

The role of human beta defensins and cathelicidins in atopic dermatitis

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Purpose of review

Atopic dermatitis is a chronic inflammatory skin disease associated with significant barrier disruption, T-helper type 2 mediated skin inflammation, and an impaired innate immune response. These characteristics increase the susceptibility of atopic dermatitis patients to recurrent skin infections, some of which may have potentially fatal implications. The mechanisms resulting in this increased propensity for skin infections have been an area of active investigation.

Recent findings

Antimicrobial peptides are an integral component of the innate immune response due to their broad spectrum activity against invading pathogens. Recent studies have shown that these peptides are effective at killing *Staphylococcus aureus*, herpes simplex virus, vaccinia virus, and the *Malassezia* species, pathogens associated with significant morbidity in patients with atopic dermatitis. Additionally, these peptides are deficient in the skin of atopic dermatitis patients, suggesting that the increased propensity of patients towards skin infection is due to the lack of antimicrobial peptide expression.

Summary

The current review will examine recent literature on the role of antimicrobial peptides in atopic dermatitis in an effort to improve our understanding of why patients with the condition suffer from recurrent infections.

Keywords

antimicrobial peptides, atopic dermatitis, infection, innate immunity

Abbreviations

ADEH	eczema herpeticum
AMP	antimicrobial peptide
EAD	extrinsic atopic dermatitis
HBD	human- β -defensin
HSV	herpes simplex virus
IAD	intrinsic atopic dermatitis
Ig	immunoglobulin
IL	interleukin
LL-37	cathelicidin
Th	T helper
TNF	tumor necrosis factor

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Introduction

Atopic dermatitis is a highly pruritic, chronic inflammatory skin disease that is often seen as the first step of the 'atopic march'. Atopic skin disease is associated with significant morbidity and occupational disability affecting nearly 17% of children and 2% of adults. The skin of these patients is characterized by T helper type 2 (Th2)-mediated inflammation and significant barrier disruption; however, the mechanisms underlying the development and onset of atopic dermatitis are not completely understood [1,2*,3]. Recent studies have demonstrated that genetic and environmental factors play important roles [4–6].

In recent years, there has been a great deal of interest in understanding the role and function of the epidermal barrier within atopic dermatitis skin. The epidermal barrier serves as the first line of defense against invading pathogens and allergens. When the protective barrier is compromised, however, further invasion is prevented by the innate immune response. In atopic dermatitis skin, the protective epidermal barrier is significantly compromised and the innate immune response is impaired, leading to increased allergen sensitization and recurrent skin infections, respectively [1,3,7].

Antimicrobial peptides (AMPs) are an integral component of the innate immune response due to their broad spectrum activity against bacteria, viruses, and fungi. The current review will examine the role of AMPs in the skin of atopic dermatitis patients.

Overview of antimicrobial peptides

AMPs are endogenous small molecular weight proteins that are produced primarily by keratinocytes in the

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epidermis. These proteins are primarily cationic in nature, with hydrophobic and hydrophilic regions that allow solubility and permeability in aqueous and lipid-rich environments. Two major classes of AMPs have been identified in mammalian skin: β -defensins [8,9] and cathelicidins [10]. Currently, there are four known human β -defensins (HBDs) which contain six cysteine residues that form three disulfide bridges. Whereas HBD-1 is constitutively expressed in epithelial tissue, HBD-2 is upregulated in inflamed skin [11]. HBD-3 was originally isolated from psoriatic scales and is expressed in a variety of tissues [8]. HBD-4 was identified by screening the human genome database and is inducible in human keratinocytes [12]. Cathelicidins are characterized by a highly conserved N-terminal cathelin domain and a C-terminal cationic domain. Currently, only one cathelicidin LL-37 has been identified in humans and is expressed in various epithelia and induced in the keratinocytes of inflamed skin [13].

The primary function of these two families of AMPs is to protect the skin from invasion by foreign pathogens. The microbial killing mechanism is dependent on the charge and structure of the AMPs. AMPs are cationic and therefore interact with the anionic components of bacteria, fungi, and viruses to permeabilize the microbial membrane. Permeabilization of the membrane disrupts the ion gradient leading to cell lysis [14]. Studies using animal models have demonstrated the importance of the AMPs in host defense against bacterial [15] and viral [16] infection.

In addition to their microbicidal function, AMPs also modulate immune functions. β -defensin 2 recruits immature dendritic cells and memory T cells to the site of inflammation by binding to CCR6 [17]. The cathelicidin LL-37 binds to formyl peptide receptor-like 1 to recruit neutrophils, monocytes and T cells [18]. Using DNA microarray analysis, it was recently shown that LL-37 upregulates chemokines and cytokines involved in inflammation in human keratinocytes [19]. A recent article by Niyonsaba *et al.* [20^{*}] extensively evaluated the effect of β -defensins and LL-37 on keratinocytes. They demonstrated that β -defensins and LL-37 induce the production of cytokines [interleukin (IL)-6, IL-10] and chemokines (interferon inducible protein-10, monocyte chemoattractant protein-1, macrophage inflammatory protein-3 α , RANTES) in keratinocytes. HBD-3 and LL-37 induced similar patterns of cytokine and chemokine expression to a greater extent than those levels observed for HBD-1, 2, and 4. It was also shown that β -defensins and LL-37 mobilize calcium and trigger keratinocyte migration through the activation of epidermal growth factor receptor, STAT-1, and STAT-3. Calcium is essential for the differentiation of keratinocytes within the

epidermis and plays an important role in the regulation of numerous proteins within the epidermal differentiation complex. Therefore, there could be an important relationship between AMP expression in the epidermis and barrier integrity.

Clinical relevance of antimicrobial peptides in the skin

Differential expression of AMPs in the skin plays an important role in skin susceptibility to recurrent skin infections. Atopic dermatitis and psoriasis are the two most common inflammatory skin diseases. Both skin diseases are characterized by significant skin barrier disruption [21], however, a comparison of atopic dermatitis and psoriasis showed that 30% of atopic dermatitis patients suffer from skin infections whereas only 6.7% of psoriasis patients had this complication [22]. One potential explanation is that the reduced prevalence of infections in psoriasis, but not atopic dermatitis, may be associated with the increased production of AMPs [23]. Ong *et al.* [24] were the first to demonstrate that HBD-2 and LL-37 expression are reduced in the skin of atopic dermatitis patients compared with the levels seen in patients with psoriasis [24]. A follow-up paper [7] demonstrated that HBD-3 and other innate immune response genes were reduced in lesional skin from atopic dermatitis patients compared with those with psoriasis. Atopic dermatitis can be separated into two subsets. Extrinsic atopic dermatitis (EAD) patients have elevated serum immunoglobulin E (IgE) and allergic sensitization and comprise approximately 70–80% of atopic dermatitis patients. Intrinsic atopic dermatitis (IAD) or nonatopic eczema patients make up 20–30% of patients and exhibit normal serum IgE levels and lack allergen-specific sensitization [25]. Despite these differences, both EAD and IAD patients present classically and suffer from recurrent skin infections. The original studies demonstrating reduced AMP expression in atopic dermatitis skin primarily used patients with EAD; however, recent studies by Howell *et al.* [26,27] have demonstrated that HBD-2, HBD-3, and LL-37 expression is reduced in the skin of both EAD and IAD patients. Atopic dermatitis skin is characterized by the overexpression of IL-4, IL-10 and IL-13 [27–29]. In contrast, psoriasis skin is characterized by the overexpression of proinflammatory [tumor necrosis factor- α (TNF- α), IL-6] and Th1 [interferon (IFN- γ)] cytokines that have been shown to induce AMP expression [7,30]. The relationship of these cytokines and AMP expression was investigated and it was determined that reduced AMP expression in atopic dermatitis skin was due to the direct inhibitory effects of IL-4 and IL-13 on TNF- α and IFN- γ stimulation in keratinocytes; and the indirect effects of the immunomodulatory cytokine IL-10 on proinflammatory cytokine production by infiltrating cells [26,27].

Role in staphylococcal infections

Staphylococcus aureus is a Gram-positive bacteria that colonizes approximately 90% of atopic dermatitis patients [3]. In contrast, *S. aureus* is found on less than 5% of normal individuals. Whereas *S. aureus* is usually low in number and mainly confined to the intertriginous areas when present on normal skin; super-infection with *S. aureus* can reach 10^7 organisms per square centimeter on acute exudative atopic dermatitis skin lesions. This suggests that atopic dermatitis skin supports the colonization and proliferation of *S. aureus*. While the increased binding affinity of atopic skin for *S. aureus* could account for a slight increase in bacterial numbers, an impaired innate immune response is necessary for the exponential increase in *S. aureus* colonization seen in AD skin. LL-37 and HBD-3 have been shown to individually exhibit potent antibacterial activity against *S. aureus*; however, HBD-2 must work synergistically with additional AMPs to kill *S. aureus* [8,9,24,31]. Stimulation of normal keratinocytes with *S. aureus* has been shown to induce the expression of HBD-2, HBD-3, and LL-37 [32–34]. The induction of these AMPs in normal keratinocytes and the potent antibacterial activity against *S. aureus* explains, in part, why such a small percentage of normal individuals are colonized with *S. aureus*. In contrast, the overexpression of Th2 cytokines in the skin of atopic dermatitis patients inhibits the induction of the AMPs, thereby potentiating bacterial colonization and infection. This is particularly concerning with the emergence of methicillin-resistant *S. aureus*, which poses a significant community health problem for atopic dermatitis patients since treatment is difficult.

Role in viral infections

Eczema herpeticum (ADEH) is a disseminated herpes simplex virus (HSV) 1 or 2 infection that develops in a subset of atopic dermatitis patients [35] and can be potentially fatal if left untreated [36]. The relationship between AMP expression and HSV infection is not completely understood, so a recent study investigated the role of LL-37 in the susceptibility to ADEH [37^{••}]. Initially, it was demonstrated that LL-37 exhibits potent antiviral activity against HSV and that cathelicidin expression is necessary to control HSV pathogenesis. Additionally, it was determined that there is an inverse relationship between serum IgE levels and LL-37 expression. ADEH patients characteristically exhibit serum IgE levels higher than those observed for the general atopic dermatitis population [35]. This suggests that serum IgE and LL-37 expression could be markers for identifying those at risk of developing ADEH.

Patients with atopic dermatitis are additionally at risk of developing eczema vaccinatum following inoculation with the smallpox vaccine, vaccinia virus [38]. The seriousness of this complication has prompted the Centers

for Disease Control and Prevention to exclude patients with atopic dermatitis, and anyone who comes into close contact with them, from voluntary smallpox vaccination [39]. Several recent studies have demonstrated that the AMPs play an important role in the innate immune response against vaccinia virus. A key investigation demonstrated that atopic dermatitis skin biopsies support vaccinia virus replication to a greater extent than skin from normal individuals or psoriasis patients. This corresponded with the reduced ability to express LL-37 following stimulation with vaccinia virus. Further investigation demonstrated that IL-4 and IL-13 inhibit vaccinia virus-mediated induction of LL-37 through STAT-6 [40^{••}]. LL-37 is known to exhibit antiviral activity against vaccinia virus [16]; combined with a recent study which demonstrates the antiviral activity of HBD-3 against vaccinia virus [41], the impaired AMP response in atopic dermatitis skin may predispose these patients to adverse skin reactions following smallpox vaccination.

Role in fungal infections

Malassezia (Pityrosporum ovale) is a lipophilic yeast commonly present in the seborrheic areas of the skin such as the scalp, face, neck and upper part of the chest. Similar levels of *Malassezia* colonization are seen in normal individuals and atopic dermatitis patients [42]; however, recent studies demonstrate that IgE antibodies against the *Malassezia* species are more common in atopic dermatitis patients [43,44]. It has recently been shown that LL-37 and the β -defensins exhibit activity against *Malassezia* [45,46]. AMPs serve as the second line of defense against invading pathogens and prevent further penetration. Since AMPs are deficient in the skin of atopic dermatitis patients due to the overexpression of Th2 cytokines, it is postulated that the enhanced penetration of the *Malassezia* species may predispose atopic dermatitis patients to IgE and hypersensitivity responses. This is supported by reports that treatment with antifungal agents improves the skin of atopic dermatitis patients with IgE antibodies against the *Malassezia* species [47[•]].

Conclusion

Atopic dermatitis is a chronic inflammatory skin disease complicated by barrier disruption and deficient innate immune response. These complications affect the development and severity of the disease and are influenced by genetic and environmental factors. The skin innate immune response is highlighted by the presence of AMPs which participate in several immunological functions. The primary function of the AMPs is to prevent cutaneous infection, directly and indirectly. AMPs exhibit potent activity against a variety of Gram-positive and Gram-negative bacteria, viruses, and fungi to directly prevent skin infection. Additionally, the interaction between AMPs and their cognate receptors recruits target cells to the site of infection, thereby amplifying the

antimicrobial response to the invading pathogen. The deficiency of these AMPs in atopic dermatitis skin predisposes atopic dermatitis patients to recurrent skin infections with pathogens that may have potentially fatal implications. Further understanding the role of the AMPs in the innate immune response will provide additional insight into the treatment and management of atopic dermatitis patients.

Acknowledgements

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- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 455).

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