ORIGINAL REPORT

Antimicrobial Peptide Loss, Except for LL-37, is not Characteristic of Atopic Dermatitis

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Atopic dermatitis is an inflammatory skin disease characterized by significant permeability barrier damage. Regulation and maintenance of permeability and antimicrobial skin barriers are strongly connected. There is a lack of comprehensive studies of the expression of all 5 major antimicrobial peptide functional groups in atopic dermatitis. The aim of this study was to investigate the major antimicrobial peptide functional groups in lesional atopic dermatitis, non-lesional atopic dermatitis, and healthy control samples, using real-time quantitative PCR and immunohistochemistry. Lesional psoriatic skin was also examined as a diseased control. No differences in mRNA levels were detected between non-lesional atopic dermatitis and healthy control skin, and, at the protein level, the only change was the significantly decreased LL-37 in non-lesional atopic dermatitis. In lesional atopic dermatitis, several antimicrobial peptides were significantly altered at the mRNA level, while, at the protein level, all antimicrobial peptides were significantly upregulated or unchanged, except for LL-37, which decreased, compared with healthy controls. Antimicrobial peptides were similarly elevated in lesional atopic dermatitis and lesional psoriatic skin, with somewhat higher expression in lesional psoriatic skin, except for LL-37. In conclusion, LL-37 was the only antimicrobial peptide that was impaired in both non-lesional and lesional atopic dermatitis, highlighting its potential pathogenetic or exacerbating role in the initial stages of the disease.

Key words: antimicrobial peptide; atopic dermatitis; skin barrier; psoriasis.

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SIGNIFICANCE

Data regarding antimicrobial peptides in atopic dermatitis are incomplete, and many discrepancies exist, which may be because the expression of antimicrobial peptides has often been compared with psoriatic rather than control skin. This study comprehensively analysed the main antimicrobial peptide representatives, at both the mRNA and protein levels, in clinically asymptomatic and symptomatic skin from patients with atopic dermatitis, and examined diseased control (psoriatic) and healthy control samples. The facts that the only impairment was a lack of induction of LL-37, and that LL-37 is associated with all the major pathogenic features of atopic dermatitis, indicate a driver role for LL-37 in the pathophysiology of atopic dermatitis, and raise the possibility of its therapeutic potential.

The skin is the primary line of defence of the human body. In addition to providing a physical barrier, the skin senses, transmits, and responds to signals from the outside world via its antimicrobial and immunological barriers (1, 2). The antimicrobial barrier is formed by antimicrobial peptides (AMPs), which have both antimicrobial activity and immunomodulatory effects under homeostatic and inflammatory conditions (3). AMPs are classified into 5 groups based on their functions: classic AMPs, AMPs with protease inhibitor or enzymatic activity, AMPs with chemokine activity, AMPs with neuropeptide activity, and AMPs that do not fit into any other group (4).

AMPs are key factors in the pathogenesis of several immune-mediated skin diseases, including Th1/Th17-driven psoriasis vulgaris (PsV) and rosacea (5). AMP levels are notably increased in these skin diseases. In contrast, the role of AMPs in the pathogenesis of Th2/Th22-driven atopic dermatitis (AD) is less obvious, there are several uncertainties in this topic. Some contradictions have arisen because AD has been compared with PsV samples without healthy controls in several studies. Furthermore, only mRNA levels were measured in some studies, non-lesional AD (AD NL) samples were not involved in many studies, and the role of AMP functional groups in AD was not examined.

The aim of the current study was to comprehensively analyse AMPs in AD NL and lesional AD (AD L) skin compared with healthy controls. Lesional PsV (PsV L) samples were also analysed as diseased controls. Expression of the keratinocyte-expressed representative members of all 5 main functional AMP groups was investigated at the mRNA level by real-time quantitative PCR (RT-qPCR) and at the protein level by immunohistochemistry (IHC) combined with quantification following whole-slide imaging.

Table I. Characteristics of healthy individuals and patients included in the study

Subjects	Sex	Age, years	Localization
Healthy individuals ($n=10$)			
HC 1	F	51	Upper arm
HC 2	F	45	Thigh
HC 3	F	50	Upper arm
HC 4	M	34	Upper arm
HC 5	M	44	Shin
HC 6	F	52	Thigh
HC 7	M	48	Upper arm
HC 8	M	39	Thigh
HC 9	F	43	Upper arm
HC 10	F	63	Forearm
Mean age±SD		46.90±7.95	
Atopic dermatitis individuals ($n=10$)			
AD1	M	33	Back
AD2	F	49	Knee
AD3	M	39	Waist
AD4	M	39	Arm
AD5	F	25	Forearm
AD6	M	27	Forearm
AD7	F	50	Waist
AD8	F	33	Upper arm
AD9	M	29	Elbow
AD10	F	19	Forearm
Mean age±SD		34.3±10.06	
Psoriasis vulgaris individuals ($n=5$)			
PsV1	M	32	Forearm
PsV2	F	35	Forearm
PsV3	M	48	Waist
PsV4	M	63	Elbow
PsV5	F	61	Elbow
Mean age±SD		47.8±14.3	

AD: atopic dermatitis; PsV: psoriasis vulgaris; SD: standard deviation.

MATERIALS AND METHODS

Skin biopsies

Biopsies were collected from the lesional and non-lesional skin of 10 AD patients with chronic symptoms, from the lesional skin of PsV patients, and from the corresponding skin regions of 10 healthy individuals (**Table I**), as distinct healthy skin regions have different immune activity (2, 6, 7). Written, informed consent was obtained, according to the principles of the Declaration of Helsinki, and the study was approved by the local ethics committee (Regional Institutional Research Ethics Committee, Clinical Center, University of Debrecen, Debrecen, Hungary; study i.d.: IV/2072-2/2020/EKU). One part of the biopsies was stored in RNAlater (Qiagen, Hilden, Germany) at –70°C until RNA isolation for RT-qPCR, the other part of the biopsies was formalin-fixed and paraffin-embedded and used for IHC.

Real-time quantitative PCR

Sample preparation and reactions were performed as described previously (2, 6). The oligo sets used are shown in Appendix S1.

Immunohistochemistry

Freshly prepared paraffin-embedded sections of skin from AD and PsV patients and healthy controls were used. IHC experiments and quantification were performed as described previously (2, 6). The primary and secondary antibodies used are shown in Appendix S1.

Statistical analysis

Statistical significance was determined by 1-way analysis of variance (ANOVA) and Newman-Keuls post hoc tests. Graphs show the means and the corresponding 95% confidence intervals (95% CI) (boxes) and maximum/minimum values of protein levels (Figs 1 and 2).

RESULTS

Antimicrobial peptide mRNA expression in non-lesional atopic dermatitis and lesion atopic dermatitis skin

First, the study detected and compared the mRNA levels of AMPs in AD NL and healthy control skin samples. Significant change was barely detectable; only 2 AMPs were found to be significantly differentially expressed at the mRNA level, namely *RNASE7* with enzymatic activity was downregulated, while secretory leukocyte peptidase inhibitor (*SLPI*) was expressed at higher levels in AD NL samples vs healthy controls (**Fig. 1**, Table SI). Other AMPs were present at similar levels in the 2 sample groups (Fig. 1, Table SI).

Next, AD L samples were compared with control skin. The classic AMPs, the expression of *DEFB4B* and *DEFB104A* (encoding human beta defensin (hBD)-2 and hBD-4, respectively) were significantly higher in AD L skin compared with control skin (Fig. 1, Table SI). In contrast, *DEFB1* (encoding hBD-1) mRNA showed an opposite trend, with significant differences between AD L and control skin. Gene expression levels of *DEFB103A/DEFB103B* and *CAMP* (encoding hBD-3 and cathelicidin/LL-37, respectively) were not significantly different between the sample groups (Fig. 1, Table SI). Regarding AMPs with protease inhibitor or enzymatic activity, peptidase inhibitor 3 (*PI3*) and lysozyme (*LYZ*), were significantly higher, while angiogenin (*RNASE5/ANG*) and *RNASE7* mRNA levels were significantly lower in AD L skin compared with the levels in healthy controls. *SLPI* gene expression levels were similar in AD L and healthy skin (Fig. 1, Table SI). Regarding AMPs with chemokine activity, the mRNA expression levels of S100 calcium-binding protein A molecules were significantly higher in AD L skin compared with the levels in control skin (Fig. 1, Table SI). No significant difference in *C-C motif chemokine ligand (CCL)20* was observed between the sample groups. The gene expression levels of adrenomedullin (*ADM*), which has neuropeptide activity, were significantly lower in AD L skin vs controls. Finally, *lipocalin-2 (LCN2)* gene expression levels were significantly elevated in AD L skin (Fig. 1, Table SI).

Protein expression of antimicrobial peptides in non-lesional atopic dermatitis and lesion atopic dermatitis skin

As proteins are the functional forms of molecules, and mRNA and protein expression do not always coincide due to transcriptional modifications, representatives of all AMP functional groups were subsequently determined and quantified at the protein level, using IHC following whole-slide imaging.

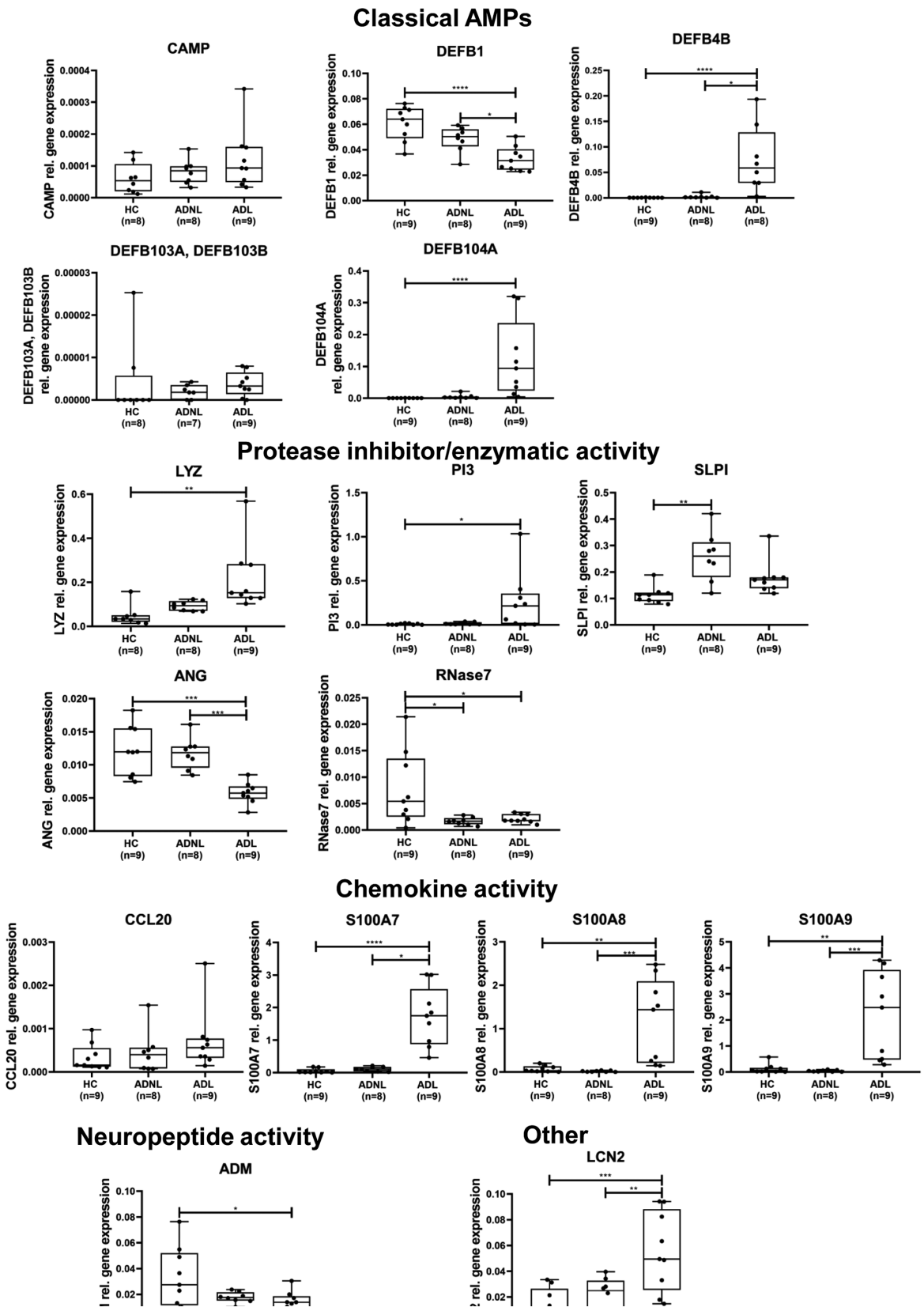

Fig. 1. Antimicrobial peptide (AMP) mRNA levels detected by real-time quantitative PCR (RT-qPCR) in atopic dermatitis lesional (AD L), AD non-lesional (NL), and healthy control (HC) skin samples AMPs are classified into functional groups. The graphs show the median ±95% confidence interval (95% CI) (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, determined by 1-way analysis of variance (ANOVA) 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). DEFB: defensin beta; CAMP: cathelicidin antimicrobial peptide; LYZ: lysozyme; ANG: angiogenin; SLPI: secretory leukocyte peptidase inhibitor; CCL: chemokine (C-C motif) ligand; S100: S100 calcium-binding protein; ADM: adrenomedullin; LCN. lipocalin.

When comparing AD NL and control groups, in line with the mRNA findings, similar protein levels were detected for almost all AMPs (hBDs, RNase7, CCL20, S100A8, ADM, LCN2), except LL-37 levels, which were significantly lower in AD NL skin compared with control skin (**Fig. 2**, Table SII).

When comparing AD L and healthy controls, most investigated AMPs (6 of 9) were significantly upregulated in AD L skin compared with healthy control skin, in agreement with the mRNA results (Fig. 2, Table SII). Two AMPs were present at similar levels, while only 1 AMP was significantly lower in AD L skin compared with control skin. Concerning classic AMPs, hBDs were present at significantly higher levels, while LL-37 was significantly downregulated in AD L skin compared with controls (Fig. 2, Table SII). The protein levels of CCL20 and S100A8 were significantly higher in AD L skin compared with healthy control skin. No significant differences in RNase7 and ADM protein levels were detected between the AD L and healthy skin samples. However, LCN2 level was significantly higher in AD L skin vs controls (Fig. 2, Table SII).

Regarding the staining pattern of the investigated AMPs, it was found that LCN2, hBD-4, ADM, and hBD-4 were homogeneous. LCN2 and hBD-4 staining was strong in AD L samples, while ADM staining was weak and LL-37 staining was barely detectable in AD samples. Concerning hBD-1, hBD-2, S100A8, and LCN2, they were localized mainly in the apical part of the epidermis, with decreasing levels towards the basal keratinocytes in all sample groups. Strong staining for RNase7 was observed in the stratum corneum and the upper granular epidermal layers in all sample groups.

In summary, only LL-37 showed significantly decreased levels in AD NL vs controls, while other AMPs were not induced. AMPs with significantly decreased mRNA levels did not show a decrease at the protein level in AD L samples. AMP protein levels in AD L were significantly increased in most cases, 2 were unchanged, and LL-37 was the only AMP that was lower in AD L vs control.

Comparison of antimicrobial peptide protein levels in lesion atopic dermatitis and lesional psoriasis vulgaris skin

It was hypothesized that some inconsistencies in the literature may be due to AD AMP levels being compared with PsV skin instead of healthy skin. Thus, immunostainings of AMPs in PsV L samples were performed to clarify this discrepancy.

It was found that PsV L skin had highly and significantly elevated protein expression compared with the control skin for most AMPs. ADM and RNase7 exhibited prominent staining, but the levels were not significantly different in PsV L skin compared with healthy control skin (Fig. 2).

When comparing AD L and PsV L, interestingly, the protein levels of most AMPs were similar in AD L and PsV L skin (Fig. 2). In contrast, LL-37 and CCL20 were highly and significantly upregulated in PsV L vs AD L skin, while ADM showed a similar trend with prominently higher levels in PsV L vs AD L (Fig. 2).

DISCUSSION

AMPs play a prominent role in the pathogenesis of several immune-mediated skin diseases. In PsV and rosacea, the production of these molecules is highly induced in keratinocytes, and some AMPs play an initial role in disease pathogenesis (5, 8, 9).

The impaired skin permeability barrier has been demonstrated as a major disease-driver factor in AD (10–12). The literature also suggests that the regulation of the skin permeability and antimicrobial barriers is closely connected (3, 13, 14). However, the examination of AMPs in AD is incomplete, and many discrepancies exist regarding the extent of AMP expression. These may partly be because AMP expression has often been compared with PsV rather than normal controls (**Table II**). In addition, in many cases, AMPs have been studied only at the mRNA level, which can be misleading, as proteins are the functional forms of the molecules. In most cases, studies did not include AD NL skin samples, which could provide critical data concerning the initial steps of disease pathophysiology (Table II). Lastly, to date, no study has investigated AMPs in AD by considering which functional subgroup each AMP belongs to.

The current study comprehensively analysed the 5 functional AMP groups, both at the mRNA and protein levels. AD NL (clinically asymptomatic) and AD L (clinically symptomatic) skin of patients with AD were compared with that of healthy controls. PsV is a chronic inflammatory skin disease with clinical and immunological characteristics which are completely distinct from those of AD; barrier damage is probably not the driver of this disease, thus this study also examined PsV L samples as a diseased control.

When comparing AMP levels in AD NL and control samples, no prominent difference was detected at the mRNA level, and only 2 AMPs showed significant alteration. At the protein level, only LL-37 showed alteration, as it was significantly decreased in AD NL. Limited data are available on AMPs' protein expression in AD NL skin, and no studies have covered most AMPs at the same time. In line with the current findings, no prominent differences have been detected in AD NL vs controls (Table II).

Several AMPs were significantly altered at the mRNA level in AD L skin, and most AMPs were elevated compared with controls. At the protein level, AMP expression was prominently increased in AD L skin compared with controls, while 2 AMPs were unchanged and LL-37 was the only AMP with a highly decreased level in AD

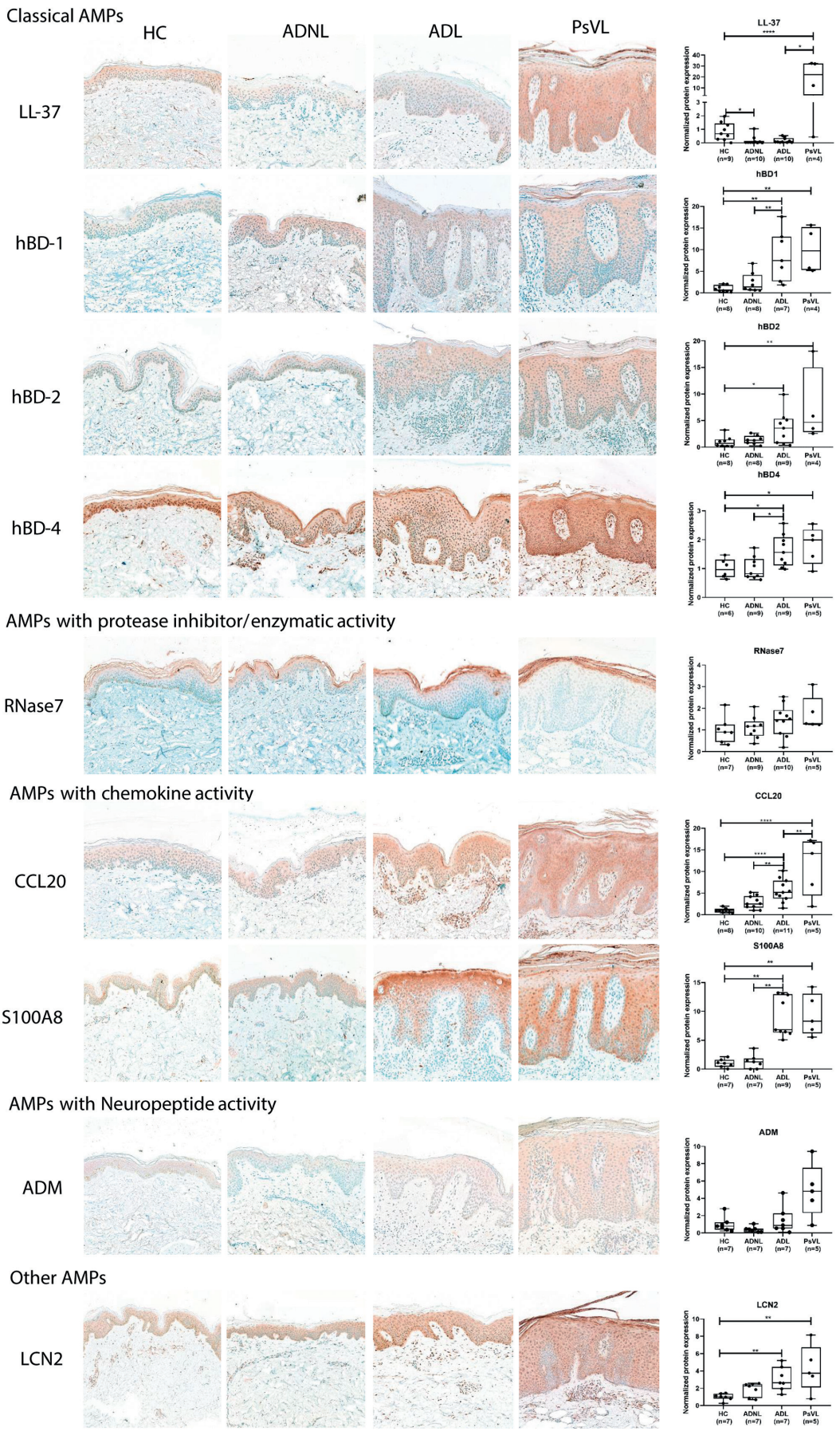

Fig. 2. Representative images for immunostaining and epidermal quantification of antimicrobial peptides (AMPs) classified into functional groups in atopic dermatitis lesional AD L, AD non-lesional (NL), psoriasis vulgaris lesional (PsV L), and healthy control (HC) skin samples. The graphs show the median ± 95% confidence interval (95% CI) of measured protein levels ($*p < 0.05$; $**p < 0.01$; $***p < 0.001$, as determined by 1-way analysis of variance (ANOVA) followed by Sidak'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). hBD: human beta-defensin; CCL: chemokine (C-C motif) ligand; S100: S100 calcium-binding protein; ADM: adrenomedullin; LCN: lipocalin. original magnification: 200x.

Table II. Currently available literature about antimicrobial peptides (AMPs) in atopic dermatitis (AD) and psoriasis vulgaris (PsV)

	AD NL vs healthy		AD L vs healthy		PsV L vs AD L	
	mRNA (RT-qPCR, MA, RNAseq)	protein (TS, IHC, WF)	mRNA (RT-qPCR, MA, RNAseq)	protein (TS, IHC, WF)	mRNA (RT-qPCR, MA, RNAseq)	protein (TS, IHC, WF)
hBD-1	nd	nd	↓ in AD L (RT-qPCR) (24) no difference (RT-qPCR) (18) ↑ in AD L (RT-qPCR) (25)	nd	NS (RT-qPCR) (18, 25) ↓ in AD L (MA) (18, 26)	nd
hBD-2	NS (RT-qPCR) (27) NS (TS/RT-qPCR) (28) ↑ AD NL (TS/RNAseq) (28) ↑ in AD NL (TS-RT-qPCR) (29)	NS (IHC) (30) NS (TS/WB) (31) NS (TS/ELISA) (32, 33) NS (WF) (30)	↑ in AD L (RT-qPCR) (18, 24, 25) NS (RT-qPCR) (34, 35) ↑ AD L (TS/RT-qPCR) (28, 29) ↑ AD L (TS/RNAseq) (28)	↑ in AD L (IHC) (30, 35) NS (IHC) (18, 19, 34) ↑ in AD L (TS/ELISA) (31–33) ↑ AD L (TS/WB) (31) ↑ in AD L (WF) (30)	↓ in AD L (RT-qPCR) (18, 24, 35, 36) ↓ in AD L (MA) (18, 26, 37) ↓ AD L (TS/RT-qPCR) (28) ↓ AD L (TS/RNAseq) (28) NS (RT-qPCR) (27)	↓ in AD L (IHC) (18, 19, 30, 35) NS (TS) (31) ↓ in AD L (WF) (30)
hBD-3	NS (RT-qPCR) (27)	↑ in AD NL (IHC) (30) NS (TS/ELISA) (33) not detectable (WF) (30)	↑ in AD L (RT-qPCR) (18, 25) NS (RT-qPCR) (24, 26, 36)	↑ in AD L (IHC) (30) NS (IHC) (18, 26) NS (TS/ELISA) (33) not detectable (WF) (30)	↓ in AD L (RT-qPCR) (26) NS (RT-qPCR) (18, 25, 27) NS (MA) (18)	↓ in AD L (IHC) (26, 30) NS (IHC) (18) not detectable (WF) (30)
hBD-4	nd	nd	nd	nd	nd	nd
LL-37	NS (RT-qPCR) (27, 36, 38, 39) NS (TS/RT-qPCR) (28) ↑ AD NL (TS/RNAseq) (28) NS (TS/RT-qPCR) (29)	NS (IHC) (38) not detectable (TS/ELISA) (33)	↑ in AD L (RT-qPCR) (38) NS (RT-qPCR) (18, 24, 25, 34-36, 39) ↑ AD L (TS/RT-qPCR) (28, 29) ↑ AD L (TS/RNAseq) (28)	↑in AD L (IHC) (38) NS (IHC) (19, 36) NS (WB) (34) not detectable (TS/ELISA) (33) NS (IF) (34) not detectable (WB) (19)	↓ in AD L (RT-qPCR) (19, 35, 36, 39) NS (RT-qPCR) (18, 25, 27) NS (MA) (18) NS (TS/RT-qPCR) (28) NS (TS/RNAseq) (28)	↓ in AD L (IHC) (19)
RNase7	nd	NS (TS/ELISA) (33) NS (WF) (30) NS (IHC) (30)	↑ in AD L (RT-qPCR) (25)	NS (IHC) (30) NS (TS/ELISA) (33) ↑ in AD L (WF) (30)	↑ in AD L (RT-qPCR) (25)	NS (IHC) (30) NS (WF) (30)
Psoriasin	↑ AD NL (TS/RNAseq) (28)	↑ in AD NL (IHC) (40) NS (TS/ELISA) (33) ↑ in AD NL (WF) (30, 40) NS (IHC) (30)	↑ in AD L (RT-qPCR) (25) NS (RT-qPCR) (35) ↑ AD L (TS/RNAseq) (28)	↑ in AD L (IHC) (30, 35, 40) NS (TS/ELISA) (33) ↑ in AD L (WF) (30, 40)	↓ in AD (RT-qPCR) (18) NS (RT-qPCR) (25) ↓ in AD L (MA) (18) NS (TS/RNAseq) (28)	↓ in AD L (IHC) (30, 35, 40) ↓ in AD L (WF) (30)
Dermcidin	nd	nd	nd	nd	nd	nd
RNase5	nd	nd	nd	nd	nd	nd
ADM	nd	nd	nd	nd	nd	nd
Elafin	↑ AD NL (TS/RT-qPCR) (28) ↑ AD NL (TS/RNAseq) (28)	nd	↑ AD L (RT-qPCR) (18) NS (RT-qPCR) (35) ↑ AD L (TS/RT-qPCR) (28) ↑ AD L (TS/RNAseq) (28)	NS (IHC) (18)	↓ in AD L (RT-qPCR) (35) NS (RT-qPCR) (18) ↓ in AD L (MA) (18, 37) ↓ AD L (TS/RNAseq) (28)	↓ in AD L (IHC) (18) ↓ AD L (TS/RT-qPCR) (28)
SLPI	nd	nd	nd	NS (IHC) (18)	nd	↓ in AD L (IHC) (18)
LYZ	nd	nd	nd	nd	nd	nd
CCL20	↑ AD NL (TS/RT-qPCR) (28, 29) ↑ AD NL (TS/RNAseq) (28)	nd	↑ AD L (TS/RT-qPCR) (28, 29) ↑ AD L (TS/RNAseq) (28)	NS (IHC) (35)	↓ AD L (TS/RT-qPCR) (28) ↓ AD L (TS/RNAseq) (28)	↓ in AD L (IHC) (35)
S100A8	↑ AD NL (TS/RNAseq) (28)	nd	↑ AD L (RT-qPCR) (18) ↑ AD L (TS/RNAseq) (28)	↑ AD L (IHC) (18)	↓ in AD L (MA) (18) NS (RT-qPCR) (18) NS (TS/RNAseq) (28)	NS (IHC) (18)
S100A9	↑ AD NL (TS/RT-qPCR) (28, 29) ↑ AD NL (TS/RNAseq) (28)	nd	↑ AD L (RT-qPCR) (18) ↑ AD L (TS/RT-qPCR) (28, 29) ↑ AD L (TS/RNAseq) (28)	↑ AD L (IHC) (35)	↓ in AD L (RT-qPCR) (18) ↓ in AD L (MA) (18) ↓ AD L (TS/RT-qPCR) (28) ↓ AD L (TS/RNAseq) (28)	↓ in AD L (IHC) (35)
LCN2	NS (TS/RNAseq) (28)	nd	NS (RT-qPCR) (35) NS (TS/RNAseq) (28)	NS (IHC) (35)	↓ in AD L (RT-qPCR) (35) ↓ AD L (TS/RNAseq) (28) ↓ AD L (MA) (37)	↓ in AD L (IHC) (35)

Arrows indicate direction of change.
AD L: lesional atopic dermatitis skin; AD NL: non-lesional AD skin; IHC: immunohistochemistry; MA: microarray; nd: not determined; NS: no significant difference; PsV L: lesional psoriatic skin; TS: tape-stripping; WB: Western blot; WF: washing fluid; nd: not detected; hBD: human beta defensin; ADM, adrenomedullin; SLPI: secretory leukocyte Peptidase inhibitor; LYZ: lysozime; CCL20: C-C Motif Chemokine Ligand 20; S100A: S100 calcium-binding protein A; LCN2: lipocalin 2.

L skin. The published data are contradictory, despite the relatively high number of studies. Review articles emphasize that AMP expression is generally decreased in AD L skin; however, original studies showed predominantly elevated or unchanged AMP levels (Table II). Most data are available at the mRNA level, and the quantification of IHC results was either missing or subjective in most cases. Regarding acute AD, limited studies are available, which suggest that AMPs (S100A7, hBD-2, RNase7) are already highly induced and are not substantially enhanced in the acute-chronic transition (Table II).

When comparing AD L with PsV L samples, most AMPs were prominently induced in both diseases, with similar expression patterns; however, in many cases, AMP levels in AD did not reach the levels found in PsV. LL-37 showed the most striking difference between the 2 diseases, as the level was significantly increased in PsV lesions, but significantly decreased in AD skin vs controls. In line with the current results, the elevation of most AMPs in both diseases has also been demonstrated in other studies (Table II).

Reviewing the literature, data related to LL-37 in AD appear to be controversial. Only 1 study showed that LL-37 levels are higher in AD L compared with controls (Table II); however, the authors were unable to detect LL-37 by IHC in several AD L samples, which coincide with the current findings. Some investigations failed to detect significant differences in the LL-37 protein levels between AD L and controls (Table II). Finally, in several studies, LL-37 was below the limit of detection, in agreement with the current finding (Table II).

The finding in the current study that LL-37 was the only decreased AMP raises the question of whether LL-37 may play an important role in the pathogenesis of AD. Interestingly, literature data suggest that LL-37 is associated with all 3 major pathogenetic features of AD, including barrier damage, Staphylococcal hypercolonization, and Th2 inflammation. Under healthy conditions, a

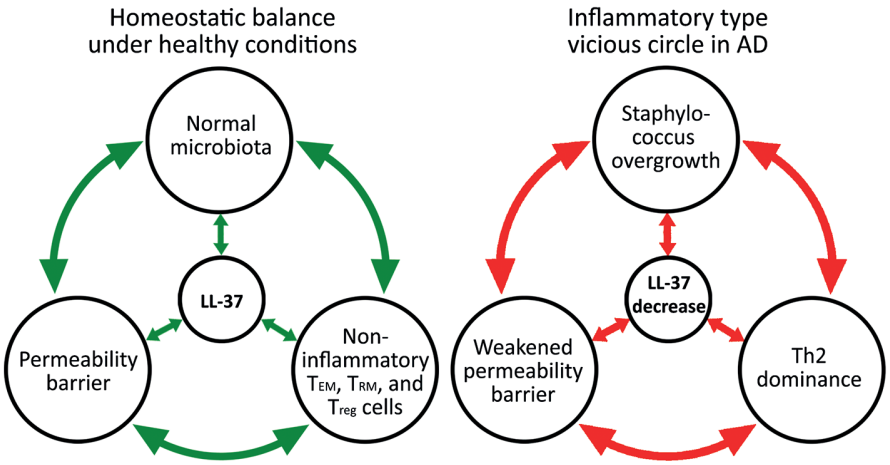

Fig. 3. The possible link between the 3 main pillars of atopic dermatitis (AD) pathogenesis. *Left panel:* Under steady-state conditions, the permeability barrier, the antimicrobial barrier, and the microbiota colonizing the skin (including *Staphylococcus* species) are in dynamic equilibrium. The homeostatic LL-37 level prevents the overgrowth of *Staphylococcus* species, and the intact permeability barrier limits their penetration into the skin. In addition, LL-37 enhances the function of the permeability barrier by promoting the expression of tight junction molecules. This homeostatic environment maintains a non-inflammatory effector memory (TEM), resident memory T cell (TRM), and Treg milieu. *Right panel:* Based on that LL-37 was the only AMP that showed significantly reduced levels already in non-lesional AD skin and in a disease-specific manner, we propose that LL-37 decrease could be one of the initial steps of disease pathophysiology. LL-37 loss leads to an increased ratio of *Staphylococcus* species. Staphylococcal proteolytic activity cleaves LL-37 and the desmosome component DSG1 protein, which can further enhance Staphylococcal colonization. As LL-37 levels decrease, positive feedback from the permeability barrier-maintaining capacity of LL-37 is abolished, resulting in a decreased expression of molecules composing tight junctions. This leads to further barrier damage, which promotes the hypercolonization of *Staphylococcus* species. Barrier damage also promotes alarmin production by keratinocytes, which mediators initiate the maturation and proliferation of inflammatory Th2 cells, leading to a vicious circle.

homeostatic balance is established between LL-37, the permeability barrier, and the microbiota, which maintains a non-inflammatory T cell (effector and resident memory) and Treg environment. However, literature data suggest that permeability barrier damage is strongly connected with LL-37 loss and Staphylococcal overgrowth. Decreased LL-37 levels lead to weakened tight junctions and impaired skin barrier function, since LL-37 is known to enhance the expression of tight junction molecules (e.g. claudins) that maintain skin permeability barrier (15). In addition, Staphylococcal overgrowth can damage both the antimicrobial and permeability barriers, as the cysteine protease (EcpA) activity of *S. epidermidis* was demonstrated to cleave LL-37 and a major desmosome component DSG1 *in vitro* (16). These findings are even more important considering that Staphylococcal density is substantially increased even on AD NL skin (17). Moreover, LL-37 loss may increase the susceptibility to *Staphylococcus* hypercolonization in AD (5, 18, 19), since LL-37 is highly effective and more potent against *Staphylococcus* species and biofilms than other AMPs, such as hBDs (20–22). In addition, during barrier damage, keratinocytes produce alarmins; mediators that initiate the promotion of Th2 cells. Finally, AD-specific Th2 cytokine milieu is known to inhibit the induction of LL-37 *in vitro*, which is in line with the decreased LL-37 levels in AD lesions in the current study *in situ* (11, 23).

In conclusion, AMPs were generally unchanged or increased in AD lesions compared with controls. The lack of LL-37 induction was the only impairment related to AMPs at the protein level in AD. The pattern and extent of AMP expression showed remarkable similarities in AD and PsV, except for LL-37. A prominent role of LL-37 in AD pathogenesis can be easily envisioned, as LL-37 is associated with all 3 major pathogenetic features of AD, including barrier damage, Staphylococcal hypercolonization, and Th2 inflammation (**Fig. 3**). The significantly decreased levels of LL-37 in AD NL skin indicate that LL-37 may play a driver role in the pathogenesis of AD, and raises the potential of LL-37 as a therapeutic target in the treatment of AD.

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