Expert Opinion

Peptides, Proteins & Antisense

Antimicrobial peptides and the skin

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In recent years, hundreds of naturally occurring peptide antibiotics have been discovered based on their ability to inhibit the growth of microbial pathogens. These antimicrobial peptides (AMPs) participate in the innate immune response by providing a rapid first-line defence against infection. This review discusses the biology and clinical relevance of the two major families of AMPs, cathelicidins and defensins, with emphasis on their function in mammalian skin and their association with skin pathology. Current evidence shows that cathelicidins and defensins act as both natural antibiotics and as signalling molecules that activate host cell processes involved in immune defence and tissue repair. Alterations in the expression pattern of AMPs have been associated with a variety of pathological processes. Ongoing and future studies are likely to implicate AMPs in several unexplained human inflammatory disorders and to provide novel therapeutic approaches for the treatment of these diseases.

Keywords: antimicrobial peptides, cathelicidins, defensins, innate immunity, skin

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1. Introduction

Antimicrobial peptides (AMPs) are a diverse group of small polypeptides classified together due to their capacity to inhibit the growth of microbes. The existence of AMPs has been known for > 20 years, but their function in the mammalian immune response has been recognised only recently. As effectors of innate immunity, AMPs directly kill a broad spectrum of bacteria, fungi and certain viruses. In addition, these peptides modify the local inflammatory response and activate mechanisms of adaptive immunity.

Most AMPs maintain certain common structural features, including a cationic charge and the ability to interact with bacterial membranes through hydrophobic amino acids. Based on their structural similarities, mammalian AMPs have been divided into several families. In the skin, two major groups, cathelicidins and defensins, have been most extensively studied, although several other antimicrobial peptides and proteins are known. Both cathelicidins and defensins are expressed in keratinocytes and have been implicated in a variety of skin conditions. This review will concentrate on these two major families of AMPs and their role in the skin.

2. Antimicrobial peptides

2.1 Cathelicidins

Cathelicidins derive their name from their highly conserved 'cathelin' precursor domain [1]. They are characterised by an N-terminal signal peptide, a highly conserved prosequence resembling the protease inhibitor cathelin and a structurally variable cationic AMP at the C-terminus. Cathelin was first isolated from porcine neutrophils as an inhibitor of cathepsin L, hence its name [2]. Humans and mice have only one cathelicidin gene, whereas other mammals, such as pigs and cattle, have a

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variety of cathelicidin genes with similar precursor domains, but very different C-terminal peptides. These C-terminal peptides were among the first mammalian AMPs to show rapid, potent and broad spectrum antimicrobial activity [3,4]. They have been assigned a variety of names based on their unique sequence and have been studied more intensively because of their potent antimicrobial activity. The activities of the other part of the parent protein, the cathelin domain, are less well characterised. At least in humans, it appears to function as both a protease inhibitor and an antimicrobial [5].

The human cathelicidin, LL-37/hCAP18, was cloned from cDNA isolated from human bone marrow [6]. The mature AMP is referred to as LL-37 because, after processing by neutrophil proteases, it begins with two leucine residues and is 37 amino acids long. The term hCAP18 was independently coined to recognise human cathelicidin as a cationic AMP whose mass before proteolytic cleavage is ~ 18 kDa [7]. LL-37 has a highly hydrophobic N-terminal domain and an α-helical domain most pronounced in the presence of negatively charged lipids. Processing of LL-37 from the cathelicidin precursor is essential for activation of its antimicrobial activity and is accomplished by enzymes such as protease 3 [8,9]. LL-37 is constitutively expressed in a variety of tissues [10,11] and is also present in squamous epithelia, where its expression is regulated in several inflammatory conditions [6,12-16]. LL-37 has direct antimicrobial activity, works well in synergy with other AMPs, acts as a chemoattractant and stimulates endothelial cell proliferation by binding to formyl peptide receptor-like 1 (FPRL-1) [17,18]. LL-37 can recruit mast cells [19] and can be produced by mast cells [20], thereby participating in innate immunity both by direct antimicrobial activity and by recruitment of cellular defences. This multifunctionality is a fundamental property of many AMPs.

Many non-human cathelicidins are known and have been useful for modelling human cathelicidin function and for their potential use in novel drug design. Studies of mouse cathelin-related AMP [21] have provided direct evidence of the importance of cathelicidins in immune defence [20,22]. PR-39, a proline- and arginine-rich peptide with broad spectrum antibacterial properties, which was originally purified from porcine intestine [4], induces proteoglycan synthesis critical for wound repair, exhibits chemoattractant activity for leukocytes, has anti-inflammatory activity *in vivo* and was one of the first AMPs to demonstrate such broad functionality in mammals [23].

2.2 Defensins

Defensins are a diverse family of AMPs. They are small cationic peptides, which contain 6 – 8 cysteine residues that form characteristic disulfide bridges. The alignment of disulfide bridges and their molecular structure classify defensins into three distinct subfamilies: α-defensins, β-defensins and θ-defensins, the latter group being absent in humans. Defensins are found in mammals (distantly related peptides are found in insects and plants) [24] and exhibit antimicrobial activity against bacteria, fungi and enveloped viruses [25,26].

2.2.1 α-Defensins

α-Defensins have three disulfide bridges in a 1-6, 2-4, 3-5 alignment. Human neutrophils express four α-defensins (1, 2, 3, 4) referred to as human neutrophil peptides 1 – 4 [27], or altenratively as human defensins (HDs) 1 – 4. The other two known α-defensins, HD-5 and -6, are abundantly expressed in Paneth cells of the small intestinal crypts [28,29] and in epithelial cells of the female urogenital tract [30]. Homologous peptides, referred to as cryptdins (crypt defensins), were found in Paneth cells of the mouse small intestine [31]. In humans, defensins are stored in azurophilic granules of neutrophils as fully processed, mature peptides and as propeptides in the Paneth cells. Like cathelicidins, α-defensins exert action on both microbes and the host. For example, HD-1 – 3 have been shown to increase the expression of tumour necrosis factor (TNF)-α and IL-1 in human monocytes that have been activated by bacteria, or reduce expression of the vascular cell adhesion molecule-1 in human umbilical vein endothelial cells activated by TNF-α [32].

2.2.2 β-Defensins

β-Defensins likewise contain three disulfide bridges, but these are spaced differently than those in α-defensins, in a 1-5, 2-4, 3-6 pattern. β-Defensins have been identified from many different cell types, including epithelial cells and peripheral blood mononuclear cells [33-35]. There are four known human β-defensins (HBDs), 1 – 4. HBD-1 is highly expressed in the kidney and considered to be constitutively expressed in epithelial organs [36-38], whereas HBD-2 is only expressed at very low levels in epithelia and highly upregulated in inflamed skin [39,40]. HBD-3 was purified from human psoriatic scales and calluses [41], is expressed in a variety of tissues and is also inducible (IL-1β induces its expression in gingival keratinocytes and fetal lung tissue). In human respiratory epithelial cells, HBD-4 is considered to be inducible as well, although a low level of expression was noted in other tissues [42]. Recent genomic analysis suggests that many β-defensins have yet to be discovered [43] and several have already been identified through novel computational gene discovery strategies, leading to the development of new nomenclature for β-defensins (DEFB101, 102, etc.) [43,44]. β-Defensins have a broad spectrum of antimicrobial activity under optimal culture conditions and additional immune-related cellular functions. For example, HBD-2 binds to CCR-6 and is chemotactic for immature dendritic cells and memory T cells [45], and also promotes histamine release and prostaglandin D2 production in mast cells, suggesting a potential immunotherapeutic role as a vaccine adjuvant to enhance antibody production [46]. The upregulation of HBD-2 by human keratinocytes illustrates the important role defensins play in host defence against cutaneous pathogens. HBD-2 is essentially not

Table 1. Tissue distribution and function of the most common antimicrobial peptides in humans.

Peptide	Tissue distribution	Functions
Cathelicidin LL-37/hCAP18	Neutrophils Mast cells Epithelia (skin, lungs, gastrointestinal, oral, urogenital) Sweat Seminal fluid	Antimicrobial Chemoattractant
α-Defensins HNP-1 – 4 or HD-1 – 4 HD-5 and 6	Neutrophils Epithelia (intestinal, Paneth cells, genital, oral)	Antimicrobial Chemotactic
β-Defensins HBD-1 – 4	Peripheral blood mononuclear cells, monocytes, alveolar macrophages, monocyte-derived dendritic cells Epithelia (skin, oral, mammary, lung, urinary, eccrine ducts, ocular) Adult heart, skeletal muscle, placenta, fetal thymus Testes, gastric antrum and low level in neutrophils, uterus, thyroid, lung, kidney	Antimicrobial Chemotactic Induce histamine release

HBD: Human β-defensin; HD: Human defensin; HNP: Human neutrophil peptide.

present in normal skin and its expression in human keratinocytes requires cytokine (e.g., IL-1) or bacterial (e.g., *Pseudomonas aeruginosa*) stimuli [47,48].

3. Antimicrobial peptides in the skin

AMPs are found in a variety of tissues (Table 1). Their expression pattern and regulation in each cell type is specific for the defensive role that AMPs play in different organs, which in turn provides insight into their function.

AMPs were first observed in mammalian skin when the cathelicidin PR-39 was discovered in porcine wound fluid [23]. Subsequently, human cathelicidin LL-37 was observed in epidermal keratinocytes [12,13]. HBD-2 and -3 were first cloned from the skin and were shown to be inducible rather than constitutively expressed [39,41,49]. HBD-1 was found in the epidermis as well, but is not inducible [36,50]. A unique peptide, dermcidin, is secreted into sweat [51], where it combines with LL-37, HBD-1 and HBD-2 and provides a constitutive source of antimicrobial activity [50,52]. Other peptides with antimicrobial activity, such as adrenomedullin [53], cystatin [54], secretory leukocyte protease inhibitor [55] and neutrophil gelatinase-associated lipocalin [56], have also been observed in skin.

A wide variety of skin conditions have been examined for changes in the expression pattern of AMPs. Cathelicidin is differentially expressed in several inflammatory skin disorders. LL-37 is induced in human keratinocytes during psoriasis, lupus erythematosus and contact dermatitis [12] and is downregulated in atopic dermatitis [57]. In the inflammatory skin lesions of erythema toxicum neonatorum, LL-37 expression is upregulated, with immunolocalisation of the peptide within CD15-expressing neutrophils, EG-2-expressing eosinophils and CD1a-expressing dendritic cells [58]. LL-37 is also induced within the epidermis during development of *Verruca vulgaris* and *Condyloma accuminata*, suggesting it represents a component of the immunological response to papillomavirus infection [59]. HBD-1 and -2 are upregulated in the lesions of acne vulgaris, suggesting that they may be involved in the pathogenesis and/or resolution of this condition [60]. HBD-2 and -3 are not present in normal skin, but are upregulated in the keratinocytes of inflamed psoriatic lesions [39,41], pointing to their role in this chronic inflammatory skin condition [61].

The different roles of AMPs in the skin are well illustrated by their expression patterns. Differential expression of AMPs appears to play a role in the susceptibility of chronic inflammatory skin disorders to infectious complications. In psoriasis, cathelicidin and β-defensin levels are elevated and secondary infection is rare, whereas in atopic dermatitis expression of the same AMPs is deficient and bacterial or viral superinfection is common [57,61]. The difference in AMP expression in these two disorders is even more relevant given the known activity of human cathelicidin against *Streptococcus pyogenes* [13] and the synergistic activity of human cathelicidin and β-defensin against *Staphylococcus aureus* [57], the leading agents of skin infections in humans.

Cathelicidin is produced in high levels in the skin after wounding [13] and is strongly expressed in healing skin epithelium [62]. After wounding of skin, growth factors are produced to stimulate tissue regeneration until the physical barrier protecting against microbial infections is re-established. It has recently been shown that growth factors important in skin wound healing, such as insulin-like growth factor I and transforming growth factor-alpha, induce the expression of cathelicidins and defensins in human keratinocytes [63]. LL-37 antibodies inhibit postwounding re-epithelialisation in a dose-dependent manner. In chronic ulcers, cathelicidin levels are low or absent in the ulcer-edge epithelium [62]. LL-37 is also able to induce angiogenesis, a process

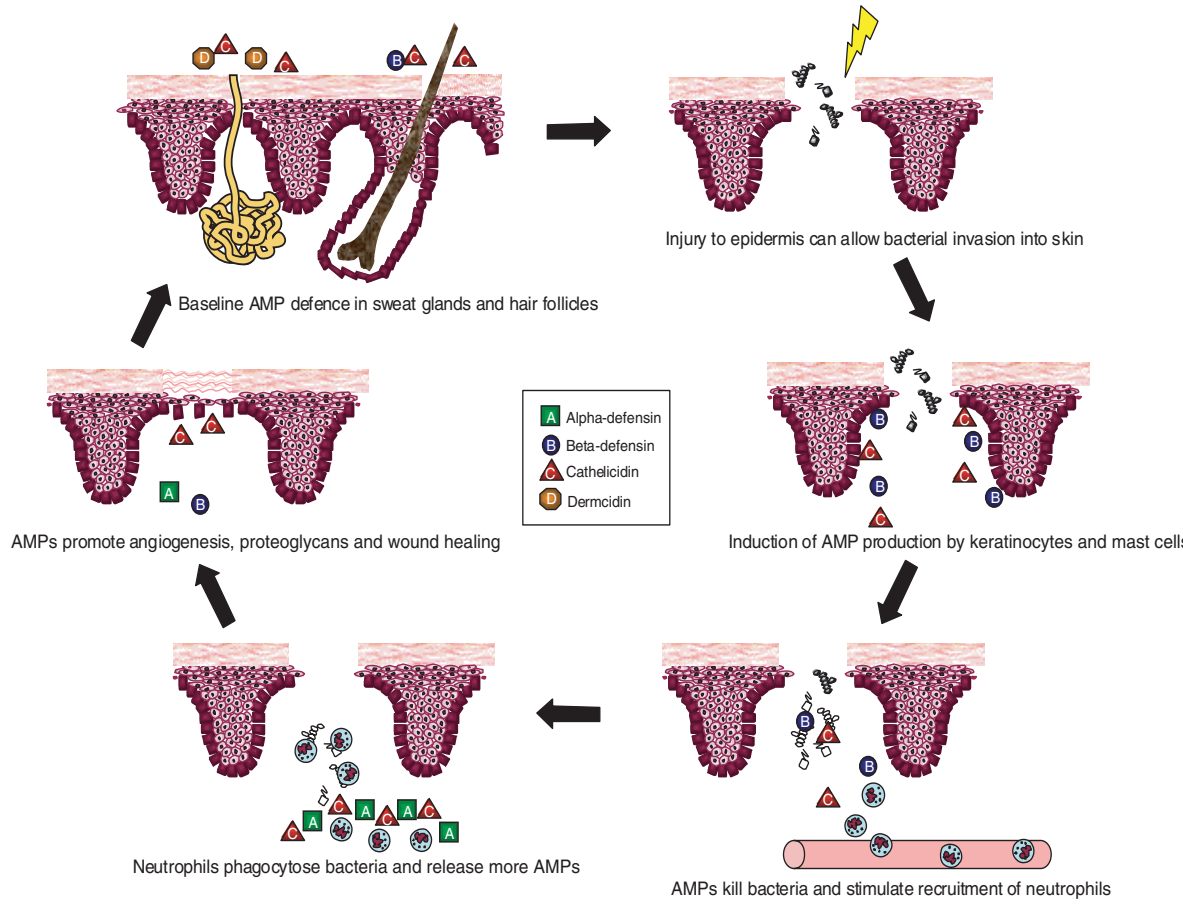

Figure 1. A working model of AMP expression and function in the skin. Upper left and proceeding clockwise – under resting conditions, some AMPs are present in sweat and cover the stratum corneum as a constitutive defence system. After injury or infection, keratinocytes increase AMP production and combine with resident mast cells and recruited neutrophils to rapidly increase several AMPs. Select AMPs have chemotactic activity and further amplify recruitment of inflammatory cells, thus eliminating microbial challenge. Cathelicidin AMPs also participate in repair process and facilitate wound repair.
AMP: Antimicrobial peptide.

important for wound healing and tissue repair [18]. Cathelicidin is, therefore, important in successful wound closure, and its downregulation in wounds can be correlated with development of chronic ulcers. Expression of β-defensin is absent in full-thickness burn wounds and blister fluid from partial thickness burns [64], evidence of an innate immune defect that may contribute to the greatly increased risk of burn wound infection and sepsis.

The immune defence function of the skin is greatly enhanced by an inducible soluble peptide barrier to microbial proliferation. This barrier is activated after the detection of a failure in the skin's physical barrier to microbial invasion. Under resting conditions, sites of potential microbial entry in the skin, such as follicular structures and sweat glands, produce small amounts of AMPs, which are likely to provide an impediment to infection where a physical barrier is absent (Figure 1). AMP production is also increased in the skin of neonates, likely to compensate for the developmental immaturity of adaptive immune responses [65,66]. After injury, the AMP levels in the skin rise rapidly due to increased synthesis in keratinocytes and deposition of AMPs from degranulation of recruited neutrophils. The chemoattractant properties of LL-37 and HBD-2 may then further amplify this process through their interaction with FPRL-1 and CCR6 leukocyte surface receptors, respectively. Thus, the AMP defence system can act directly and indirectly to effect the killing of pathogenic microbes.

4. Therapeutic implications/expert opinion

Published data show that AMPs have important and diverse functions, especially in the skin, where AMPs have already been linked to a variety of disease processes.

The most widely appreciated function of AMPs is their role as naturally occurring antibiotics. Although AMPs are mammalian peptides and antibiotic resistance in bacteria occurs at a much higher rate than the rate of adaptive mutations in mammals, there is only limited resistance to AMPs

among human microbial flora. The fact that widespread bacterial resistance to mammalian AMPs is not seen, combined with the growing problem of resistance to conventional pharmaceutical antibiotics, has prompted several attempts to develop natural AMPs as therapeutic agents. Furthermore, a few important human pathogens have evolved to inactivate AMPs as a virulence mechanism [67,68]. This underscores the importance of these peptides in host defence and provides ideas for the development of new anti-infective agents, as both molecules that mimic the action of AMPs and molecules that block their degradation could have significant therapeutic potential. As the effects of AMPs on host cellular function are diverse and substantial, systemic administration of AMPs in their native form may be associated with substantial toxicity and requires careful drug design. Topical delivery of AMPs is more likely to have the most immediate applications, although further insight into the mechanism of action of these diverse molecules is likely to reveal many additional therapeutic possibilities. Clinical applications currently under study include infected or chronic diabetic foot ulcers, oral mucositis, stomach and intestinal disorders, meningococcal meningitis, catheter infections, acne and fungal infections.

More recent understanding of AMP molecular processing and regulation suggests that selective inhibition of some of their functions may not only be possible, but may prove clinically useful. For example, the chemotactic and angiogenic activity of cathelicidins may contribute to the pathogenesis of disorders characterised by chronic inflammation. Increased AMP expression in conditions that lack an appropriate AMP response, such as atopic dermatitis, might be sufficient to control infection. The development of therapeutics derived from clinical and laboratory studies of AMPs is likely to revolutionise the treatment of many inflammatory and infectious diseases.

Despite recent progress, research on AMPs is still in its infancy. Several issues remain to be addressed and further studies will be necessary to elucidate many aspects of their biology. Additional structure–function studies of AMPs are likely to increase the understanding of their precise mechanisms of action and, therefore, generate new ideas for therapeutic design. Species specificity for the action of the AMPs has been ignored in much prior work that considered only the microbial membrane as the AMP target. With recent appreciation of the importance of host cell- and receptor-mediated responses, the mammalian- or human-derived peptides may be of increased therapeutic importance. Furthermore, identification of factors involved in the regulation of AMPs could provide us with a better understanding of the innate immune response and the relationship between the innate and acquired branches of the immune system. The study of AMPs helps us understand how the skin has evolved to represent such a formidable innate barrier to infection. Supplementation of AMPs to skin compromised by injury or by certain inflammatory conditions (e.g., atopic dermatitis) will probably possess a high therapeutic index through direct microbial killing, augmentation of leukocyte recruitment and promotion of wound healing pathways.

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