ORIGINAL REPORT

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

Lilla SZABÓ[1,2†], Anikó KAPITÁNY[1,3†], Orsolya SOMOGYI[1–3], Iman ALHAFEZ[1], Krisztián GÁSPÁR[1], Réka PALATKA[1,2], Lilla SOLTÉSZ[1,2], Dániel TÖRŐCSIK[1,3], Zoltán HENDRIK[4], Zsolt DAJNOKI[1#] and Andrea SZEGEDI[1,3#]

[1]Department of Dermatology, Centre of Excellence, Faculty of Medicine, University of Debrecen, Debrecen, Hungary, [2]Gyula Petrányi Doctoral School of Allergy and Clinical Immunology, University of Debrecen, Debrecen, Hungary, [3]ELKH-DE Allergology Research Group, Debrecen, Hungary and [4]Department of Forensic Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

[†]These authors contributed equally to this work. [#]These authors contributed equally to this work.

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.

Accepted May 30, 2023; Published Jun 30, 2023

Acta Derm Venereol 2023; 103: adv9413.

DOI: 10.2340/actadv.v103.9413

Corr: Andrea Szegedi, Department of Dermatology, Faculty of Medicine, University of Debrecen, HU-4032 Debrecen, Hungary. E-mail: aszegedi@med.unideb.hu

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.