

In vivo expression of antimicrobial peptides in atopic dermatitis

Maja-Lisa Clausen¹, H-C Slotved², Karen A. Krogh², Paal Skytt Andersen^{2,3} and Tove Agner¹

¹Department of Dermatology, Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark; ²Department of Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark; ³Veterinary Disease Biology, University of Copenhagen, Copenhagen, Denmark
Correspondence: Maja-Lisa Clausen, Department of Dermatology D92, Bispebjerg Hospital, 2. Tværvej, opg. 9 – 2. Sal, Bispebjerg Bakke 23, 2400 Copenhagen NV, Denmark, Tel.: +45 3531 6005, Fax: +45 3531 6010, e-mail: mlclausen@dadlnet.dk

Abstract: The aim of this review is to present findings on expression of antimicrobial peptides (AMPs) in atopic dermatitis (AD) skin, focusing only on *in vivo* studies, and to discuss differences in results obtained using various skin sampling techniques and different methodology for analysis of AMPs. The review also includes a discussion of the effect of frequently used treatments on AMP expression. Many studies have shown a reduced level of AMPs in lesional AD skin when compared to psoriatic skin, explaining the high frequency of AD-related infections. Interestingly, however, non-lesional AD skin has shown the same upregulation of AMPs after barrier disruption as non-lesional psoriatic skin. Various methods have been used to analyse AMP expression in the skin, and when comparing these methods, differences are revealed in AMP expression depending on the method used for sampling and analysis. Comparisons indicate that analyses of mRNA levels of AMPs may find greater differences in expression than analyses of protein levels. Few studies evaluate the

effect of topical treatments on the expression of AMPs, and these indicate an inhibition of AMP expression, particularly after use of corticosteroids. AMPs are important components of the skin as a defense against infections, and despite much research, the clinical importance of the effect of common treatments, including systemic treatments for AD and the interplay between AMPs and the skin microbiome, is still largely unknown.

Abbreviations: AD, Atopic Dermatitis; AMP, Antimicrobial Peptide; ELISA, Enzyme-Linked Immunosorbent Assay; hBD-1, human Beta-Defensin-1; hBD-2, human Beta-Defensin-2; hBD-3, human Beta-Defensin-3; HC, Healthy Controls; MRSA, Methicillin Resistant *S. aureus*; mRNA, messenger Ribonucleic Acid; PV, Psoriasis Vulgaris; RT-PCR, Real-time Polymerase Chain Reaction; TEWL, Trans Epidermal Water Loss.

Key words: AD – AMP – antimicrobial peptides – atopic dermatitis

Accepted for publication 28 July 2015

Introduction

Atopic dermatitis (AD) affects up to 20% of children and 2–10% of adults worldwide (1). It is a multifactorial skin disease, including genetic, environmental, allergic and immunological elements, associated with a defective skin barrier and an increased susceptibility to skin infections (1,2). Despite extensive research, the pathogenesis of AD is still unclear.

The discovery of filaggrin mutations has led to renewed interest in the skin barrier as a key element in the pathogenesis of AD, although only around 40% of AD patients with moderate/severe disease carry filaggrin mutations (3–5). In AD, the skin barrier is compromised in several ways by the following: decreased ceramides, altered packaging of keratinocytes, decreased numbers of tight junction proteins, aberrant protease activity and altered antimicrobial activity, thus leaving the patient susceptible to skin infections, allergen sensitisation and continued inflammation (6). The increased susceptibility to infections, in particular, may cause repeated flares and sustained activity of the disease; therefore, attention has been drawn to the antimicrobial skin barrier, and AMPs have been considered as a possible new element in AD treatment.

The first AMP was discovered in the 1920s by Alexander Fleming, with the antimicrobial activity of lysozyme (7). Since then, numerous AMPs have been classified, and today, more than 2000 are registered in the antimicrobial peptides database (APD, <http://aps.unmc.edu/AP/main.php>). Most AMPs are small cationic peptides with a broad antimicrobial activity, present in epithelial tissue throughout the body (8). They play an important role in both

the innate and adaptive immune response and show direct antimicrobial activity as well as essential chemotactic and immunomodulating properties (9–13). AMPs are believed to kill the bacteria by binding with anionic components in the cell membrane of microbes, resulting in disruption of the membrane and cytoplasmic leakage (14,15).

Many studies have shown decreased levels of AMPs in AD skin compared with psoriatic skin, which is attributed to elevated Th2 cytokines in AD skin (16,17). Animal studies of beagles with AD have also shown altered AMP expression in AD skin (18–20).

The aim of this review is to summarise the literature on *in vivo* expression of AMP in AD, focusing on human studies from a clinical perspective, highlighting different sample techniques and analytical methods in relation to findings.

Search

Literature search performed in PubMed.

('Dermatitis, Atopic'[Mesh] AND 'Antimicrobial Cationic Peptides'[Mesh]) OR (('dermatitis, atopic'[Mesh Terms] OR ('dermatitis'[All Fields] AND 'atopic'[All Fields]) OR 'atopic dermatitis'[All Fields] OR ('atopic'[All Fields] AND 'dermatitis'[All Fields])) AND 'antimicrobial peptides'[All Fields] NOT Medline [sb]).

Additional relevant papers selected from references.

Antimicrobial peptides of importance for atopic dermatitis

In normal skin, the main source of AMP production is keratinocytes, which produce a low constitutive level of many AMPs.

Upon infection or injury, AMP synthesis in keratinocytes is induced; however, infiltrating cells such as neutrophils and mast cells will contribute to the majority of AMPs at the site of inflammation (12,14,21–24). Many antimicrobial agents exist in the skin, both from migrating cells and from resident cells, including elafin, adrenomedullin, lysozyme as well as lipids (25–28) (S1). However, in this review focusing on AMPs produced in keratinocytes, the most commonly discussed and investigated AMPs in relation to AD will be assessed for their *in vivo* expression: defensins, LL-37, RNase7, psoriasin as well as dermcidin (29–32).

Defensins in humans are divided into two main classes α - and β -defensins which both show immune-modulating properties (10,33). β -defensins are vital for the antimicrobial defense of the skin (34–40). Human beta-defensin-1 (hBD-1) is expressed constitutively, whereas hBD-2 and hBD-3 are induced by infection, pro-inflammatory cytokines and injury, and present in healthy skin only in very low concentrations (16,38,41–43). Despite hBD-1 showing weak antimicrobial activity on its own (Table S1), studies have shown increased effect when hBD-1 acts in synergy with LL-37 (34,44). Furthermore, in contrast to oxidised hBD-1, reduced hBD-1 has shown potent activity against *Candida albicans* (*C. albicans*) as well as anaerobic pathogens (45) (Figs 1 and 2).

In keratinocytes, LL-37 is expressed constitutively in low concentrations (46–48) and is induced by injury and inflammation (49). In migrating cells such as neutrophils and mast cells, LL-37 is constitutively expressed as the precursor hCAP18 and stored in granules, which can rapidly increase local concentration at the site of inflammation (24). LL-37 has broad antimicrobial activity (Table S1) (34,50–55) and demonstrates important chemotactic properties by recruiting neutrophils, T cells, mast cells and monocytes to sites of infection as well as promote angiogenesis (11,12,33,56,57). Psoriasin is expressed constitutively in healthy skin and induced by inflammatory conditions and barrier disruption (58–60). Psoriasin also has immune-modulating properties and is an active agent against *Escherichia coli* (*E. coli*) and other bacteria (61–63) (Table S1). RNase7 is also expressed constitutively in healthy skin (58) and is induced by pro-inflammatory

cytokines (64). RNase7 is a highly potent agent against *S. aureus* in micromolar concentrations (64–66) (Table S1). Dermcidin is constitutively and specifically expressed in sweat glands, but unlike the above mentioned, it is not found or induced in keratinocytes (21,67). It is active against *Staphylococcus aureus* (*S. aureus*) and *C. albicans* in an acidic environment resembling human sweat (68) (Table S1).

Methods used for assessment of AMP expression in AD skin

Sample techniques used for AMP quantification include full-thickness skin biopsies, tape strips and washing fluid from the skin. Analysing techniques include the following: immunohistochemical staining detecting protein expression (skin biopsies), RT-PCR or microarray analysis for mRNA expression (skin biopsies), immunoassays (ELISA and Western blot) for protein expression in skin from tape strips or skin washing fluid.

The use of skin biopsies (47,48) has been predominant (Table S2) and has the advantage of ensuring enough material for thorough analysis. However, biopsies also contain the deeper layers of the skin including capillaries and migrating cells, and the expression of AMPs could therefore represent AMPs from migrating cells in the blood rather than AMPs locally present in the skin. Tape stripping (69,70) has the advantages over skin biopsies of collecting only stratum corneum and thereby minimising interfering substances from blood vessels and other cells. Isolating washing fluids derived from the surface of the skin is also a useful corneum stratum-specific technique (58,59) (Table S2). Tape stripping and washing fluid of the outer layers of the skin are minimally invasive techniques and therefore enable multiple testing of the same patient over time. The disadvantage of these methods is the limited amount of material collected, which, in turn, might make protein analysis difficult.

RT-PCR on skin biopsies (48,71) is a very sensitive and reproducible method for quantitative assessment of the expressed mRNA of a given AMP. However, measurements of mRNA may not necessarily reflect the local protein environment in the stratum corneum. Furthermore, results are normalised to different housekeeping genes (48,55,72), making comparison between reports more difficult. Microarrays allow for analysis of multiple

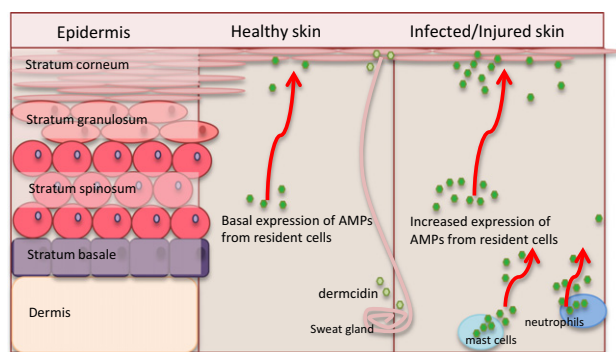


Figure 1. AMPs are produced at low constitutive levels in healthy skin. Upon injury, inflammation or infection, AMPs are generally upregulated to create a sufficient antimicrobial defense as well as to attract immune cells to the site of the infection/injury. In AD, altered upregulation of AMPs in lesional skin compared with psoriasis may leave the patient more susceptible to skin infections. In psoriasis, the levels of AMPs in lesional skin are induced. One study (68) has shown similar upregulation of AMPs in non-lesional skin of AD and psoriasis, resembling healthy skin.

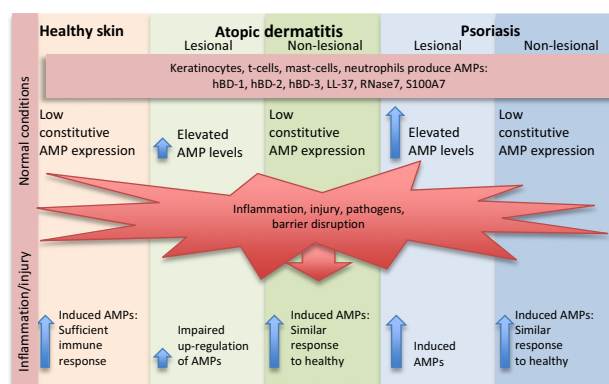


Figure 2. Expression of AMPs in healthy and injured skin. Basal constitutive AMP expression is found for the majority in the stratum basale/spinosum and stratum corneum. Dermcidin is expressed constitutively from sweat glands. Upon infection or injury, resident keratinocytes will increase expression of AMPs, and migrating cells like neutrophils and mast cell will contribute substantially with AMP stored in lamellar bodies.

genes in a single sample; however, like RT-PCR, the presence of mRNA does not necessarily directly reflect the current amount of protein in the skin (73,74). Immunohistochemical staining of skin biopsies illustrates the actual protein content in the skin at a given point in time and furthermore indicates the localisation of AMPs in the tissue. This method is only considered semiquantitative, and the scoring of staining tends to be a subjective assessment. AMP protein quantification by immunoassays (ELISA and Western blot) have been used on tape strips and washing fluid (69,70). ELISAs have high specificity and sensitivity and can quantify the amount of protein present in a given sample, although collecting sufficient material and retrieving the proteins from the tape might be challenging. So far, tape strips and washing fluid have only been used for protein quantification.

In brief, the results are influenced by sampling techniques (biopsies, tape strips or washing fluid) as well as the method used for analysis (whether it is of gene or protein expression) and this may hamper direct comparison between studies.

Expression of antimicrobial peptides in AD skin

Expression of AMPs in AD skin has in most of the recent studies been reported to be significantly lower in lesional AD skin compared with lesional psoriatic skin (Table 1). Interestingly, the differences in AMP quantity between AD and psoriasis appear to vary depending on the method used. While studies using assessment of mRNA expression reported a decrease of up to 20 times in hBD-2 in AD (47,48,55), microarray studies found hBD-2 to be 4–8 times decreased (16,47), and studies using immunohistochemical staining found hBD-2 levels 2–3 times decreased in AD compared with psoriatic skin (47,55). When focusing on the protein expression of hBD-2 in AD skin compared with psoriatic skin, one study using Western blot analysis on tape strips from epidermis found no difference in protein expression (64). Another study using washing fluid from epidermis found greatly decreased levels of hBD-2 protein in AD (10-fold) compared with psoriatic skin (58) (Table 1).

These differences suggest that the applied methodology plays a crucial role, and should be considered when interpreting the results of AMP expression in the skin. However, the overall tendency is clearly a decreased level of AMPs in AD skin as compared to psoriatic skin.

When comparing AMPs in AD skin with skin from healthy controls, data are based mostly on mRNA assessments and immunohistochemical staining (17,46,48,75,76), while only two

studies provide data of protein expression in the stratum corneum (Table 2) (70,77). Most studies show increased levels of AMPs in lesional AD skin as compared to healthy skin (48,58,59,78), while in non-lesional AD skin, AMP levels do not seem to differ significantly from healthy skin (46,58,71,78).

Skin barrier disruption

In general, barrier disruption causes increased expression of AMPs (hBD-2, hBD-3, LL-37, psoriasin) in both non-lesional AD skin and healthy skin (Table 3) (58,79). A similar response is seen in animal studies with increased AMP expression after tape stripping, which was reduced by occlusion (80). Interestingly, upregulation of hBD-2, hBD-3 and psoriasin mRNA in non-lesional skin after skin barrier disruption (tape stripping) shows a similar response in AD and psoriasis (79), indicating that non-lesional AD skin has the same capacity as non-lesional psoriatic skin for producing AMPs when induced by barrier disruption alone. This is supported by another study, which showed upregulation of the LL-37 precursor hCAP18 mRNA after injury in non-lesional AD skin (81), whereas this upregulation was inhibited in lesional skin. In psoriasis, upregulation of LL-37 is found to be higher in lesional injured skin, than non-lesional injured skin (82). These two latter studies indicate that cytokine differences between AD and psoriasis and local inflammatory milieu of lesional AD skin might inhibit sufficient AMP production in AD.

AMPS and disease severity

Whether AMPs can be used as biomarkers for disease severity is still unclear. Analysis of stratum corneum collected by tape strips showed a positive correlation between hBD-2 protein expression and disease severity as assessed by SCORAD (69,70), and a positive correlation between serum levels of hBD-2 and SCORAD was also reported (83,84). This correlation was, however, not confirmed in other studies where no correlation was found when examining skin washing fluid (51) and skin biopsies (17,78).

AMPS and infection

The clinical significance of AMPs on skin infections is still not completely established, but hBD-3 and RNase7, in particular, have been shown to be of importance in the protection and clearance of *S. aureus* infections (85,86). From *in vitro* studies, it is documented that *S. aureus* and *Staphylococcus epidermidis* (*S. epidermidis*) both upregulate the expression of hBD-2, hBD-3, LL-37 and partly hBD-1 (44,87,88).

Few *in vivo* studies exist and these comprise healthy individuals. Zanger *et al.* (85,86) performed two studies on mRNA expression

Table 1. AMP expression in AD skin compared with psoriasis, lesional (ls) and non-lesional (nls) skin

	mRNA (biopsies)	Protein staining (biopsies)	Gene (biopsies)	Protein (tape strips)	Protein (washing fluid)
hBD-1	no difference ls(47,48)	n.d.	↓ in AD ls (16,47)	n.d.	n.d.
hBD-2	↓ in AD ls (17,47,48,55) no difference nls (10)	↓ in AD ls (47,55,58)	↓ in AD ls (16,47)	no difference ls (69)	↓ in AD ls + nls (58)
hBD-3	↓ in AD ls (16,53) no difference ls (47,48,76)	↓ in AD ls (16,58) no difference ls (47)	no difference ls (47)	n.d.	not detectable ls (58)
LL-37	↓ in AD ls (17,55,81) no difference ls (47,48) no difference nls (76,81)	n.d.	no difference ls (47)	n.d.	n.d.
RNase7	↑ in AD ls (48)	no difference AD ls (58)	n.d.	n.d.	no difference ls + nls (58)
Psoriasin	↓ in AD (47) no difference ls (48)	↓ in AD ls (58)	↓ in AD ls (47)	n.d.	↓ in AD ls (58) ↑ in AD nls
Dermcidin	n.d.	Not expressed in keratinocytes, (21)	n.d.	n.d.	n.d.

AD, Atopic dermatitis; ↑, induced; ↓, reduced; n.d., not determined.

in skin biopsies of healthy volunteers. One study examined the levels of AMP mRNA in *S. aureus*-infected skin compared with non-infected skin of matching controls (85), and the other study related levels of AMPs in the skin to the severity of the infection (86). Low basal expression of RNase7 was associated with *S. aureus* skin infections, an association not seen for hBD-2 or hBD-3 which, nevertheless, were both significantly increased in infected skin. Regarding the clinical outcome, participants with low increase in hBD-3 had more recurrences and greater frequency of skin infections in contrast to individuals with a high increase in hBD-3 who had a better recovery rate and less severe symptoms. HBD-2 mRNA showed a remarkable induction in response to *S. aureus* on skin; however, no correlation was found between hBD-2 and clinical outcome of skin infections (86). These findings support *in vitro* studies (39,65,89,90) that hBD-3 and RNase7 are particularly important in the protection against *S. aureus*.

In relation to viral infections, AD is associated with eczema vaccinatum, an adverse reaction to vaccinia virus vaccination. One study analysed non-lesional skin biopsies from AD and found enhanced replication of vaccinia virus and decreased LL-37 expression compared with biopsies from psoriatic and healthy skin (91). When neutralising IL-4/IL-13 in AD skin biopsies, the authors found increased LL-37 together with inhibited vaccinia virus replication, indicating an important role for LL-37 in fighting vaccinia virus.

AMPs also show antifungal activity (S1). The clinical significance of this in relation to AD patients and fungal infections is greatly warranted and needs to be addressed in future studies.

There is no doubt that AMPs are essential in keeping skin free of infections and play a key role in the defense against invading pathogens. As bacteria such as *S. aureus* and *S. epidermidis* both upregulate the expression of AMPs (42,92,93), it would seem that a symbiosis between AMPs and the microflora of the skin is important in balancing an AMP response as well as regulating a constitutive level of AMP defense (94). Future studies in the microbiome of diseased skin and AMPs will deepen our understanding of the important relationship between commensal bacteria and innate antimicrobial defense.

Effect of treatment

Common topical treatments for AD include corticosteroids, calcineurin inhibitors and UV irradiation, which have all been shown to influence the expression of AMPs. So far, no studies of the effect of systemic treatments on AMPs have been undertaken.

Topical corticosteroids

Corticosteroids reduce the level of AMPs; however, the clinical importance of this is not known. Treatment with corticosteroids for a 3-week period significantly reduced mRNA of hBD-2, hBD-3, LL-37, RNase7 and psoriasin, as measured by RT-PCR in skin biopsies of AD skin, to levels significantly lower than found in healthy skin. The effect was most profound for hBD-2 and psoriasin (95).

Topical calcineurin inhibitors

Pimecrolimus treatment over a 3-week period was found to reduce mRNA of hBD-2, hBD-3, LL-37 and psoriasin in AD skin biopsies compared with untreated skin; however, AMP levels were

Table 2. AMP expression in AD skin compared with healthy controls

	mRNA (biopsies)	Protein staining (biopsies)	Gene (biopsies)	Protein (tape strips)	Protein (washing fluid)
hBD-1	↓ in AD Is (75) ↑ in AD Is (48)	n.d.	n.d.	n.d.	n.d.
hBD-2	↑ in AD Is (48,75) no difference Is (17) no difference nls (46,71)	↑ in AD Is (58) no difference nls (46,58)	n.d.	↑ in AD Is (70)	↑ in AD Is no difference nls (58)
hBD-3	↑ in AD Is (48) no difference Is (72,75) no difference nls (71)	↑ in AD Is + nls (58)	n.d.	n.d.	not detectable (58)
LL-37	↑ in AD Is (78) no difference Is (17,48,75) no difference nls (46,71,78,81)	↑ in AD Is (78) no difference nls (46,78)	n.d.	n.d.	n.d.
RNase7	↑ in AD Is (48)	↑ in AD Is (58)	n.d.	n.d.	↑ in AD Is no difference nls (58)
Psoriasin	↑ in AD I (48)	↑ in AD Is ¹ + nls (59)	n.d.	n.d.	↑ in AD Is + nls (58,59)
Dermcidin	n.d.	n.d.	n.d.	n.d.	↓ in AD sweat (67)

AD, Atopic dermatitis; ↑, induced; ↓, reduced; n.d., not determined.

Table 3. AMP expression in skin after impaired barrier function

	mRNA (biopsies)	Protein staining (biopsies)	Gene (biopsies)	Protein (tape strips)	Protein (washing fluid)
hBD-1	n.d.	n.d.	n.d.	n.d.	n.d.
hBD-2	induced equally in AD and PV, induced higher in HC (79)	induced in HC (58) induced equally in AD and HC (79)	n.d.	n.d.	induced in HC (58)
hBD-3	induced equally in AD, PV and HC (79)	induced in HC (58)	n.d.	n.d.	n.d.
LL-37	induced in AD, induced higher in HC, and highest in PV (79)	induced in AD nls lower induction in AD Is (81)	n.d.	n.d.	n.d.
RNase7	n.d.	No difference (58)	n.d.	n.d.	induced in HC (58)
Psoriasin	induced equally in AD and HC, lower induction in PV (79)	induced equally in AD and HC (79) induced in HC (59)	n.d.	n.d.	induced in HC (59)
Dermcidin	n.d.	n.d.	n.d.	n.d.	n.d.

AD, Atopic dermatitis; PV, psoriasis vulgaris; HC, healthy controls; n.d., not determined.

not suppressed to the same extent as with corticosteroid treatment. After Pimecrolimus treatment of AD skin, AMP levels in the skin resembled the levels found in healthy controls (95). On the contrary, tacrolimus treatment applied twice a day for a 4-week period was reported to induce expression of hBD-2 and LL-37 mRNA in AD skin biopsies along with normalisation of the skin barrier function in a study comprising skin from 3 individuals (96).

UV

In AD, UV treatment induced mRNA of hBD-1, and decreased hBD-2 but had no significant effect on hBD-3 and LL-37 (75). These changes could be a direct effect of UV irradiation, or due to immunosuppressive effects caused by UV. In healthy skin, however, UV was reported to induce protein expression of hBD-3, RNase7 and psoriasin but not hBD-2, analysing skin biopsies (97).

In general, topical treatment with corticosteroids and calcineurin inhibitors decreases AMP levels in the skin. This may be a direct effect of the medication, or secondary to an improved skin barrier function following the treatment. It would appear that corticosteroids significantly suppress AMP production, whereas calcineurin inhibitors and UV irradiation have a more modulating effect on AMP expression, although still preserving endogenous AMP production. Hence, steroid treatment leads to reduced AMP production to a significantly greater extent than treatment with calcineurin inhibitors. Both corticosteroids and calcineurin inhibitors have been shown to impair barrier function in healthy skin, which together with reduced AMP expression might leave patients more susceptible to skin infections (98,99).

Conclusions

When comparing multiple studies, great differences exist in the reporting on AMP expression in AD skin. However, it is generally accepted that expression of certain AMPs in lesional AD skin is decreased compared with psoriatic skin, but increased compared with non-lesional skin and healthy controls (Table 1). Some of these differences might be explained by the applied method and the specific AMP being investigated (Table 1). RT-PCR and microarray analyses tend to report greater differences in mRNA expression, than those reported by immunohistochemical staining, which could be explained by post-transcriptional modulation. Furthermore, immunostaining can only be interpreted as a semiquantitative measurement, making quantitative comparison challenging.

Some studies report results from small sample sizes, which could explain the different conclusions, and at times, there is a lack of clarity regarding numbers of participants as well as data on whether samples are taken from acute or chronic lesions. The cytokine profile is crucial for AMP production (16,79,100) and, consequently, distinguishing between acute or chronic lesions is important in the interpretation of data. The observation that wounded psoriasis lesional skin upregulates AMP expression even more than wounded non-lesional skin, and the opposite is found for AD skin, supports the hypothesis that the local inflammatory milieu might hinder upregulation of AMPs in AD lesional skin (81,82).

What remains to be determined is whether the AMP response to bacterial infections is insufficient in AD skin, compared with the response in infected healthy skin. Another important question is whether the *in vitro* findings of antibacterial effects of AMPs can be applied in an *in vivo* context as many studies have shown

differentiated antimicrobial activity of AMPs, depending on strain of bacteria, native or recombinant AMP, pH, culturing conditions and oxidised or reduced form of AMP (Table S1) (38,45,101–103). These conditions all play a role in the clinical outcome and make *in vitro* studies hard to apply for *in vivo* relevance. The synergistic effect of AMPs has also been shown to be of importance for antimicrobial effect, and this could have significant relevance *in vivo* in relation to low expression of AMPs in AD (34,104). Furthermore, proteolytic processing of precursors and AMPs could also alter *in vivo* efficiency, as proteases belonging to the kallikrein family have been shown to regulate and process hCAP18/LL-37 (105). This might also provide an explanation for the differences found between mRNA and protein levels.

Factors other than AMP are relevant for the frequent infections seen in AD. Barrier properties such as skin pH (106), flaggrin expression and its breakdown products (107,108), sphingosine levels (109), and barrier disruption (110,111) allowing for penetration of bacteria and virus, are all important factors in relation to skin infections.

The microbial composition of the skin also plays an important role in maintaining healthy skin and is shown to regulate AMP production (42,87,112). Future research into the possible beneficial effects of a balanced microbiome and a description of the microbiome of AD and healthy skin will help us understand the regulatory mechanisms in controlling skin infections and maintaining a well-balanced antimicrobial shield in the skin.

Treatments in AD may modulate AMP expression and, topical corticosteroids, in particular, have been reported to affect AMP expression. This could be an important element in the reduced AMP expression seen in AD. More studies are needed to gain insight into the effects of AD treatments on the antimicrobial defense of the skin, and the relation to clinical outcome and disease severity. In particular, more studies of the immune-modulating treatments, used both topically and systemically, in relation to AMP expression are needed. Future research will also reveal the impact of genetic variations, as AD has been associated with single nucleotide polymorphisms in the DEFB1 gene (113,114).

In conclusion, AMPs are crucial for maintaining healthy skin, free of infections, and for mobilising sufficient immune response upon pathogen invasion. In AD, there is evidence of reduced expression of certain AMPs compared with psoriasis (16,115), probably due to the cytokine milieu of AD as well as the bacterial flora of the skin, which both play a role in modulating the AMP response. More studies on AMPs 'the body's own antibiotics' might lead to the development of new therapeutic approaches in the treatment and prevention of skin infections (116,117).

Acknowledgement

Clausen M-L wrote the paper, including literature search and analysis and interpretation of data. Slotved H-C, Andersen P, Krogfelt K A and Agner T all contributed to the interpretation and analysis of literature search as well as carefully and critically revising and approving the final manuscript.

Conflict of interests

The authors have no conflict of interest to disclose.

Supporting Information

Additional supporting data may be found in the supplementary information of this article.

Table S1. AMPs - Their localization, concentration and antimicrobial function.

Table S2. Important publications on AMP in AD (methodology, participants and AMPs analysed).

References

*References are cited in online only supporting information.

- 1 Bieber T. *Ann Dermatol* 2010; **22**: 125–137.
- 2 Boguniewicz M, Leung D Y. *Immunol Rev* 2011; **242**: 233–246.
- 3 Palmer C N, Irvine A D, Terron-Kwiatkowski A et al. *Nat Genet* 2006; **38**: 441–446.
- 4 Morar N, Cookson W O C M, Harper J I et al. *J Invest Dermatol* 2007; **127**: 1667–1672.
- 5 O'Regan G M, Irvine A D. *Clin Exp Allergy* 2010; **40**: 965–972.
- 6 Agrawal R, Woodfolk J A. *Curr Allergy Asthma Rep* 2014; **14**: 433.
- 7 Gallo R L. *Exp Dermatol* 2013; **22**: 517.
- 8 Ganz T, Lehrer R I. *Curr Opin Immunol* 1998; **10**: 41–44.
- 9 Lai Y, Gallo R L. *Trends Immunol* 2009; **30**: 131–141.
- 10 Yang D, Chertov O, Bykovskaia S N et al. *Science* 1999; **286**: 525–528.
- 11 Koczulla R, Von Degenfeld G, Kupatt C et al. *J Clin Invest* 2003; **111**: 1665–1672.
- 12 Di Nardo A, Vitiello A, Gallo R L. *J Immunol* 2003; **170**: 2274–2278.
- 13 Yang D, Chen Q, Chertov O et al. *J Leukoc Biol* 2000; **68**: 9–14.
- 14 Izadpanah A, Gallo R L. *J Am Acad Dermatol* 2005; **52**: 381–382.
- 15 Zasloff M. *Nature* 2002; **415**: 389–395.
- 16 Nomura I, Goleva E, Howell M D et al. *J Immunol* 2003; **171**: 3262–3269.
- 17 Howell M D, Novak N, Bieber T et al. *J Invest Dermatol* 2005; **125**: 738–745.
- 18 Santoro D, Bunick D, Graves T K et al. *Vet Dermatol* 2013; **24**: 39–47 e10.
- 19 Santoro D, Marsella R, Bunick D et al. *Vet Immunol Immunopathol* 2011; **144**: 382–388.
- 20 Van Damme C M M, Willemse T, van Dijk A et al. *Mol Immunol* 2009; **46**: 2449–2455.
- 21 Rieg S, Garbe C, Sauer B et al. *Br J Dermatol* 2004; **151**: 534–539.
- 22 Lee D-Y, Yamasaki K, Rudisil J et al. *J Invest Dermatol* 2008; **128**: 1863–1866.
- 23 Agerberth B, Charo J, Werr J et al. *Blood* 2000; **96**: 3086–3093.
- 24 Vandamme D, Landuyt B, Luyten W et al. *Cell Immunol* 2012; **280**: 22–35.
- 25 Braff M H, Bardan A, Nizet V et al. *J Invest Dermatol* 2005; **125**: 9–13.
- 26 Harder J, Schroder J M. *Chem Immunol Allergy* 2005; **86**: 22–41.
- 27 Elias P M. *Semin Immunopathol* 2007; **29**: 3–14.
- 28 Brogden N K, Mehalick L, Fischer C L et al. *Ski Pharmacol Physiol* 2012; **25**: 167–181.
- 29 Metz-Boutigue M H, Shooshtarizadeh P, Prevost G et al. *Curr Pharm Des* 2010; **16**: 1024–1039.
- 30 Afshar M, Gallo R L. *Vet Dermatol* 2013; **24**: 32–39.
- 31 Schitteck B. *Curr Probl Dermatol* 2011; **41**: 54–67.
- 32 Schröder J M, Harder J. *Cell Mol Life Sci* 2006; **63**: 469–486.
- 33 Oppenheim J J, Yang D. *Curr Opin Immunol* 2005; **17**: 359–365.
- 34 Chen X, Niyonsaba F, Ushio H et al. *J Dermatol Sci* 2005; **40**: 123–132.
- 35 Fritz P, Beck-Jendroschek V, Brasch J. *Med Mycol* 2012; **50**: 579–584.
- 36 Feng Z, Jiang B, Chandra J et al. *J Dent Res* 2005; **84**: 445–450.
- 37 Harder J, Bartels J, Christophers E et al. *J Biol Chem* 2001; **276**: 5707–5713.
- 38 Harder J, Bartels J, Christophers E et al. *Nature* 1997; **387**: 861.
- 39 Kisich K O, Carspecken C W, Fiéve S et al. *J Allergy Clin Immunol* 2008; **122**: 62–68.
- 40 García J R, Jaumann F, Schulz S et al. *Cell Tissue Res* 2001; **306**: 257–264.
- 41 Gallo R L, Murakami M, Ohtake T et al. *J Allergy Clin Immunol* 2002; **110**: 823–831.
- 42 Lai Y, Cogen A L, Radek K A et al. *J Invest Dermatol* 2010; **130**: 2211–2221.
- 43 Wittersheim M, Cordes J, Meyer-Hoffert U et al. *Exp Dermatol* 2013; **22**: 364–366.
- 44 Midorikawa K, Ouhara K, Komatsuzawa H et al. *Infect Immun* 2003; **71**: 3730–3739.
- 45 Schroeder B O, Wu Z, Nuding S et al. *Nature* 2011; **469**: 419–423.
- 46 Goo J, Ji J H, Jeon H et al. *Pediatr Dermatol* 2010; **27**: 341–348.
- 47 De Jongh G J, Zeeuwen P L J M, Kucharekova M et al. *J Invest Dermatol* 2005; **125**: 1163–1173.
- 48 Gambichler T, Skrygan M, Tomi N S et al. *Int Arch Allergy Immunol* 2008; **147**: 17–24.
- 49 Frohm M, Agerberth B, Ahangari G et al. *J Biol Chem* 1997; **272**: 15258–15263.
- 50 Howell M D, Jones J F, Kisich K O et al. *J Immunol* 2004; **172**: 1763–1767.
- 51 Braff M H, Hawkins M A, Di Nardo A et al. *J Immunol* 2005; **174**: 4271–4278.
- 52 Turner J, Cho Y, Dinh N et al. *Antimicrob Agents Chemother* 1998; **42**: 2203–2214.
- 53 Howell M D, Wollenberg A, Gallo R L et al. *J Allergy Clin Immunol* 2006; **117**: 836–841.
- 54 Murakami M, Ohtake T, Dorschner R A et al. *J Invest Dermatol* 2002; **119**: 1090–1095.
- 55 Ong P Y, Ohtake T, Brandt C et al. *N Engl J Med* 2002; **347**: 1151–1160.
- 56 Yang D, Oppenheim J J. *Arthritis Rheum* 2004; **50**: 3401–3403.
- 57 Kahlenberg J M, Kaplan M J. *J Immunol* 2013; **191**: 4895–4901.
- 58 Harder J, Dressel S, Wittersheim M et al. *J Invest Dermatol* 2010; **130**: 1355–1364.
- 59 Gläser R, Meyer-Hoffert U, Harder J et al. *J Invest Dermatol* 2009; **129**: 641–649.
- 60 Gläser R, Köten B, Wittersheim M et al. *J Dtsch Dermatol Ges* 2011; **9**: 897–902.
- 61 Gläser R, Harder J, Lange H et al. *Nat Immunol* 2005; **6**: 57–64.
- 62 Zheng Y, Niyonsaba F, Ushio H et al. *Immunology* 2008; **124**: 357–367.
- 63 Jinquan T. *J Invest Dermatol* 1996; **107**: 5–10.
- 64 Harder J, Schroder J-M. *J Biol Chem* 2002; **277**: 46779–46784.
- 65 Simanski M, Dressel S, Gläser R et al. *J Invest Dermatol* 2010; **130**: 2836–2838.
- 66 Köten B, Simanski M, Gläser R et al. *PLoS ONE* 2009; **4**: e6424.
- 67 Rieg S, Steffen H, Seeber S et al. *J Immunol* 2005; **174**: 8003–8010.
- 68 Schitteck B, Hipfel R, Sauer B et al. *Nat Immunol* 2001; **2**: 1133–1137.
- 69 Asano S, Ichikawa Y, Kumagai T et al. *Br J Dermatol* 2008; **159**: 97–104.
- 70 Clausen M L, Jungerstedt J M, Andersen P S et al. *Br J Dermatol* 2013; **169**: 587–593.
- 71 Hata T R, Kotol P, Boguniewicz M et al. *Br J Dermatol* 2010; **659**: 659–661.
- 72 Howell M D, Boguniewicz M, Pastore S et al. *Clin Immunol* 2006; **121**: 332–338.
- 73 Maier T, Güell M, Serrano L. *FEBS Lett* 2009; **583**: 3966–3973.
- 74 Vogel C, Marcotte E M. *Nat Rev Genet* 2012; **13**: 227–232.
- 75 Gambichler T, Skrygan M, Tomi N S et al. *Br J Dermatol* 2006; **155**: 1275–1278.
- 76 Hata T R, Kotol P, Boguniewicz M et al. *Br J Dermatol* 2010; **163**: 659–661.
- 77 Dressel S, Harder J, Cordes J et al. *Exp Dermatol* 2010; **19**: 628–632.
- 78 Ballardini N, Johansson C, Lilja G et al. *Br J Dermatol* 2009; **161**: 40–47.
- 79 De Koning H D, Kamsteeg M, Rodijk-Olthuis D et al. *J Invest Dermatol* 2011; **131**: 263–266.
- 80 Ahrens K, Schunck M, Podda G-F et al. *J Invest Dermatol* 2011; **131**: 443–452.
- 81 Mallbris L, Carlén L, Wei T et al. *Exp Dermatol* 2010; **19**: 442–449.
- 82 Hata T R, Afshar M, Miller J et al. *Exp Dermatol* 2013; **22**: 599–601.
- 83 Kanda N, Watanabe S. *Immunobiology* 2012; **217**: 436–445.
- 84 Jansen P A M, Rodijk-Olthuis D, Hollox E J et al. *PLoS ONE* 2009; **4**: e4725.
- 85 Zanger P, Holzer J, Schleucher R et al. *J Infect Dis* 2009; **200**: 1907–1915.
- 86 Zanger P, Holzer J, Schleucher R et al. Severity of *Staphylococcus aureus* infection of the skin is associated with inducibility of human beta-defensin 3 but not human beta-defensin 2. *Infect Immun* 2010; **78**: 3112–3117.
- 87 Percoco G, Merle C, Jaouen T et al. *Exp Dermatol* 2013; **22**: 800–806.
- 88 Menzies B E, Kenoyer A. *Infect Immun* 2005; **73**: 5241–5244.
- 89 Sawamura D, Goto M, Shibaki A et al. *Gene Ther* 2005; **12**: 857–861.
- 90 Kisich K O, Howell M D, Boguniewicz M et al. *J Invest Dermatol* 2007; **127**: 2368–2380.
- 91 Howell M D, Gallo R L, Boguniewicz M et al. *Immunity* 2006; **24**: 341–348.
- 92 Wanke I, Steffen H, Christ C et al. *J Invest Dermatol* 2011; **131**: 382–390.
- 93 Dinulos J G H, Mentelle L, Fredericks L P et al. *Clin diagnostic Lab Immunol* 2003; **10**: 161–166.
- 94 Simanski M, Gläser R, Harder J. *Exp Dermatol* 2014; **23**: 230–231.
- 95 Jensen J M M, Ahrens K, Meingassner J et al. *Exp Dermatol* 2011; **20**: 783–788.
- 96 Park K Y, Kim D H, Jeong M S et al. *J Korean Med Sci* 2010; **25**: 766–771.
- 97 Felton S, Navid F, Schwarz A et al. *Photochem Photobiol Sci* 2013; **12**: 29–36.
- 98 Kao J S, Fluhr J W, Man M Q et al. *J Invest Dermatol* 2003; **120**: 456–464.
- 99 Kim M, Jung M, Hong S-P et al. *Exp Dermatol* 2010; **19**: 501–510.
- 100 Kanda N, Watanabe S. *Eur J Immunol* 2008; **38**: 1287–1296.
- 101 Routsias J G, Karagounis P, Parvulescu G et al. *Peptides* 2010; **31**: 1654–1660.
- 102 Maisetta G, Di Luca M, Esin S et al. *Peptides* 2008; **29**: 1–6.
- 103 Nuding S, Zabel L T, Enders C et al. *Microbes Infect* 2009; **11**: 384–393.
- 104 Abou Alaiwa M H, Reznikov L R, Gansemer N D et al. *Proc Natl Acad Sci* 2014; **111**: 18703–18708.
- 105 Yamasaki K, Schaubert J, Coda A et al. *FASEB J* 2006; **20**: 2068–2080.
- 106 Rippke F, Schreiner V, Doering T et al. *Am J Clin Dermatol* 2004; **5**: 217–223.
- 107 Mijalovic H, Fallon P G, Irvine A D et al. *J Allergy Clin Immunol* 2010; **126**: 1184–1190 e3.
- 108 Van Drongelen V, Haisma E M, Out-Luiting J J et al. *Clin Exp Allergy* 2014; **44**: 1515–1524.
- 109 Arikawa J, Ishibashi M, Kawashima M et al. *J Invest Dermatol* 2002; **119**: 433–439.
- 110 Jinnestäl C L, Belfrage E, Bäck O et al. *Int J Dermatol* 2014; **53**: 27–33.
- 111 Wanke I, Skabytska Y, Kraft B et al. *Exp Dermatol* 2013; **22**: 153–155.
- 112 Sørensen O E, Thapa D R, Rosenthal A et al. *J Immunol* 2005; **174**: 4870–4879.
- 113 Prado-Montes de Oca E, García-Vargas A, Lozano-Inocencio R et al. *Int Arch Allergy Immunol* 2007; **142**: 211–218.
- 114 Kim E, Lee J-E, Namkung J-H et al. *J Dermatol Sci* 2009; **54**: 25–30.
- 115 Albanesi C, Fairchild H R, Madonna S et al. *J Immunol* 2007; **179**: 984–992.
- 116 Harder J, Gläser R, Schröder J-M. *Endocr Metab Immune Disord Drug Targets* 2007; **7**: 75–82.
- 117 Nakatsuji T, Gallo R L. *J Invest Dermatol* 2012; **132**: 887–895.
- 118 *Wang Z, Wang G. *Nucleic Acids Res* 2004; **32**: D590–D592.
- 119 *Duits L A, Ravensbergen B, Rademaker M et al. *Immunology* 2002; **106**: 517–525.
- 120 *Fulton C, Anderson G M, Zasloff M et al. Expression of natural peptide antibiotics in human skin. *Lancet* 1997; **350**: 1996–1997.

- 121 *Ali R S, Falconer A, Ikram M *et al.* *J Invest Dermatol* 2001; **117**: 106–111.
- 122 *Supp D M, Karpinski A C, Boyce S T. *Burns* 2004; **30**: 643–648.
- 123 *Krishnakumari V, Rangaraj N, Nagaraj R. *Antimicrob Agents Chemother* 2009; **53**: 256–260.
- 124 *Oren A, Ganz T, Liu L *et al.* *Exp Mol Pathol* 2003; **74**: 180–182.
- 125 *Harder J, Schroder J M. *J Leukoc Biol* 2005; **77**: 476–486.
- 126 *Lai Y, Villaruz A E, Li M *et al.* *Mol Microbiol* 2007; **63**: 497–506.
- 127 *Hoover D M, Wu Z, Tucker K *et al.* *Antimicrob Agents Chemother* 2003; **47**: 2804–2809.
- 128 *Sahly H, Schubert S, Harder J *et al.* *Antimicrob Agents Chemother* 2003; **47**: 1739–1741.
- 129 *Maisetta G, Batoni G, Esin S *et al.* *Antimicrob Agents Chemother* 2006; **50**: 806–809.
- 130 *Braff M H, Di Nardo A, Gallo R L. Keratinocytes store the antimicrobial peptide cathelicidin in lamellar bodies. *J Invest Dermatol* 2005; **124**: 394–400.
- 131 *Larrick J W, Hirata M, Shimomura Y *et al.* Antimicrobial activity of rabbit CAP18-derived peptides. *Antimicrob Agents Chemother* 1993; **37**: 2534–2539.
- 132 *Regenhard P, Leippe M, Schubert S *et al.* *Vet Microbiol* 2009; **136**: 335–340.
- 133 *Michalek M, Gelhaus C, Hecht O *et al.* *Dev Comp Immunol* 2009; **33**: 740–746.
- 134 *Lai Y-P, Peng Y-F, Zuo Y *et al.* *Biochem Biophys Res Commun* 2005; **328**: 243–250.
- 135 *Schitteck B. *J Innate Immun* 2012; **4**: 349–360.
- 136 *Schalkwijk J, Van Vlijmen-Willems I M J J, Alkemade J A C *et al.* *J Invest Dermatol* 1993; **100**: 390–393.
- 137 *Alkemade J A, Molhuizen H O, Ponc M *et al.* *J Cell Sci* 1994; **107**: 2335–2342.
- 138 *Simpson A J, Maxwell A I, Govan J R W *et al.* *FEBS Lett* 1999; **452**: 309–313.
- 139 *Martinez A, Elsasser T H, Muro-Cacho C *et al.* *Endocrinology* 1997; **138**: 5597–5604.
- 140 *Allaker R P, Zihni C, Kapas S. *FEMS Immunol Med Microbiol* 1999; **23**: 289–293.
- 141 *Allaker R P, Grosvenor P W, McAnerney D C *et al.* *Peptides* 2006; **27**: 661–666.
- 142 *Niyonsaba F, Ogawa H. *J Dermatol Sci* 2005; **40**: 157–168.
- 143 *Ogawa H. *J Dermatol* 1975; **2**: 45–50.
- 144 *Ogawa H, Miyazaki H, Kimura M. *J Invest Dermatol* 1971; **57**: 111–116.
- 145 *Garcia J R C, Krause A, Schulz S *et al.* *FASEB J* 2001; **15**: 1819–1821.

Supplementary material:

In Vivo Expression of Antimicrobial Peptides in Atopic Dermatitis

S1: AMPs - Their localization, concentration and antimicrobial function

AMP	Cellular source	Epidermal location	Tissue concentration	Antimicrobial potency	Reference
hBD-1	Keratinocytes Monocytes Dendritic cells	Malpighian layer/suprabasal layers and stratum corneum. Sweat ducts Sebaceous glands	nd	<i>S. aureus</i> : LD ₅₀ 6.5µg/ml <i>E. coli</i> : LD ₅₀ 30µg/ml <i>C. albicans</i> : MFC 7µM	(34,118–123)
hBD-2	Keratinocytes Monocytes	Malpighian layer/suprabasal layers and stratum corneum.	10-50µg /g <i>psoriatic</i> skin	<i>S. aureus</i> : bacteriostatic at 100 µg/ml. <i>S. aureus</i> : LD ₉₀ 10µg/ml <i>S. epidermidis</i> : nd <i>E. coli</i> : LD ₉₀ 10-20 µg/ml <i>C. albicans</i> : LD ₉₀ 25µg/ml	(34,38,55,119,121–125,101)
hBD-3	Keratinocytes	Cornified layers	15µg / 112 g SC	<i>S. aureus</i> : LD ₉₀ 10 µg/ml <i>S. aureus</i> : LD ₅₀ 0.2-1.6 µg/ml <i>S. aureus</i> : bacteriocidal 4-8 µg/ml <i>S. epidermidis</i> : LD ₉₀ 70 µg/ml <i>S. epidermidis</i> : LD ₅₀ 0.4 µg/ml <i>E. coli</i> : LD ₉₀ 6 µg/ml <i>E. coli</i> : LD ₅₀ 0.1 µg/ml <i>C. albicans</i> : LD ₉₀ 17 µg/ml	(34,37,122,123,126–129)
LL-37	Keratinocytes Neutrophils Monocytes Lymphocytes Mast cells	Basal and suprabasal layers. Stratum corneum.	304 µM in <i>psoriatic</i> skin	<i>S. aureus</i> : LD ₉₀ >5 µg/ml <i>S. epidermidis</i> : LD ₉₀ 5 µg/ml <i>E. coli</i> : LD ₉₀ 2.5 µg/ml <i>S. pyogenes</i> : LD ₉₀ 2.5 µg/ml	(34,55,118,126,130,131)
RNase7	Keratinocytes	nd	200-300 µg/ 50g SC 4-8 µg/g SC	<i>S. aureus</i> : LD ₉₀ 0.5-1 µM <i>C. albicans</i> : LD ₉₀ 1 µM <i>E. coli</i> : LD ₉₀ 0.5 µM	(64,125)
Psoriasin	Keratinocytes	Malpighian epidermal layers	5-20 ng/cm ² 1-20 ng/ml	<i>S. aureus</i> : LD ₉₀ >100 µg/ml <i>C. albicans</i> : LD ₉₀ 25 µg/ml (bovine psoriasin) <i>E. coli</i> : LD ₉₀ 25µg/ml	(59,61,125,132,133)
Dermcidin	Eccrine sweat glands, not keratinocytes	Secretory coils of eccrine sweat glands	1.25 µg/ml sweat	<i>S. aureus</i> : LD ₉₀ 1-10 µg/ml <i>C. albicans</i> : LD ₉₀ 1-10 µg/ml <i>S. epidermidis</i> : LD ₉₀ 10µg/ml <i>E. coli</i> : LD ₉₀ 45-85 µg/ml	(21,67,68,126,134,135)
Elafin	Keratinocytes	Stratum spinosum and granulosum	nd	<i>S. aureus</i> : LD ₅₀ 25 µM <i>E. coli</i> : No effect	(136–138)
Adreno-medullin	Keratinocytes	Stratum basale and spinosum	nd	<i>S. aureus</i> : MBC 25 µg/ml <i>C. albicans</i> : No effect	(118,139–141)
Lysozyme	Keratinocytes Neutrophils Monocytes Macrophages	Upper epidermal layers	60 -120 µg/ g wet skin 5ng/cm ² skin surface	<i>S. aureus</i> : ED ₅₀ 2.6 µg/ml	(34,118,142–144)
hBD-4	Not yet detected in human skin	nd	nd	<i>S. aureus</i> : MIC > 100 µg/ml <i>E. coli</i> : MIC > 100 µg/ml	(125,145)

nd: not determined, LD₅₀: Lethal dose killing 50% of bacteria, LD₉₀: Lethal dose killing 90% of bacteria, ED₅₀: dose that inhibited 50% growth of bacteria, MBC: minimal bactericidal concentration, MIC: minimal inhibitory concentration.

S2: Important publications on AMP in AD (methodology, participants and AMPs analysed)

Skin sampling method	Participants	Detection method	Detection	AMP	Group
Biopsies	10 AD ls 10 PV ls 6 HC	RT-PCR (n=10) Gene-array (n=6) IHC staining (n=6)	Protein mRNA Gene	hBD-1, 2, 3	Nomura 2003
Biopsies	6 AD ls 6 PV ls 6 HV	RT-PCR	mRNA	hBD-2 LL-37	Howell 2005
Biopsies	7 AD nls 7 HC	RT-PCR Western blot IHC staining	Protein mRNA	hBD-2 LL-37	Goo 2010
Biopsies Purified epidermal cells	16 AD chr ls 20 PV chr ls 11 HC	RT-PCR (n=6) Microarray IHC staining (n=10)	protein mRNA gene	hBD-1, 2, 3	De Jong 2005
Biopsies	42 AD chr ls 35 PV chr ls 18 HC	RT-PCR	mRNA	hBD-1, 2, 3 LL-37 RNase7 Psoriasis	Gambichler 2008
Biopsies	10 AD ls 10 ADEH ls	RT-PCR	mRNA	LL-37	Howell 2006
Biopsies	8 AD ls 11 PV ls 6 HC	RT-PCR Western blot IHC staining	mRNA protein	hBD-2 LL-37	Ong 2002
Washing fluid Biopsies (n=4)	24 AD chr ls+nls 14 AD acu ls+nls 8 PV ls+nls 34 HC	ELISA IHC staining	Protein	hBD-2, -3 RNase7 Psoriasis	Harder 2010
Washing fluid Biopsies	12 AD ls+nls 12 HC	ELISA IHC staining (n=2)	Protein	Psoriasis	Gläser 2009
Tape-stripping	100 AD ls+nls 76 HC 15 PV ls+nls	Western blot	Protein	hBD-2	Asano 2002
Tape-strips	25 AD 11 HC	ELISA	Protein	hBD-2	Clausen 2013
Biopsies	21 AD ls+nls 26 PV ls+nls 25 HC	RT-PCR	mRNA	hBD-2, 3 LL-37	Hata 2010
Biopsies	7 IAD + EAD ls 8 PV ls 6 HC	RT-PCR	mRNA	hBD-3	Howell 2006
Biopsies (treatment)	12 AD 12 HC	RT-PCR	mRNA	hBD-1, 2, 3 LL-37	Gambichler 2006
Biopsies	20 AD ls+nls 10 HC	RT-PCR	mRNA	LL-37	Ballardini 2009
Biopsies	6 AD nls 6 PV nls 25 HC	RT-PCR IHC staining	mRNA	hBD-2, 3 LL-37 Psoriasis	De Koning 2011
Biopsies	14 AD ls+nls 28 PV ls+nls 4 HC	RT-PCR Western blot	mRNA protein	LL-37	Mallbris 2010
Biopsies (Treatment)	15 AD ls	Micro-array IHC staining	Protein mRNA	hBD-2, 3 LL-37 RNase7 Psoriasis	Jensen 2011
Biopsies (treatment)	3 AD ls	RT-PCR Western blot	mRNA protein	hBD-2 LL-37	Park 2010
Sweat	17 AD 17 HC	ELISA	Protein	Dermcidin	Rieg 2005

AD: Atopic dermatitis, PV: psoriasis vulgaris, HC: healthy controls, chr: chronic, acu: acute, ls: lesional skin, nls: non lesional skin, RT-PCR: Real time polymerase chain reaction; IHC: Immunohistochemical