



ORIGINAL ARTICLE

Free human DNA attenuates the activity of antimicrobial peptides in atopic dermatitis

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Funding information

This project was funded by DFG grants WE1289/10-1 and HA 3386/8-1 and by LOM of Hannover Medical School. The work of AB and BS has been funded by the Deutsches Zentrum für Infektionsforschung (DZIF; German Center for Infection Research, TTU Infections of the Immunocompromised Host), and the Niedersachsen Research Network on Neuroinfectiology (N-RENNT) of the Ministry of Science and Culture of Lower Saxony, Germany. The work of BS, VK, JZ, and TW has been funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy – EXC 2155 “RESIST” – Project ID 390874280. VK and SD had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Abstract

Background: The high susceptibility of AD patients to microbial skin infections has been attributed to a deficient antimicrobial peptide (AMP) expression, which is contradicted by a growing amount of recent studies clearly demonstrating that AMP expression is not impaired in lesional skin of AD patients. The reasons for the high susceptibility of AD patients to microbial infections are still unknown.

Methods: The influence of self-DNA on the antimicrobial activity of RNase 7, LL-37, and hBD2 has been investigated using antibacterial and antiviral assays. The amount of self-DNA on skin has been analyzed by skin rinsings and subsequent quantification using dsDNA assays. DNA source was identified by qPCR.

Results: Complex formation of the AMPs with self-DNA significantly impaired their antibacterial activity against *Staphylococcus aureus* and their antiviral activity against *HSV-1*. The inhibition of the antibacterial activity was dependent on the DNA concentration but not on the length of the DNA molecules. Of note, we detected significant higher amounts of cell-free self-DNA in skin rinses taken from lesional AD skin compared to skin rinses from non-lesional skin and from normal skin of healthy donors.

Verena Kopfnagel and Sylvia Dreyer contributed equally to this article and should be considered joint first author.

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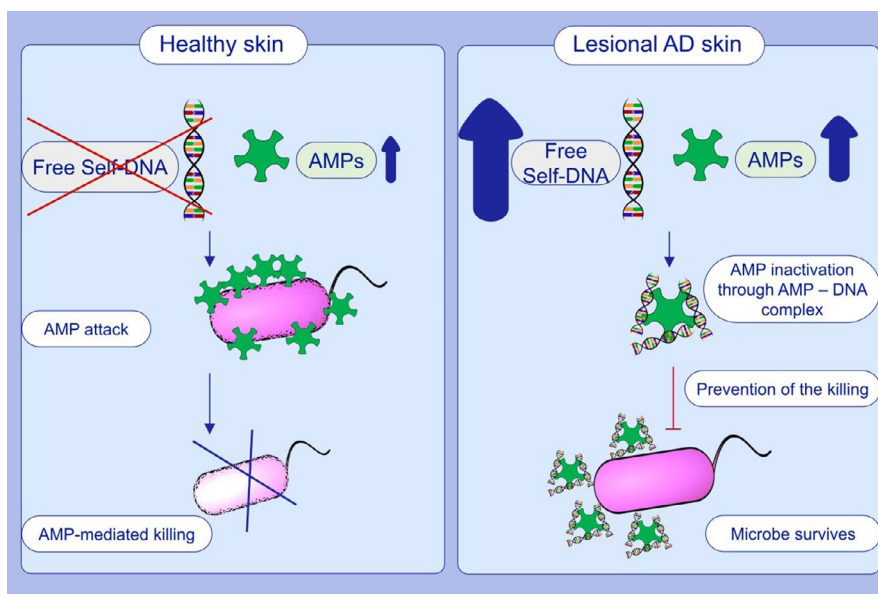
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Consequently, rinse solution from AD lesional skin prevented antibacterial activity of LL-37.

Conclusion: Our study indicates that extracellular self-DNA is released in considerable amounts in AD skin lesions and AMP-self-DNA-complex formation leads to a significant loss of antibacterial and antiviral activity in atopic dermatitis. Studies on strategies to reduce the amount of extracellular DNA in AD are needed to identify possible methods relevant in clinical settings.

KEYWORDS

antimicrobial peptides, atopic dermatitis, DNA, HSV-1, *Staphylococcus aureus*



GRAPHICAL ABSTRACT

AMP-DNA complex formation impairs antimicrobial activities of AMPs. Inhibitory effect of DNA depends on the amount of DNA not on the length of the molecule. Analyses from skin rinses reveal a significantly higher amount of free self-DNA in lesional AD skin compared to non-lesional or healthy skin.

1 | INTRODUCTION

Atopic dermatitis (AD) is a common chronic inflammatory skin disease with a complex pathogenesis, which is mainly characterized by a disturbed skin barrier and an imbalance of the skin immune system. These conditions favor the microbial colonization of the skin, particularly with *Staphylococcus aureus* (*S. aureus*).¹ More than 90% of AD patients show colonization with *S. aureus* in lesional skin with simultaneous decrease in skin microbiome diversity.^{1,2} *S. aureus* plays an important role in the pathogenesis of AD.³⁻⁶ The severity of the skin disease correlates with bacterial load and the expression of *S. aureus* virulence factors. Besides bacterial burden, up to 10% of AD patients suffer from viral infections, mainly with herpes simplex virus type 1 (HSV-1) which may result in extensive cutaneous manifestations, a condition called *Eczema herpeticum* (EH).⁷ EH may lead to severe complications like keratoconjunctivitis, meningitis, encephalitis, or

secondary bacterial sepsis. An infection with *S. aureus* predisposes AD patients to EH.^{8,9}

Antimicrobial peptides and proteins (AMPs) show a broad spectrum of antimicrobial activity against bacteria, viruses, fungi, and protozoa. AMPs are highly cationic small endogenous proteins and peptides which range between only 5 to up to 100 amino acids in size.¹⁰ They are ubiquitously expressed in nature and several have been identified in humans.¹¹ The AMPs of the human skin, like RNase 7, LL-37, and human beta-defensin 2 (hBD2), play an essential role in the innate immune response against invading pathogens.¹²⁻¹⁶ The cationic charge of these AMPs is considered crucial for their antibacterial action.¹⁷ In addition to their direct antibacterial activity, many AMPs have also been described to exhibit immuno-regulatory functions that play an important role in antimicrobial defense. One function of several skin-associated AMPs is to enable keratinocytes and immune cells to sense extracellular self-DNA. The cationic

AMPs form complexes with negatively charged DNA molecules, mediate the uptake by immune cells and keratinocytes¹⁸ and trigger activation of DNA sensing receptors.^{19,20} For the first time, these complex formations and its impact on the immune system have been described in 2007 by Lande et al. using atomic force microscopy.²¹ Subsequently, other studies showed the same effect on other AMPs, including a study on RNase 7.^{22,23} In a study from Schmidt et al. 2015, they combined several techniques to demonstrate the activation of the immune response by the AMP-DNA complex formation.²⁴

A deficiency of AMP expression has long been assumed to contribute to the high susceptibility of AD patients to microbial infections. However, recent studies demonstrate that AMP expression is enhanced and not decreased in lesional skin of AD patients compared to healthy skin.²⁵⁻²⁷ Thus, the mechanism of enhanced susceptibility to *S. aureus* colonization has remained unclear. We hypothesized that self-DNA may decrease the antimicrobial effects of cationic AMPs, and therefore investigated the impact of cell-free DNA on the function of keratinocyte-derived AMPs. Furthermore, we determined the amount of extracellular self-DNA in lesional skin of AD patients. Our study demonstrates for the first time that AD skin lesions contain a high amount of extracellular self-DNA, and that the complex formation of AMPs with self-DNA significantly reduces their antibacterial and antiviral potency in AD.

2 | MATERIALS AND METHODS

2.1 | Study design

After initiation, the skin from AD patients and healthy volunteers was rinsed to determine the DNA concentration. In addition, the impact of free self-DNA on the antimicrobial activity of keratinocyte-derived cationic AMPs was investigated. Data inclusion/exclusion criteria: All patients with a clinical diagnosis of moderate-to-severe AD with obvious lesions and no other chronic cutaneous comorbidity (eg, Psoriasis) and no acute indication of superinfection were included. Patients did not undergo treatment immediately prior to rinsing. For antibacterial assays: Experiments which resulted in an AMP-mediated killing efficiency of less than 20% have been excluded from analysis. For antiviral assays: Experiments with an infection rate below 20% or a LL-37-mediated killing efficiency of less than 3% were excluded from analysis.

2.2 | Antimicrobial peptides and reagents

The origin of the AMPs and the used reagents are described in the supplement.

2.3 | Isolation of human genomic keratinocyte DNA (self-DNA)

The isolation of DNA is described in the supplement.

2.4 | Antibacterial assay

The antibacterial assay was carried out with *Staphylococcus aureus* (ATCC 29213, type 17) as described in the supplement.

2.5 | Antiviral assay with Herpes Simplex Virus type 1

HSV-1-GFP was prepared as recently described.²⁸ For details refer to the supplement.

2.6 | Skin rinsing

Skin rinses were collected from 32 volunteers (controls n = 10, patients n = 22 (mean SCORAD 44.56)). The diagnosis of AD was confirmed by the diagnostic criteria of AD defined by Hanifin and Rajka.²⁹ For details refer to the supplement.

2.7 | Quantification of dsDNA

For details refer to the supplement

Analysis of skin-derived DNA from skin rinsings by PCR and qPCR. Human DNA abundance was measured by qPCR as previously described.³⁰ For details refer to the supplement.

2.8 | Statistics

Averages of the samples are presented as mean or median. Statistical analysis was performed with GraphPad Prism software version 8 (GraphPad software, Inc., La Jolla, CA, USA). Gaussian distribution was verified using Anderson-Darling test, D'Agostino-Pearson omnibus normality test, Shapiro-Wilk normality test, and Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lillie for *p* value. For statistical evaluation of Gaussian distributed data, the paired *t* test and RM ANOVA were used. For asymmetrically distributed data, the Wilcoxon signed-rank test, the Mann-Whitney test, or the Friedman test with Dunn's Multiple Comparison were used. *p*-value below .05 was regarded as significant. Two-sided testing was used with *p* < .05 is depicted with *, *p* < .01 with **, *p* < .001 with ***, and *p* < .0001 with ****.

2.9 | Study approval

The study was approved by the ethics committee of the Hannover medical school and all study participants gave written informed consent prior to inclusion in the study. Study participants received no stipend.

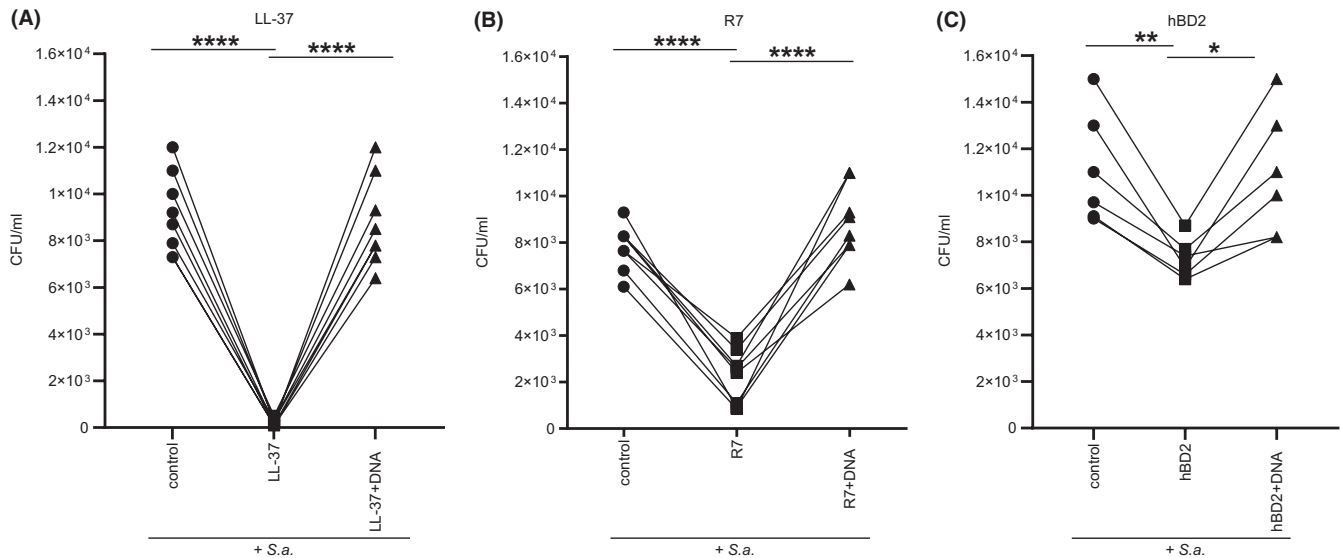


FIGURE 1 Complex formation with self-DNA attenuates antibacterial activity of AMPs. *S. aureus* (*S. a.*) cells (approximately 1×10^4 CFU/ml) were incubated for 1 h at 37°C in $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ buffer alone (control) or supplemented with (A) LL-37 0.1 μM , (B) R7 0.5 μM , (C) hBD2 0.5 μM or with the respective AMP-DNA-complex. LL-37 and hBD2 were mixed with 5 $\mu\text{g}/\text{ml}$ self-DNA each (A and C). R7 was mixed with 15 $\mu\text{g}/\text{ml}$ self-DNA (B). Self-DNA and the respective AMP were pre-incubated for 10 minutes prior to stimulation. The CFUs were quantified on blood agar plates at 37°C. Statistical significance was tested using paired t test ((A) and (B) $n = 8$, respectively, (C) $n = 6$). * $p < .05$; ** $p < .01$; *** $p < .001$; **** $p < .0001$

3 | RESULTS

3.1 | Complex formation with self-DNA attenuates antibacterial activity of AMPs

To investigate if the interaction of AMPs with DNA influences the antibacterial activity of the skin-derived AMPs LL-37, RNase 7, and hBD2, a colony-forming units (CFU) assay with *S. aureus* was performed. *S. aureus* were incubated with AMPs at a concentration known to cause significant bacterial killing in the presence or absence of cell-free DNA for 1 hr. Bacteria were then cultured overnight on blood agar plates and AMP-mediated killing was assessed by counting CFUs of surviving bacteria. The results show a significant loss of antibacterial activity in the presence of DNA compared to AMP effects in the absence of DNA (Figure 1A–C). A potential effect of the AMP solvent and self-DNA alone on the antibacterial activity was excluded by control experiments (data not shown).

3.2 | Abrogation of antibacterial activity by DNA is dose-dependent but independent of the length of the DNA molecules

To study if decreased antibacterial activity depends on the amount of applied DNA, dose-response studies for hBD2, for LL-37, and for RNase 7 were conducted. Bacteria were incubated with LL-37, hBD2, or RNase 7 complexed with increasing concentrations of self-DNA and subsequently analyzed for the number of surviving bacteria.

The inhibition of AMP-mediated antibacterial activity was clearly dependent on the concentration of self-DNA. A complete inhibition of the LL-37 function was achieved at a DNA concentration of 5 $\mu\text{g}/\text{ml}$ (Figure 2A). For hBD2, a lower DNA concentration of 2 $\mu\text{g}/\text{ml}$ was already sufficient for a complete inhibition whereas a comparatively high DNA concentration (15 $\mu\text{g}/\text{ml}$) was necessary to completely inhibit the function of RNase 7 (data not shown). In multicellular organisms, several nucleases rapidly degrade self-DNA released during necrosis.^{31,32} To investigate the effect of short DNA fragments on AMP activity, we repeated the experiment replacing self-DNA with small hexamer primer sequences and, again, observed decreased killing in the presence of DNA (Figure 2B). These results suggest that the rate of inhibition of the LL-37 activity depended on the amount of DNA available but not on the length of the DNA molecules.

3.3 | Free self-DNA influences antiviral activity of LL-37

To investigate if the presence of self-DNA also restricts the well-described antiviral activity of LL-37,^{16,33} keratinocytes were infected with a HSV-1 strain that expresses GFP as a reporter in the presence of LL-37 alone or in complex with self-DNA. The number of infected keratinocytes was then measured by flow cytometry. In accordance with the literature,^{34,35} a significant reduction of HSV-1-GFP infected cells was observed in the presence of LL-37 alone. However, in contrast, LL-37 complexed with self-DNA exhibited significantly impaired antiviral activity (Figure 3).

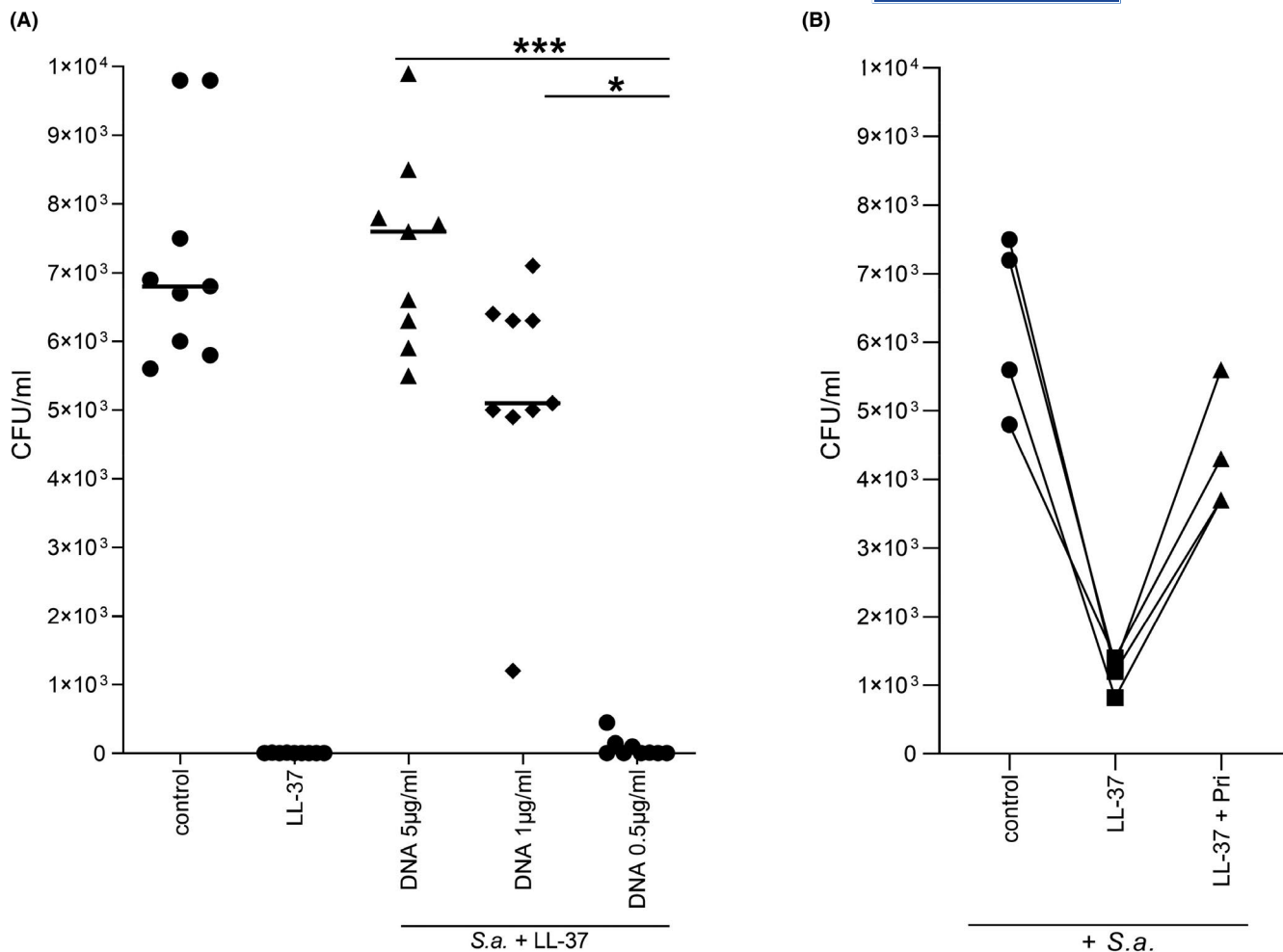


FIGURE 2 Abrogation of antibacterial activity by DNA is dose-dependent but independent of the length of the DNA molecule. *S. aureus* (*S. a.*) cells in $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ buffer (approximately 1×10^4 CFU/ml) were incubated (A) without treatment (control), with LL-37 alone ($0.1 \mu\text{M}$), or with LL-37 mixed with decreasing concentrations of DNA for 1 h at 37°C ($n = 9$) and (B) without treatment (control), with LL-37 alone ($0.01 \mu\text{M}$), or with LL-37 mixed with $5 \mu\text{g/ml}$ of DNA hexamer primer (LL-37+Pri), ($n = 4$). Nucleic acids and the respective AMP were pre-incubated for 10 minutes prior to stimulation. Data of (A) were statistically analyzed using Friedman test followed by Dunn's multiple comparison test. Bars indicate the median values. * $p < .05$; ** $p < .01$, *** $p < .001$

3.4 | Released self-DNA is elevated in lesional skin of patients with atopic dermatitis

AD is characterized by strong pruritus, xerosis, eczematous lesions, and lichenification.³⁶ We suspected that under these conditions enhanced amounts of self-DNA are released by necrotic cells following skin injury caused by scratching. Therefore, we investigated the concentration of extracellular DNA present in skin rinses taken from lesional and non-lesional skin of AD patients compared with healthy skin. We applied two different DNA quantification methods and detected significantly higher amounts of extracellular dsDNA in rinses taken from lesional AD skin when compared to non-lesional AD skin or healthy skin (Figure 4A, B). Interestingly, rinses taken from lesional psoriatic skin contained considerably less extracellular dsDNA when compared to rinses taken from lesional AD skin (median $0.01 \mu\text{g/ml}$ [range: $0.0 - 0.068 \mu\text{g/ml}$] vs median $0.04 \mu\text{g/ml}$ [range: $0.0 - 4.46 \mu\text{g/ml}$]). The lesional skin of AD patients is usually colonized with *S. aureus*.

S. aureus has recently been shown to produce biofilms on AD skin of which bacterial DNA is an integral part.³⁷⁻³⁹ Therefore, to discriminate between human and bacterial DNA, qPCR was applied using primers and protocols to specifically quantify human DNA³⁰ or bacterial DNA⁴⁰ in two selected rinses from lesional AD skin. Human DNA could be quantified in those samples as corresponding to $0.148 \pm 0.024 \mu\text{g/ml}$ (mean \pm s.d.) and $0.0127 \pm 0.0002 \mu\text{g/ml}$, respectively, whereas bacterial DNA was below the detection limit of $0.001 \mu\text{g/ml}$. These data therefore indicate the presence of increased levels of cell-free human DNA on lesional AD skin. Of note, no RNA could be detected in the skin rinses.

3.5 | Rinse solution from AD lesional skin prevents antibacterial activity of LL-37

To test for a potential biological impact, we determined if the amount of DNA in the rinse solutions collected from lesional skin impaired

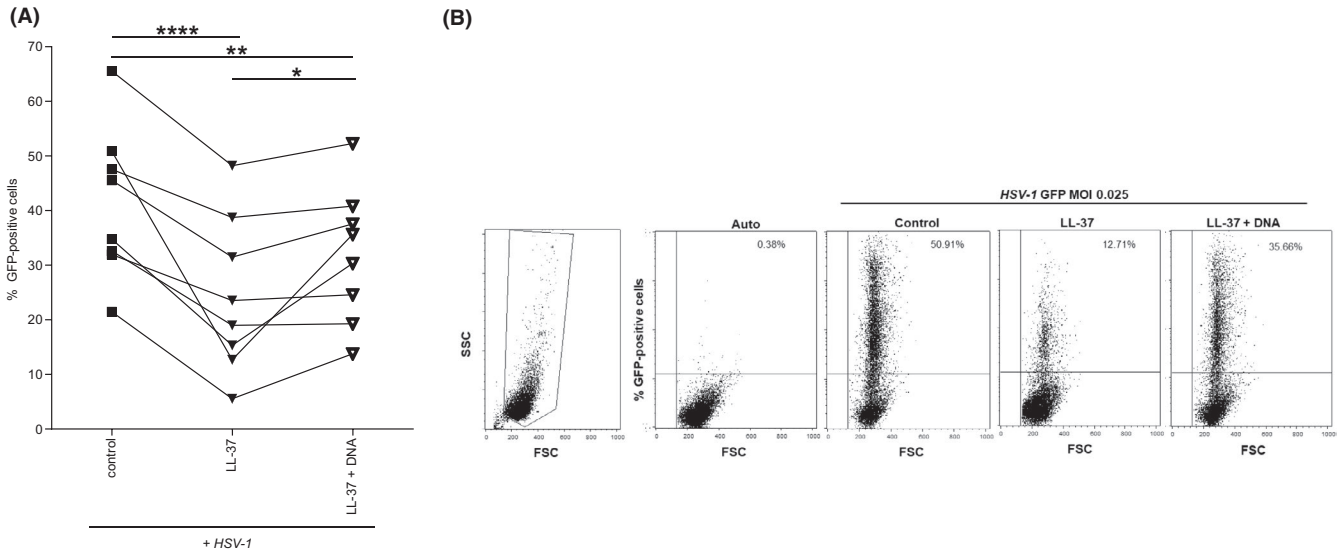


FIGURE 3 Free self-DNA influences antiviral activity of LL-37. (A) Keratinocytes were infected with HSV-1-GFP (MOI 0.025) and incubated without treatment (control), supplemented with LL-37 alone (0.5 μM), or LL-37 (0.5 μM) plus self-DNA (5 μg/ml) for 24 h. LL-37 and self-DNA were pre-incubated for 10 minutes prior to infection. The number of infected keratinocytes was analyzed by flow cytometry analysis (n = 8). (B) A representative experiment out of eight experiments is presented. HSV-1, herpes simplex virus 1; Auto, autofluorescence cell control before infection. Data were statistically analyzed using RM ANOVA. *p < .05; **p < .01; ***p < .001; ****p < .0001

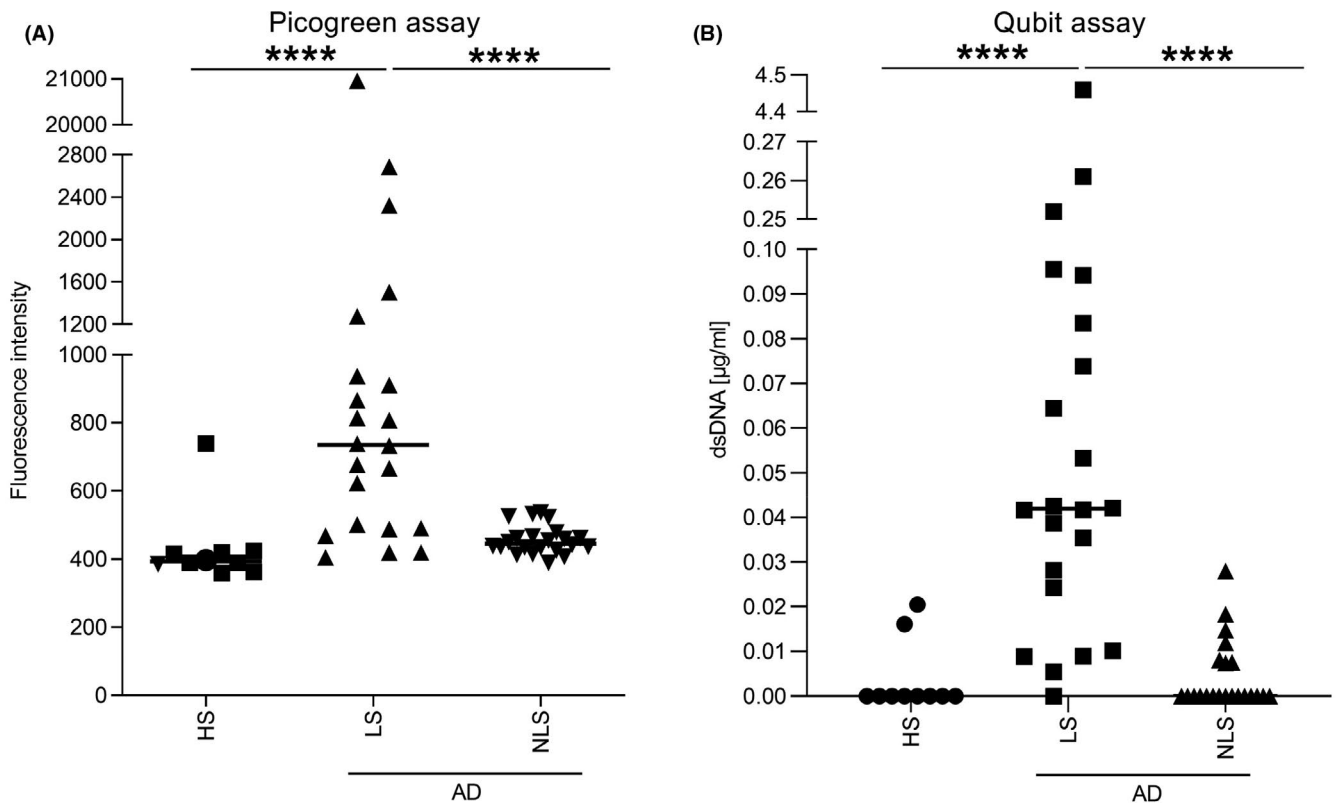


FIGURE 4 Released self-DNA is elevated in lesional skin of patients with atopic dermatitis. Skin areas (HS = healthy skin (n = 10), LS = lesional skin, NLS = non-lesional skin (n = 22, respectively)) were flushed with 1 ml of Ampuwa water for 2 minutes. The DNA concentration in rinse solutions was quantified by (A) Picogreen assay or (B) Qubit assay. Data were statistically analyzed using Mann-Whitney test and Wilcoxon matched-pairs signed rank test. Bars indicate the median values. *p < .05; **p < .01; ***p < .001; ****p < .0001

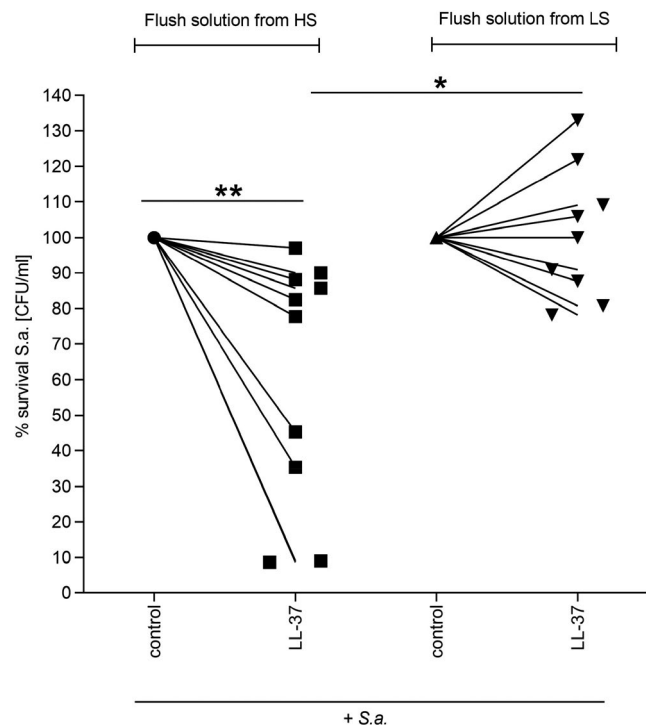


FIGURE 5 Rinse solution from AD lesional skin prevents antibacterial activity of LL-37. *S. aureus* (*S. a.*) cells were suspended in $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ buffer (10^4 CFU/ml) and further diluted 1:10 in the respective rinse solution (control) (HS =healthy skin ($n = 10$), LS =lesional skin ($n = 9$)). Where indicated, LL-37 ($0.1 \mu\text{M}$) was added and the solution was incubated for 1 h at 37°C . CFUs remaining were quantified on blood agar plates and compared to those remaining in controls. Data were statistically analyzed using Wilcoxon matched-pairs signed-rank test and Mann-Whitney test. * $p < .05$; ** $p < .01$

the antimicrobial activity of AMPs. *S. aureus* bacteria were incubated for one hour with rinse solutions (containing DNA concentrations ranging between $0.03 \mu\text{g/ml}$ and $4.46 \mu\text{g/ml}$) taken from lesional AD skin or healthy skin (see Figure 4B), alone or supplemented with LL-37. The antibacterial activity of LL-37 was then evaluated by measuring CFUs of the surviving bacteria. The results show that the antibacterial activity of LL-37 was reduced when combined with skin rinses from AD lesional skin when compared to combination with skin rinses from healthy skin (Figure 5). These data indicate a biological consequence of the DNA detected on the skin of AD patients with regard to the antimicrobial activity of AMPs.

4 | DISCUSSION

The skin of AD patients is characterized by a high susceptibility toward infections caused by *S. aureus* and other infectious threats such as *HSV-1* and *Malassezia spp.*^{8,41} Previous work suggested that an impaired expression of AMPs in AD may contribute to an impaired growth control of *S. aureus* on the skin.⁴² However, there is increasing evidence that AMPs are induced in lesional AD skin.^{25,27,43,44} Recent

studies show that several skin-derived cationic AMPs such as LL-37, hBD2, hBD3, and RNase 7 bind extracellular self-DNA and mediate sensing of normally non-immunogenic self-DNA by intracellular DNA receptors, resulting in an inflammatory immune response.²¹⁻²³ This mechanism is most likely important for the antiviral immune responses in the skin, and the pathogenesis of psoriasis. However, its role on the antimicrobial activity of cationic AMPs in inflammatory skin diseases has not yet been investigated.

The aim of this study was to investigate the hypothesis that DNA released by damaged cells in the AD environment may inhibit the antimicrobial activity of AMPs. We provide the first evidence that elevated concentrations of cell-free self-DNA, which results in a reduction of the antimicrobial functions of skin-derived cationic AMPs. In particular, LL-37, RNase 7, and hBD2, which all possess a broad spectrum of antibacterial properties,^{12,45-47} were inhibited by DNA. We used concentrations of *S. aureus*, which were in the range of published concentration for human skin in lesional atopic dermatitis.^{48,49} In our experimental setup, LL-37 showed the strongest killing activity against *S. aureus*.

AD is a skin disease characterized by an intense pruritus. Scratching of the skin results in the destruction of cells and the subsequent release of cell content, including self-DNA, into the extracellular environment. In addition, in inflammatory skin conditions, necrotic cells release self-DNA. In our comparative experiments, we detected significantly higher concentrations of extracellular DNA in skin rinses taken from lesional AD skin compared to non-lesional AD skin or healthy skin. Of note, since lesional AD skin is usually dry with scarce appearance of water,⁵⁰ we believe the extracellular DNA extracted by rinsing may have undergone up to 100-fold dilution in the rinse solution ($1000 \mu\text{l}$). Therefore, the mean DNA concentration (median $0.042 \mu\text{g/ml}$) detected in rinses taken from lesional AD skin comply with the DNA concentration used in the experiments. Furthermore, rinses taken from AD lesional skin reduce the antimicrobial activity of additionally applied LL-37. Given that the detected self-DNA is free of bound substances (eg, AMPs),²² this provides evidence that the increased amounts of extracellular self-DNA present in the rinses of AD skin could be of biological relevance, as the antimicrobial activity of the enhanced AMP levels in AD skin⁵¹ has already been neutralized by the DNA in the rinses, and even the application of additional LL-37 did not restore the antibacterial activity of the solution. The analysis of the level of antimicrobial peptides was not included here, as there are already studies available, which clearly show elevated AMP levels in AD lesional skin.²⁵⁻²⁷ This needs to be addressed in follow-up studies.

With regard to the inhibition of the antimicrobial activity of cationic AMP on AD skin, Weiner et al. have previously reported that DNA from human placenta inhibits the antibacterial activity of LL-37 against *Pseudomonas aeruginosa*.⁵² Here, we show that this phenomenon is not restricted to LL-37, to gram-negative bacteria, or to the inhibition of the antibacterial activity. The inhibitory effect of self-DNA was demonstrated for each of the used cationic AMPs and seems to be independent of structural differences of the respective AMP. As electrostatic charges between the positively charged

AMP and the negatively charged DNA mediate the formation of the AMP-DNA complex, which has been demonstrated by several studies using different techniques,^{21,22,24} we hypothesize that this DNA-mediated inhibition of the antimicrobial activity is restricted to cationic AMPs.⁵³

The DNA neutralizes the positive charges of the AMP, and sterically prevents the AMPs from binding to the microorganisms. This is in line with our finding that the abrogation of the antibacterial activity depends on the quantity of available DNA. Of note, small DNA fragments, such as hexamers, also mediated a strong reduction of the antibacterial activity of LL-37 if available in sufficient quantities. This indicates, that treatment of the DNA, for example, by DNases, would not prevent inhibition of the antimicrobial activity of AMPs by DNA. To inhibit the antibacterial activity of RNase 7 more DNA was necessary compared to LL-37 and hBD2, which has been shown by dose-response studies. It is possible that due to the higher mass of the RNase 7 peptide (R7 = 14.5 kDa,¹² LL-37 = 4.5 kDa,⁵⁴ hBD2 = 4.3 kDa⁵⁵), more DNA was necessary to cover most of the positive residues of RNase 7 which drive the interaction with the negative charge of the DNA backbone.^{21,56-58} Therefore, it seems that a threshold concentration of DNA has to be exceeded to inhibit the antibacterial activity of each AMP.

Our results also suggest that the DNA-mediated inhibition of LL-37's antiviral activity and possibly that of other AMPs, may contribute to the enhanced susceptibility of individuals with AD to infections by HSV-1.⁸ Although some AMPs, like LL-37 and human defensins, exhibit direct antiviral activities, their mechanism of action is not yet fully understood. It has been reported that human defensins inhibit viral binding or internalization into cells, hinder replication of HSV gene expression or prevent escape of HPV from endocytic vesicles.⁵⁹ LL-37 exhibits significant antiviral properties against HSV-1,³⁴ Vaccinia virus (VV),⁶⁰ and human immunodeficiency virus (HIV)-1.⁶¹ Although these studies coincide in their results, the underlying mechanism of antiviral action differed. Whereas LL-37 inhibited HIV-1 replication,⁶¹ its activity against VV has been attributed to disrupting the viral envelope.⁶¹⁻⁶³ In our setup, a pre-incubation of LL-37 with self-DNA reduced its activity against HSV-1, possibly also by neutralizing the positive charge of LL-37 or sterically hindering its binding to HSV-1. Further investigations need to investigate whether cell-free self-DNA will neutralize also the antiviral activity of LL-37 and other cationic AMPs against other viruses. A general deficiency in AMP expression has been considered to contribute to the high susceptibility of AD patients to *S. aureus* and other microbial infections including HSV-1 and *Malassezia* spp.⁴² However, several recent studies have dismissed this hypothesis.⁵¹

5 | CONCLUSIONS

In summary, our study provides evidence that the amount of extracellular self-DNA is elevated on the surface of AD lesions. The inhibition of host cationic AMP activity by released self-DNA may contribute to the high susceptibility of AD patients to *S. aureus* and

HSV-1 infections. Strategies to reduce the amount of extracellular DNA in AD may therefore improve the cutaneous antimicrobial barrier.

ACKNOWLEDGEMENTS

Author Contributions: VK and TW: Conceptualization. VK and SD: Formal analysis. TW, JH, and BS: Funding acquisition. SD, DP, and JZ: Investigation. VK, SD, DP, AB, BS, FR, and JH: Methodology. VK, SD, and TW: Project administration. TW, AB, BS, FR, and JH: Resources. TW and BS: Supervision. VK and SD: Visualization and Writing original draft. VK, SD, JZ, DP, AB, BS, FR, JH, and TW: Writing—review and editing.

CONFLICT OF INTEREST

VK has nothing to disclose. SD has nothing to disclose. JZ has nothing to disclose. DHP has nothing to disclose. AB has nothing to disclose. BS has nothing to disclose. FR has nothing to disclose. JH has nothing to disclose. TW reports grants and personal fees from Novartis, personal fees from Sanofi, grants and personal fees from Lilly, grants and personal fees from LEO, grants from Pfizer, grants from Galderma, grants and personal fees from AbbVie, outside the submitted work. The authors thank Dr. Michael Kleine and Karsten Hofmann from Planton GmbH in Kiel for providing recombinant hBD2 peptide for Figure 1C. We also thank MUDR. Michal Sapak, PhD and Paulina Syryca from the clinical team of the Dept. for Dermatology, Allergology, and Venerology at the Hannover Medical School for their support in collecting patient-derived samples. In addition, we thank Miriam Wittmann and her colleague Tom Macleod (University of Leeds, Leeds, United Kingdom) for discussing the manuscript. In addition, we thank Ilona Klug for excellent technical support.

DATA AVAILABILITY STATEMENT

All data associated with this study are available in the main text or the supplementary materials.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Kopfnagel V, Dreyer S, Zeitvogel J, et al. Free human DNA attenuates the activity of antimicrobial peptides in atopic dermatitis. *Allergy*. 2021;00:1-10. <https://doi.org/10.1111/all.14992>