein levels.

RNA isolation, reverse transcription, and real-time quantitative PCR

Total RNA was isolated using TriReagent solution (Sigma-Aldrich) with Tissue Lyser (QIAGEN, Hilden, Germany) and previously autoclaved metal beads (QIAGEN). RNA concentration and purity were assessed with a NanoDrop spectrophotometer (Thermo Scientific, Bioscience, Budapest, Hungary) and an Agilent 2100 Bioanalyser (Agilent Technologies, Santa Clara, CA, USA), respectively. Samples were treated with DNase I (Applied Biosystems, Foster City, CA, USA). The reverse transcription step (1 µg RNA) was performed using a high capacity cDNA Archive Kit (Invitrogen) according to the manufacturer's instructions and the indicated thermal protocol. The qRT-PCR measurements were conducted in triplicate using pre-designed FAM-MGB assays and TaqMan® Gene Expression Master Mix from Applied Biosystems (Life Technologies).

The following primers were used: PPIA (Hs99999904_m1), IL-17A (Hs00174383_m1), IL-1β (Hs00174097_m1), IL-12B (Hs01011518_m1), IL-23 (Hs00900829_g1), IFNγ (Hs00174143_m1), TNFα (Hs00174128_m1), CCL2 (Hs00234140_m1), CCL20 (Hs00355476_m1), S100A7 (Hs00161488_m1), S100A8 (Hs00374264_g1), S100A9 (Hs00610058_m1), DEFB4B (hBD-2) (Hs00175474_m1), LCN2 (Hs01008571_m1), FLG (Hs00856927_g1), KRT17 (Hs00356958_m1), and KRT6A (Hs01699178_g1).

Reactions were performed with a LightCycler® 480 System (Roche, Basel, Switzerland). Using either the comparative Ct method or standard curves, the relative mRNA levels were calculated and normalized to the expression of PPIA mRNA.

Statistical analysis

The Kolmogorov–Smirnov test was used to assess the distribution of data. In the case of normal distribution, independent *t*-tests were used. In the absence of normal distribution, Mann–Whitney *U* tests were used for statistical comparison of 2 experimental data. *p*-values <0.05 were considered statistically significant ($*p<0.05$; $**p<0.01$; $***p<0.001$). Statistical analyses were performed using GraphPad Prism software version 7 (GraphPad Software Inc., San Diego, CA, USA) and SPSS 25 (SPSS package for Windows, Release 25; SPSS Inc., Chicago, IL, USA).

Table SI. Summary of psoriatic patient characteristics

Patients	Sex	Age, years	Localization	PASI score	Local severity	Local PASI	Duration time (years)	DLQI
PsV-SGP ($n=6$)								
PsV-SGP1	F	59	Forearm	13.3	9	18	4	N/A
PsV-SGP2	M	57	Shin	13	9	18	19	N/A
PsV-SGP3	M	46	Forearm	17.6	8	24	28	7
PsV-SGP4	M	28	Shin	10.6	8	16	7	8
PsV-SGP5	M	52	Forearm, crook of the arm	38.4	11	33	32	29
PsV-SGP6	M	34	Forearm, crook of the arm	27.6	10	40	27	23
Mean±SD		46.0±12.6		20.1±10.8	9.2±1.2	24.8±9.7	19.5±11.7	16.7±10.9
Scalp Ps ($n=6$)								
Scalp Ps1	F	59	Scalp	13.3	8	16	4	N/A
Scalp Ps2	F	35	Scalp	11	9	18	10	17
Scalp Ps3	M	62	Scalp	13.4	6	12	12	18
Scalp Ps4	M	52	Scalp	38.4	10	30	32	N/A
Scalp Ps5	F	54	Scalp	25.8	10	30	35	13
Scalp Ps6	M	36	Scalp	13.1	6	12	14	N/A
Mean±SD		49.7±11.5		19.2±10.8	8.2±1.8	19.7±8.3	17.8±12.6	16.0±2.6

Scoring of psoriasis skin was performed according to the severity of the disease. All patients were moderate to severe.
PsV: psoriasis vulgaris; SGP: sebaceous gland poor; Ps: psoriasis; SD: standard deviation; PASI: Psoriasis Area Severity Index; DLQI: Dermatology Life Quality Index.

Table SII. Comparison of immune and barrier component expression in scalp psoriasis and psoriasis vulgaris of sebaceous gland poor (SGP) skin

	qPCR		IHC	
	p	FC Scalp Ps/ PSV-SGP	*p*	FC Scalp Ps/ PSV-SGP
Th1 markers				
IFNG	0.26	1.59	0.91	1.42
IL-12	0.23	1.48	nd	
TNFA	0.20	1.08	0.56	1.46
Th17 markers				
IL-17A	0.99	0.99	0.31	0.74
IL-23	0.83	0.66	0.17	1.52
IL-1B	0.22	2.03	nd	
Innate immune/ proinflammatory molecules				
IL-1B	0.22	2.03	nd	
TNFA	0.20	1.08	0.56	1.46
Chemokines				
CCL2	0.62	0.96	0.74	0.67
CCL20	0.12	2.31	**0.02**	**1.96**
Antimicrobial peptides				
S100A7	0.13	1.17		
S100A8	0.37	1.01	0.70	1.26
S100A9	0.47	1.27	nd	
DEFB4B	0.69	1.37	nd	
LCN2	**0.005**	**1.95**	0.25	0.44
Barrier genes				
LOR	0.53	0.96	0.48	1.06
FLG	0.14	1.45	0.79	1.04
KRT6A	0.7	0.57	nd	
KRT17	0.34	0.69	0.31	0.7

Statistical analyses between mRNA and protein levels were determined by independent t-test or Mann–Whitney U test. Data with significant differences are in bold. Six samples were examined in each group for all the investigated molecules. CCL: chemokine (C-C motif) ligand; DEFB4B: human beta defensin2; FC: fold change; FLG: filaggrin; IFNG: interferon gamma; IL: interleukin; KR: keratin; LCN: lipocalin; LOR: loricrin; PsV: psoriasis vulgaris; RT-PCR: quantitative real-time PCR; TNFA: tumour necrosis factor alpha.

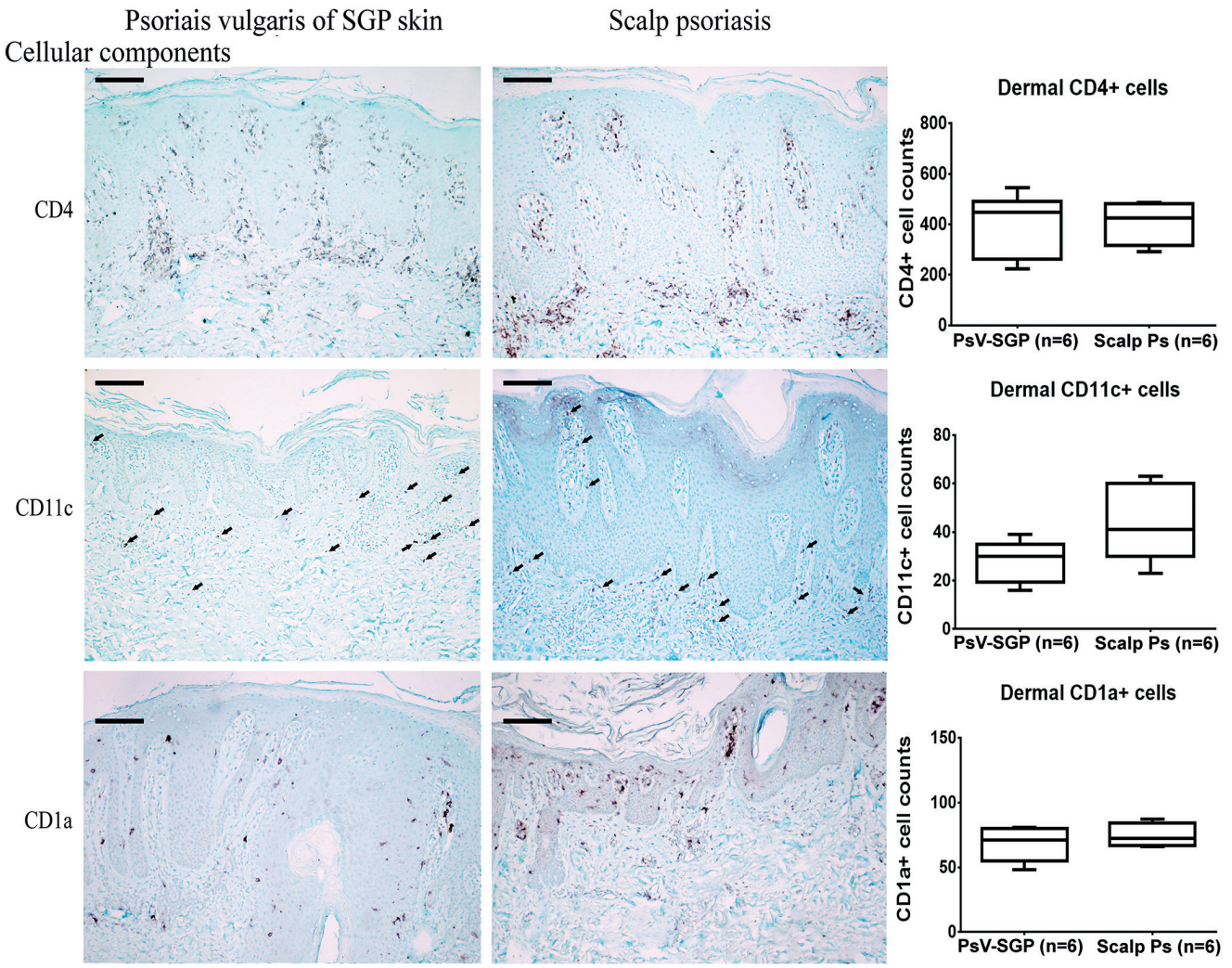

Fig. S1. Immunostaining and quantification of cellular components in psoriasis vulgaris of sebaceous gland poor (SGP) skin and scalp psoriasis samples (representative images). Regarding all the investigated molecules, 6 samples were examined in each group. Cell counts were blindly analysed by Pannoramic Viewer software. Size bars=50 μm. Arrows indicate the positive cells. The mean and the corresponding 95% upper and lower confidence intervals, as well as maximum and minimum values of protein levels, are represented by the graphs. Ps: psoriasis.

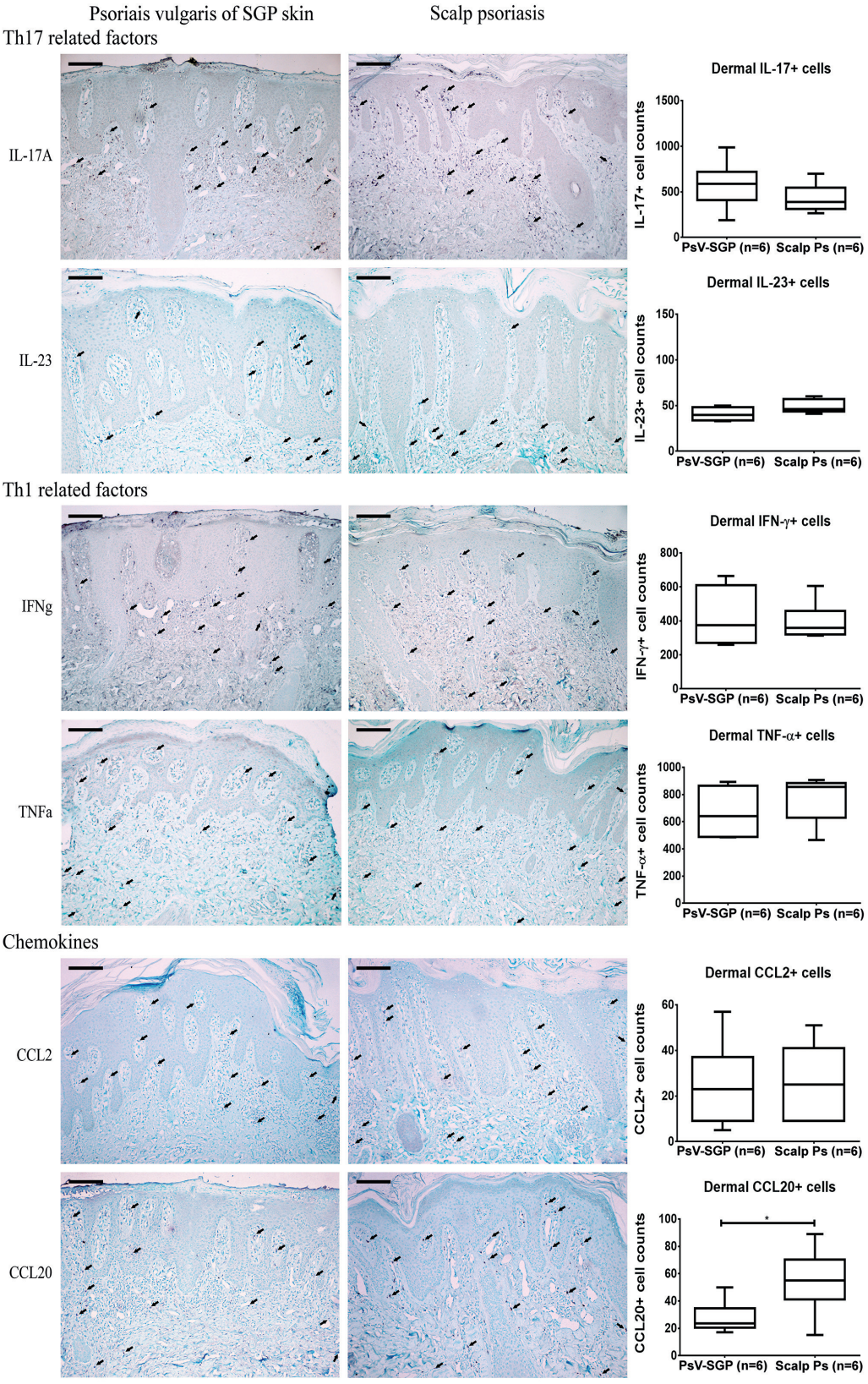

Fig. S2. Immunostaining and quantification of characteristic cytokines related to different Th subsets and Th17-related chemokines in psoriasis vulgaris of sebaceous gland poor (SGP) skin and scalp psoriasis samples (representative images). Regarding all the investigated molecules, 6 samples were examined in both groups. Cell counts were blindly analysed by Pannoramic Viewer software. Size bars=50 μm. Arrows indicate the positive cells. The mean and the corresponding 95% upper and lower confidence intervals, as well as maximum and minimum values of protein levels, are represented by the graphs. (*$p < 0.05$; as determined by independent t-test) CCL: chemokine (C-C motif) ligand; IFN-γ: interferon gamma; IL: interleukin; Ps: psoriasis; TNF: tumour necrosis factor.

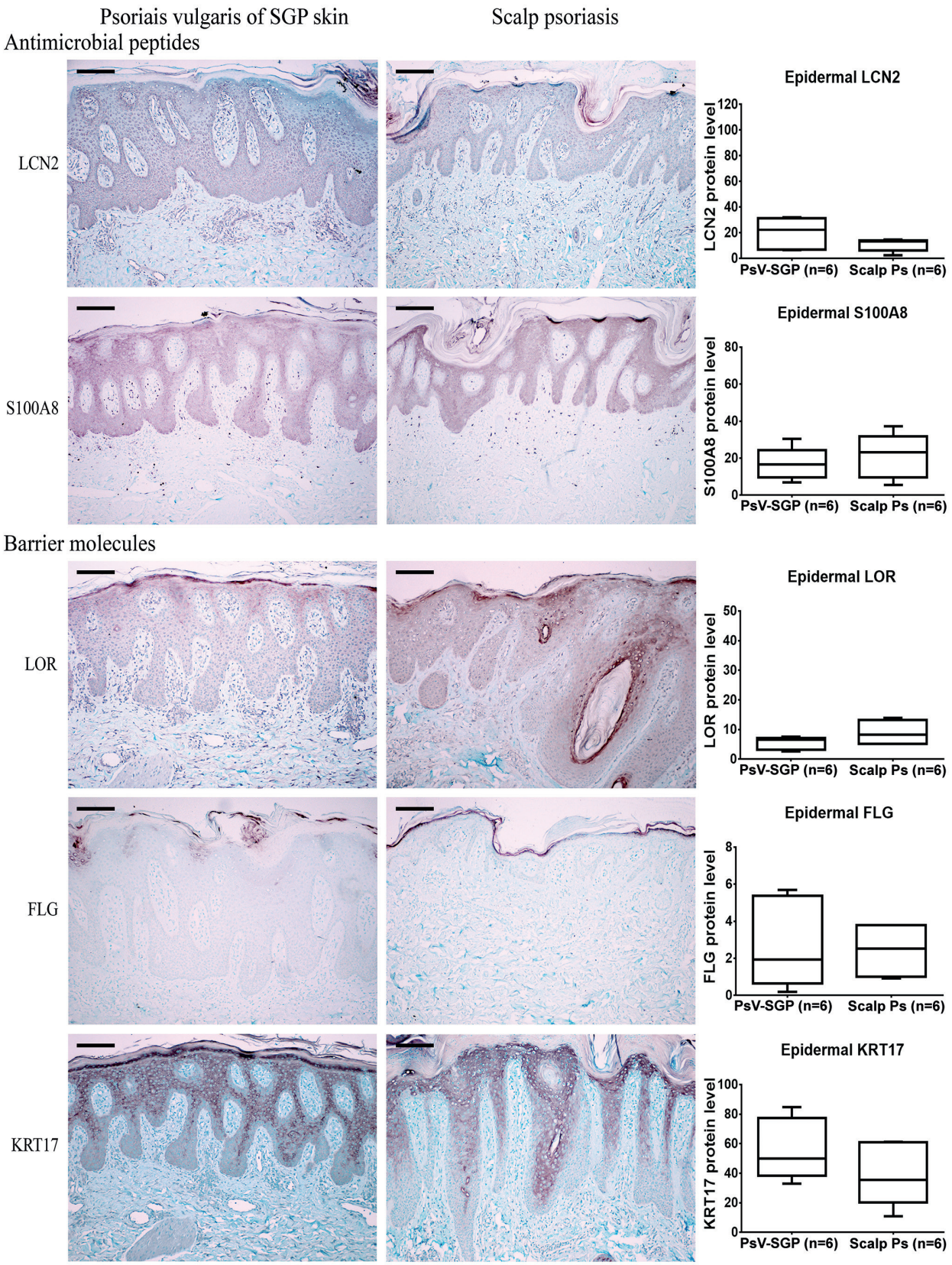

Fig. S3. Immunostaining and quantification of Th17-related AMPs and barrier molecules in psoriasis vulgaris of sebaceous gland poor (SGP) skin and scalp psoriasis samples (representative images). Regarding all the investigated molecules, 6 samples were examined in each group. Cell counts were blindly analysed by Pannoramic Viewer software. Size bars=50 μm. The mean and the corresponding 95% upper and lower confidence intervals, as well as maximum and minimum values of protein levels, are represented by the graphs. AMP: antimicrobial peptide; FLG: filaggrin; KRT: keratin; LCN: lipocalin; LOR: loricrin; Ps: psoriasis.