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Antimicrobial peptides: Old Molecules with New Ideas

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Abstract

Almost 90 years have passed since Alexander Fleming discovered the antimicrobial activity of lysozyme, the first natural antibiotic isolated from our body. Since then, various types of molecules with antibiotic activity have been isolated from animals, insects, plants and bacteria, and their use has revolutionised clinical medicine. So far, more than 1200 types of peptides with antimicrobial activity have been isolated from various cells and tissues, and it appears all living organisms employ these antimicrobial peptides (AMPs) in their host defense. In the last decade, innate AMPs produced by mammals have been shown to be essential for the protection of skin and other organs. Their importance is due to their pleiotrophic functions to not only kill microbes but also control host physiological functions such as inflammation, angiogenesis and wound healing. Recent advances in our understanding of the function of AMPs have associated their altered production with various human diseases such as psoriasis, atopic dermatitis and rosacea. In this review, we summarize the history of AMP biology and provide an overview of recent research progress in this field.

AMPs: A diverse group of molecules

The antimicrobial peptides (AMPs) have redefined the way we think about immune defense and human disease. Unfortunately, this name is misleading as the term “antimicrobial” describes more about their history of discovery than the potent influence these molecules have on cell behavior. As such, alternative terms for AMPs have also appeared. These include better descriptive terms such as “host defense peptides”, “alarmins” and even “defensins” (used in a broad context instead of the gene family). However, to appreciate the history of the discovery of these molecules as well their common unifying function to kill microbes, the term AMPs will be used in this review.

Over the last two decades more than 1200 AMPs have been identified or predicted from various organisms. For a partial list of these, see the Antimicrobial Peptide Database (APD: http://aps.unmc.edu/AP/main.php). AMPs in general consist of 10–50 amino acid residues. These peptides lack any specific consensus amino acid sequences that are associated with biological activity, but most of them maintain certain common features, such as containing

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positive charge and relatively hydrophobic and amphipathic structure. Based on their amino acid composition, size and conformational structures, AMPs can be divided into several categories, such as peptides with α-helix structures, peptides with β-sheet structures stabilized by disulfide bridges or peptides with extended or loop structures, reviewed in (Lai and Gallo, 2009). Classic AMPs, such as LL-37 and human β-defensins (hBDs), are amphipathic molecules that possess clusters of positively charged and hydrophobic charged amino acid chains. This amphipathic feature is thought to allow them to interact with negatively charged phospholipid head groups and hydrophobic fatty acid chains of microbial membranes, resulting in pore formation on the microbial membrane and release of cytosol components (Glaser *et al.*, 2005; Wimley, 2010). It is the membrane active nature of the AMPs that likely controls their function, but as we continue to study these molecules we learn that their function is much more than originally expected.

AMPs: The hot new topic preceded by 90 years of discovery

Many immunologists consider the study of AMPs as a relatively new topic that led the way towards recognition of our modern understanding of innate immunity. AMPs are a primary effector system that acts with the detection system generated by microbial pattern recognition genes such as the toll-like receptors (TLRs). However, the history of discovery of innate antimicrobials goes back much further in history. Alexander Fleming first recognized the presence of a soluble antimicrobial substance produced by humans about 90 years ago. Fleming observed the bactericidal and bacteriostatic activities of nasal secretions from a patient suffering from acute coryza when he treated bacterial culture plates with this material (Fleming, 1922). He named the activity lysozyme because of its capacity to “lyse” bacterial lawns on a dish. Subsequently, he found lysozyme activity in various human physiological fluids and tissues of animals, as well as egg whites. Such observations indicated to him that lysozyme performed a wide range of functions as a part of our immune system. In 1928, Fleming subsequently discovered that penicillin extracted from the culture of green mold, *Penicillium notatum*, stopped the growth of various bacteria (Fleming, 1929). In the 1940’s, Howard Florey and Ernst Chain brought penicillin’s potential for medical use to fruition. They shared the 1945 Nobel Prize for Medicine along with Fleming for the discovery of penicillin and its therapeutic effects. About forty years after Fleming’s lysozyme discovery, the primary structure of egg white lysozyme was characterized (Canfield, 1963), but by this point the interest in natural host antibiotics had decreased and the importance of this immune defense strategy was all but forgotten.

Antibiotics were also recognized in prokaryotic cells at an early stage in modern medicine. In 1939, René Dubos isolated antimicrobial substances, named gramicidin, from culture supernatant of soil bacteria, *Bacillus brevis*. Gramicidin exhibited bactericidal or bacteriostatic activity against a wide-range of Gram-positive bacteria *in vitro* and *in vivo* (Dubos, 1939a, b). Gramicidin was later shown to be a heterogeneous mixture of six AMPs that was identified as N-formylated polypeptides with alternating L- and D-amino acids (Sarges and Witkop, 1965a, b, c). In addition, application of gramicidin on infected wounds on guinea-pig skin rapidly disinfected pathogenic bacteria and successfully suppressed infection, indicating the therapeutic potential of gramicidin for clinical use (Gause and Brazhnicova, 1944). To our knowledge, gramicidins were the first AMPs for which the

primary structures were characterized and the first antibiotics to be commercially manufactured. This is available today as an over-the-counter antibiotic.

AMPs are now known to exist in all kingdoms. In contrast to the large size of lysozymes, relatively small antimicrobial molecules were observed to be induced in the hemolymph of wax moth larvae after challenging with *Pseudomonas aeruginosa* (Stephens and Marshall, 1962). Remarkably, normal larvae that had received hemolymph from pathogen-challenged larvae exhibited passive protection against bacterial infection, thus proving these innate protective molecules were soluble. In 1981, Hans Boman at the Karolinska Institute pioneered the field of modern innate immunity by seeking to identify the structure of innate insect antimicrobial defense molecules. Initially, Steiner *et al*., characterized primary structures of two AMPs, named cecropins A and B, in hemolymph of the cecropia silk moth, *Hyalophora cecropia* (Steiner *et al.*, 1981). Later, an AMP was isolated from pig small intestines which showed a high similarity with the insect cecropins, therefore it was named cecropin P1 (Lee *et al.*, 1989). However, the research group that discovered the peptide later corrected their original conclusion and discovered that this peptide in fact originated from parasite *Ascaris* nematodes (Andersson *et al.*, 2003).

AMPs are also an integral part of the immune systems in plants. In the early 1940's, Stuart and Harris demonstrated that a crystalline protein isolated from wheat flour exhibited antimicrobial effects against some human pathogenic bacteria and yeast (Stuart and Harris, 1942). It was a low molecular weight proteinaceous material with high sulfur content and named purothionin (Balls *et al.*, 1942). In the 1990's, a group of small basic proteins was isolated from wheat endosperm and characterized as purothionin homologs, so they are called γ-purothionins (Colilla *et al.*, 1990). However, subsequent study has established that purothionins and γ-purothionins are structurally unrelated (Bruix *et al.*, 1993). Considering that γ-purothionins show a high structural analogy with insect defensins, adapting β-sheets and a single α-helix structures stabilized by an eight cysteine-motif, they are renamed as plant defensins (Broekaert *et al.*, 1995) (see below for defensins). Transgenic plants over-expressing AMPs have been explored for decades as a mechanism for disease resistance. For example, expression of defensins in plants confers enhanced resistance to phytopathogen attacks (De Coninck *et al.*, 2010; Jha *et al.*, 2009).

The discoveries of defensins and cathelicidins led the way for the emergence of our appreciation of AMPs in mammals. Arginine-rich cationic peptides possessing potent antimicrobial activity against both Gram-positive and Gram-negative bacteria were first identified in the lysosomal fraction of guinea-pig polymorphonuclear leukocytes by mobility to the cathode (Zeya and Spitznagel, 1963). Selsted *et al.* characterized primary structures of the six cationic AMPs purified from rabbit neutrophils and named these defensins (now classified as α-defensins) (Selsted *et al.*, 1985; Selsted *et al.*, 1984). The research group subsequently identified three defensins from normal human neutrophils and also demonstrated that the peptides directly inactivated herpes simplex virus (Ganz *et al.*, 1985). These defensins have a characteristic six-cysteine motif which forms three intramolecular disulfide bonds (Cys1–Cys6, Cys2–Cys4 and Cys3–Cys5). Selsted *et al.* isolated and characterized 13 AMPs with 38–42 amino acid residues and the six-cysteine motif of defensin in bovine granulocytes (Selsted *et al.*, 1993). However, these defensin-like AMPs

differed from α-defensins by relatively longer amino acid residues and different disulfide pairings (Cys1–Cys5, Cys2–Cys4 and Cys3–Cys6), therefore they were newly classified as β-defensins. Genomic-based approach have identified 28 human and 43 mouse β-defensin genes (Schutte *et al.*, 2002). Tang *et al.*, characterized an AMP in the leukocytes of rhesus monkey, which consisted of 18 amino acid residues with the six-cysteine motif of defensin, and in which the peptide backbone is naturally cyclized (Tang *et al.*, 1999). This AMP was classified as θ-defensin. Interestingly, θ-defensin mRNA transcripts are present in human bone marrow, spleen, thymus, testis, and skeletal muscle, but a premature stop codon aborts their translation (Nguyen *et al.*, 2003). In addition to the antibacterial activity, defensins also offer effective antiviral and antifungal activities through multiple modes of action. Mammalian defensins exhibit strong viral neutralizing activities by directly interacting with viral envelope proteins (Doss *et al.*, 2009; Hazrati *et al.*, 2006). In addition, α- and θ-defensins directly interact with specific viral receptors on the host cell, antagonizing viral attachment, entry, or intracellular shuttling (Cole *et al.*, 2002; Furci *et al.*, 2007). α-defensins also kill *Candida albicans* by lysing cells, possibly in a similar way to their antibacterial activity (Lehrer *et al.*, 1985; Patterson-Delafield *et al.*, 1980).

Zanetti *et al.* cloned a full-length cDNA of Bac5, a proline-rich AMP which had previously been isolated from bovine neutrophils (Frank *et al.*, 1990), from myeloid bone marrow cell mRNA (Zanetti *et al.*, 1993). The region upstream of mature Bac5 peptide was found to share high homology to cathelin (more than 70% identity), an inhibitor of the cysteine proteinase cathepsin L isolated from pig leukocytes (Kopitar *et al.*, 1989). Bovine or porcine leukocytes both contained at least 10 structurally-diverse AMPs composed of 12–100 amino acid residues whose precursor proteins have cathelin-like domains in the N-terminal, therefore these AMPs were named cathelicidins (Zanetti *et al.*, 1995). Interestingly, the number of different cathelicidins varies substantially among species. In contrast to the multiplicity of cathelicidins in bovine and porcine neutrophils, the human and murine neutrophils express only a single cathelicidin gene (*CAMP*) that encodes an inactive precursor proteins, hCAP18 in human and CRAMP in mouse (Agerberth *et al.*, 1995; Cowland *et al.*, 1995; Gallo *et al.*, 1997). Post-transcriptional processing cleaves out the C-terminal cathelin domain from cathelicidin precursor protein and makes the active AMP. In human, for example, active AMP composed of 37 amino acids beginning with two leucines, named LL-37, is generated from hCAP18 (Zanetti, 2004). hCAP18 is cleaved extracellularly by proteinase 3 or kallikrein family serine proteases to generate the active LL-37 peptides (Sorensen *et al.*, 2001; Yamasaki *et al.*, 2006). After the cleavage, the N-terminal cathelin domain also exhibits antimicrobial activity (Zaiou *et al.*, 2003). The cathelicidin AMPs generated from *CAMP* genes found between species show little similarity to each other and are referred to as a group solely because of the similarity of the precursor protein that is predominated by the large cathelin domain. In addition, they are remarkably variable in secondary structures. Many of them adopt an α-helical structure followed or not by a hydrophobic unstructured tail (human LL-37, mouse CRAMP, rabbit CAT-18), while others are proline/arginine-rich (bovine Bac-5 and porcine PR-39), tryptophan rich (bovine indolicidin and porcine tritrpticin), and β-hairpin-like structure (porcine protegrins) (Bulet *et al.*, 2004; Lehrer and Ganz, 2002). The cathelicidins have been cloned not only mammals,

but also fish (Chang *et al.*, 2005; Maier *et al.*, 2008), chicken (van Dijk *et al.*, 2005), snakes (Wang *et al.*, 2008; Zhao *et al.*, 2008) and hagfish (Uzzell *et al.*, 2003).

AMPs in the skin: Demonstrating Relevance

As described above, in the early 1990s AMPs were being discovered in a wide range of organisms and cell types. Despite this, we were surprised to discover that AMPs were abundantly present in mammalian skin (Gallo *et al.*, 1994). These observations were first made in pig wounds with the discovery of the porcine cathelicidin PR-39. Following this discovery, Harder *et al.* described the production of hBD-2 in human skin, lung, uterus and trachea epitheliums, and elevated hBD-2 expression in human keratinocytes exposed to pathogenic bacteria (Harder *et al.*, 1997). Subsequent work from our group and others demonstrated that AMPs are induced in the skin by injury and are abundantly found in some inflammatory processes such as psoriasis (Dorschner *et al.*, 2001; Frohm *et al.*, 1997). The unique inflammatory environment of psoriasis has been subsequently exploited for discovery of multiple AMPs including α-and β-defensins, psoriasin and RNase7 (Harder and Schroder, 2005). A key advance for the field came with the capacity to not only purify and test antimicrobial activity of isolated AMPs, but also to apply modern mouse molecular genetic approaches to evaluation of their function. These studies led the way to revise perceptions of AMPs as evolutionarily ancient molecules that have relatively insignificant roles in organisms that have developed adaptive immune defense systems. The key initial finding was that targeted deletion of cathelicidin proved this AMP was essential to the normal immune response and resistance to bacterial skin infection (Nizet *et al.*, 2001). This critical role for cathelicidin has been now shown in many tissues, mucosa and body fluids, and for a wide range of pathogenic bacteria, several viruses and funguses as well as *Leishmania* (Doss *et al.*, 2010; Gallo *et al.*, 1997; Gordon *et al.*, 2005; Gutner *et al.*, 2009; Kulkarni *et al.*, 2011). Targeted deletion of other molecules involved in pattern recognition and processing of AMPs have shown similar results (Takeuchi *et al.*, 2000). Although similar *in vivo* data with defensins have been more difficult to generate due to the extensive gene duplication of the α- and β-defensin gene families, several reports have successfully demonstrated an important roles for defensins in host defense against bacteria and some viruses *in vivo*. Metalloprotease-7-deficient mice, lacking all mature α-defensins due to the loss of the protease required for proteolytic cleavage, displayed a reduced clearance of bacteria and higher mortality rates upon pathogen challenge (Wilson *et al.*, 1999). Mouse β-defensin-1 knockout mice showed earlier weight loss and higher mortality after influenza virus infection than wild-type mice (Ryan *et al.*, 2011). Conversely, transgenic over-expression and knock-in of AMPs further supported these conclusions by showing that the addition of excess AMPs can increase resistance to some microbes (Lee *et al.*, 2005; Salzman *et al.*, 2003).

Today, cathelicidins and β-defensins are the most well characterized AMPs found in the human skin (Lai and Gallo, 2009; Wiesner and Vilcinskas, 2010). hBD-1 is constitutively expressed in keratinocytes, but exhibits only minor antibiotic killing activity in comparison with other defensins (Yadava *et al.*, 2006; Zaalouk *et al.*, 2004). More recently, the reduced form of hBD-1 has been shown to become a potent antimicrobial peptide, of which reduction is catalyzed by thioredoxin expressed in the epidermis (Schroeder *et al.*, 2011).

This suggests that the redox regulation is crucial for the innate immune protection by hBD-1. The expression levels of hBD-2, hBD-3, and human cathelicidin in keratinocytes are very low at the steady state and typically upregulated during infection, inflammation and wounding (Froy, 2005; Gallo *et al.*, 2002). This suggests that with these AMPs it is the secondary response of increasing expression that serves to limit the severity of clinical symptoms when the primary line of defense (constitutive expression of AMPs) fails. Human keratinocytes also express many molecules that were first discovered for reasons other than action as an AMP, but subsequently found to also inhibit microbial growth. Once example of these are the ribonucleotidases (RNases). Of these, RNases 5 and 7 exhibit antimicrobial activity against many pathogenic microorganisms independent of the RNase activity (Abtin *et al.*, 2009; Huang *et al.*, 2007; Zanger *et al.*, 2009). The antimicrobial activity of those RNases is inhibited by RNase inhibitor protein expressed in epidermal keratinocytes, and, in turn, activated when the RNase inhibitor is cleaved by stratum corneum serine proteases (Abtin *et al.*, 2009). An antimicrobial heterodimeric complex, S100A8/S100A9 (calprotectin), is induced in epidermal keratinocytes during Gram-negative bacteria infection and sensing of bacterial flagellin by TLR5 is critical for the regulation of calprotectin (Abtin *et al.*, 2010). Cathelicidin, hBD-2 and -3 and antimicrobial histone H4 are detected in the cultured human sebocytes, and their expression levels are upregulated in the presence of Gram positive bacteria or sebum free fatty acids (Lee *et al.*, 2009a; Nagy *et al.*, 2006; Nakatsuji *et al.*, 2010). Large amounts of psoriasin antimicrobial peptide accumulate in the epidermis of sebaceous skin as well as sebaceous glands and secreted to the external skin surface (Glaser *et al.*, 2005). Sweat eccrine glands are also known as an important supplier of AMPs to the epidermal surface. Dermcidin is an AMP constitutively expressed as a small precursor protein in eccrine sweat glands and secreted into sweat where active AMPs are proteolytically generated (Schittek *et al.*, 2001).

The skin provides a rapid first-line of immune defense against invading pathogens from outside environments by constitutively and actively producing various AMPs. In human skin, the main cellular sources of AMPs are keratinocytes, mast cells, neutrophils, sebocytes and eccrine epithelial cells (Figure 1). This layered system of deployment functions well because of the dual action of AMPs. First, as we have thus far described, the secretion or release of these peptides provides innate antibiotic-like action against infectious pathogens. However, a key component of the overall defense strategy of skin and other epithelial organs is that alternate defense systems are activated to provide protection in the event that microbes evade the first system. Clearly, microbes are well adapted to develop antimicrobial resistance, and many microbes are human pathogens because they have succeeded in doing this. AMPs such as Cathelicidins and Defensins therefore appear to have maintained their relevance because they also contribute to host defense by triggering inflammatory cell recruitment and cytokine release. This system often involves signaling mediated by pattern recognition receptors such as TLRs or responses to pro-inflammatory cytokines. The AMPs amplify defense by calling for help.

AMPs and the pathophysiology of human disease

As a result of the inherent association of AMPs with inflammation, recent evidence indicates that abnormal production of AMPs affects the pathogenesis of diseases such as psoriasis,

rosacea, and atopic dermatitis (Yamasaki and Gallo, 2008). These observations have two important consequences. First, they have further demonstrated the relevance of these evolutionarily ancient genes to human health. Secondly, the role of some AMPs in human disease appears to depend more on the actions of these peptides beyond those as an antibiotic. It is in this context that the alternative terms such as “host defense peptide” or “alarmin” are most appropriate.

Cathelicidins and hBDs were well known to be strongly induced in psoriatic lesions in comparison with normal skin, and this degree of induction mimicked expression expected when normal skin was injured. However, the induction of some AMPs such as cathelicidin and hBD-2 and 3 was found to be lower in atopic dermatitis lesions than expected, despite the presence of skin inflammation (Hata *et al.*, 2010; Lande *et al.*, 2007; Mallbris *et al.*, 2010; Ong *et al.*, 2002). In contrast, RNase7 and psoriasin are induced in atopic dermatitis lesional skin and in this case AMP induction is appropriately upregulated by barrier disruption (Harder *et al.*, 2010). The defective expression of some AMPs in atopic dermatitis has been linked to a higher propensity to *Staphylococcus aureus* colonization, which is known to play important roles in the exacerbation of the infection and is correlated with its extent and severity of atopic lesions (Miller *et al.*, 2005). Thus, in this situation, a lack of the antimicrobial function of the AMP may lead to disease. An informative contrast to the observations in atopic dermatitis can be made in analysis of the diseases rosacea and psoriasis. Our group showed that an excess of cathelicidin in the form of LL-37 exists in rosacea, and this drives inflammation and abnormal blood vessel growth by mechanisms of cell activation not related to antimicrobial action (Yamasaki *et al.*, 2007). High amounts of LL-37 appear to result from the abnormal function of innate immune pattern recognition by TLRs, and proteases that process hCAP18 (Bensch *et al.*, 1995; Jugeau *et al.*, 2005; Yamasaki *et al.*, 2007; Yamasaki *et al.*, 2010). In psoriasis and systemic lupus erythematosus, the excess presence of LL-37 enables recognition of self-nucleic acids by both plasmacytoid dendritic cells (Lande *et al.*, 2011; Lande *et al.*, 2007) and keratinocytes (Morizane *et al.*, 2011). Thus, under these conditions AMPs may be exacerbating inflammation and contributing to disease by permitting auto-inflammatory signaling. Expression of AMPs is also associated with viral infectious diseases such as mollusca contagiosm (Meyer-Hoffert *et al.*, 2010), condyloma acuminatum, and verruca vulgaris (Conner *et al.*, 2002), as well as autoimmune diseases, such as cutaneous lupus erythematosus (Kreuter *et al.*, 2011).

Multifunctional roles of AMPs

As introduced by the proceeding discussion of AMPs in human diseases, these molecules have several important physiological and immunomodulatory functions. Some α- and β-defensins are chemotactic for T lymphocytes, monocytes and immature DCs, and can induce cytokine production by monocytes and epithelial cells (Yang *et al.*, 2004). hBD-2 activates immature dendritic cells through TLR4-dependent mechanisms, inducing a robust Th1 response (Biragyn *et al.*, 2002). Cathelicidin triggers inflammatory cell recruitment and cytokine release through various mechanisms. LL-37 is a potent chemoattractant for mast cells, monocytes, T lymphocytes and neutrophils through activating formyl peptide receptor–like 1(FPRL1) (De *et al.*, 2000; Niyonsaba *et al.*, 2002). The complex of LL-37

with self-DNA or self-RNA released from dead cells activates dendritic cells by triggering TLR9 or TLR7/8, respectively, leasing productions of proinflammatory cytokines and type-I interferons (Ganguly *et al.*, 2009; Lande *et al.*, 2011; Lande *et al.*, 2007). These immunomodulatory properties of AMPs can contribute to host defense against infections by attracting and activating various immune cells as well as by their direct antimicrobial activity.

LL-37 contributes to cutaneous wound healing by stimulating re-epithelialization (Heilborn *et al.*, 2003). LL-37 also induces neovascularization which is mediated by FPRL1 signaling in endothelial cells, and the cathelicidin-mediated angiogenesis is important for cutaneous wound neovascularization (Koczulla *et al.*, 2003). Furthermore, LL-37 induces proliferation and migration by human endothelial cells (Ramos *et al.*, 2011). In fact, accumulation of cathelicidin has been observed in epidermal wound and blister fluid (Dorschner *et al.*, 2001; Frohm *et al.*, 1996; Gallo *et al.*, 1994).

The AMPs of our Microbiome

A surprising recent revelation is that the AMPs that directly contribute to our skin innate immune defense are made not only by our own cells, but also in prokaryotic organisms that inhabit our epidermis. Our group has proposed that the unique peptides phenol-soluble modulin (PSM)γ and PSMδ produced by *Staphylococcus epidermidis* (*S. epidermidis)* could be beneficial to the host and thus serve as additional AMPs on normal skin surface (Cogen *et al.*, 2010b). Although we know today from 16S sequencing approaches that the microbiome of human skin is diverse (Grice *et al.*, 2009), *S. epidermidis* was of high interest because it is the predominant bacteria that can be cultured from healthy human skin, and thus known to thrive on the epidermis (Kloos and Musselwhite, 1975). PSMs caused membrane leakage and membrane perturbation in bacteria as well as classic AMPs such as LL-37 and hBDs, suggesting that these peptides function in a similar mechanistic manner as that of innate cutaneous AMPs. These peptides selectively exhibited bactericidal activity against skin pathogens, such as *Staphylococcus aureus* (*S. aureus*), Group A *Streptococcus* (GAS) and *Escherichia coli*, whereas they are not active against *S. epidermidis*. Moreover, inoculating PSMs on the mouse skin surface reduced the survival of GAS but not *S. epidermidis*. This selective activity is likely to be an important part of a normal microbial defense strategy against colonization. In addition, PSMs enhance the capacity of bacterial killing activity by human neutrophils by inducing their neutrophil extracellular traps (Cogen *et al.*, 2010a). These lines of discovery are only at the very early stages, and considering the diversity of the human microbiome it is likely that many other microbial AMPs that benefit our immune defense will be discovered.

Targeting AMPs for therapy

The important pleiotropic actions of AMPs, the many examples of relevance in animal models, and their associations with human disorders, all point towards this class of molecules as a new target for therapy. In cases where increasing AMP expression would be beneficial, such as when attempting to treat or prevent infectious disease, a simple therapy has shown promise. Vitamin D, long suspected of having health benefits in infectious

diseases such as influenza and tuberculosis, has been shown to be a potent stimulus of AMPs (Beard *et al.*, 2011). Experiments with cultured cells have shown that 1–25-dihydroxyvitamin D3 enhances the expression of cathelicidin and hBD-2 by normal human keratinocytes, resulting in an enhanced antimicrobial function against *S. aureus in vitro* (Schauber *et al.*, 2008; Wang *et al.*, 2004). Pilot studies have attempted to compensate for the defective expression of AMPs in the skin of patients suffering from atopic dermatitis by administering vitamin D (Hata *et al.*, 2008). Analysis of killing of *Mycobacterium tuberculosis* by monocytes has shown improvement with vitamin D (Liu *et al.*, 2006). However, most clinical trials have not shown a conclusive association between vitamin D and infections in humans. It is possible that this lack of an ability to observe an association reflects the influence of other variables not yet understood. In psoriasis patients, decreased expression of hBD-2 and hBD-3 were observed in lesional skin following topical administration with calcipotriol, a vitamin D analog, despite increased expression of cathelicidin (Peric *et al.*, 2009). The suppression of AMP expression was accompanied by decreased expression of interleukins-17 and -8 that play important roles to cause psoriatic inflammation. Thus, ameliorating AMP expression is also a novel therapeutic approach for the treatment of skin diseases with disturbed AMP expression such as psoriasis, rosacea or atopic dermatitis.

Conclusions

AMPs have been known for some time, but their discovery in the skin and associations with disease are a relatively recent advance that has opened a new chapter in immunology. AMPs mediate the host innate immune defense through various modes of action. A variety of factors influence AMP expression and function. For example, a recent study from our group demonstrated that a small molecule of <10 kDa secreted from *S. epidermidis*, the predominant commensal in healthy human skin, increased expression of hBDs in murine skin and human keratinocytes through activation of TLR2 signaling (Lai *et al.*, 2010). Similarly, co-cultivation of differentiated human primary keratinocytes with live *S. epidermidis*, but not heat-inactivated bacteria, enhanced production of hBD-2, hBD-3 and RNase7. In addition, keratinocytes pre-incubated with *S. epidermidis*-conditioned media strongly enhanced AMP production induced by *S. aureus*, suggesting that *S. epidermidis* sensitizes human keratinocytes toward pathogenic bacteria and amplifies the innate immune response (Wanke *et al.*, 2011). These results suggest that the recognition of *Staphylococcal* molecules by TLR2 may be involved in the steady-state production of AMPs in keratinocytes and enhances resistance to infection by bacterial, fungal and viral pathogens. Thus, the correlation between AMP expression and commensal microbiota may be very important to maintaining skin homeostasis. Imbalanced skin microflora would alter AMP expression in the skin, which is critical in the pathogenesis of psoriasis, rosacea, and atopic dermatitis. Indeed, many lines of evidence suggest a role for the imbalanced cutaneous microflora with pathogenicity of these disorders (Gallo and Nakatsuji, 2011). Understanding and control of AMPs is an exciting new part of the immunological revolution taking place today.

References

Abtin A, Eckhart L, Glaser R, Gmeiner R, Mildner M, Tschachler E. The antimicrobial heterodimer S100A8/S100A9 (calprotectin) is upregulated by bacterial flagellin in human epidermal keratinocytes. J Invest Dermatol. 2010; 130:2423–30. [PubMed: 20555353]

Abtin A, Eckhart L, Mildner M, Ghannadan M, Harder J, Schroder JM, et al. Degradation by stratum corneum proteases prevents endogenous RNase inhibitor from blocking antimicrobial activities of RNase 5 and RNase 7. J Invest Dermatol. 2009; 129:2193–201. [PubMed: 19262607]

Agerberth B, Gunne H, Odeberg J, Kogner P, Boman HG, Gudmundsson GH. FALL-39, a putative human peptide antibiotic, is cysteine-free and expressed in bone marrow and testis. Proc Natl Acad Sci U S A. 1995; 92:195–9. [PubMed: 7529412]

Ali RS, Falconer A, Ikram M, Bissett CE, Cerio R, Quinn AG. Expression of the peptide antibiotics human beta defensin-1 and human beta defensin-2 in normal human skin. J Invest Dermatol. 2001; 117:106–11. [PubMed: 11442756]

Andersson M, Boman A, Boman HG. Ascaris nematodes from pig and human make three antibacterial peptides: isolation of cecropin P1 and two ASABF peptides. Cell Mol Life Sci. 2003; 60:599–606. [PubMed: 12737319]

Balls AK, Hale WS, Harris TH. A crystalline protein obtained from a lipoprotein of wheat flour. Cereal Chem. 1942; 19:279–88.

Beard JA, Bearden A, Striker R. Vitamin D and the anti-viral state. J Clin Virol. 2011; 50:194–200. [PubMed: 21242105]

Belaaouaj A, Kim KS, Shapiro SD. Degradation of outer membrane protein A in Escherichia coli killing by neutrophil elastase. Science. 2000; 289:1185–8. [PubMed: 10947984]

Bensch KW, Raida M, Magert HJ, Schulz-Knappe P, Forssmann WG. hBD-1: a novel beta-defensin from human plasma. FEBS Lett. 1995; 368:331–5. [PubMed: 7628632]

Biragyn A, Ruffini PA, Leifer CA, Klyushnenkova E, Shakhov A, Chertov O, et al. Toll-like receptor 4-dependent activation of dendritic cells by beta-defensin 2. Science. 2002; 298:1025–9. [PubMed: 12411706]

Broekaert WF, Terras FR, Cammue BP, Osborn RW. Plant defensins: novel antimicrobial peptides as components of the host defense system. Plant Physiol. 1995; 108:1353–8. [PubMed: 7659744]

Bruix M, Jimenez MA, Santoro J, Gonzalez C, Colilla FJ, Mendez E, et al. Solution structure of gamma 1-H and gamma 1-P thionins from barley and wheat endosperm determined by 1H-NMR: a structural motif common to toxic arthropod proteins. Biochemistry. 1993; 32:715–24. [PubMed: 8380707]

Bulet P, Stocklin R, Menin L. Anti-microbial peptides: from invertebrates to vertebrates. Immunol Rev. 2004; 198:169–84. [PubMed: 15199962]

Caccavo D, Pellegrino NM, Altamura M, Rigon A, Amati L, Amoroso A, et al. Antimicrobial and immunoregulatory functions of lactoferrin and its potential therapeutic application. J Endotoxin Res. 2002; 8:403–17. [PubMed: 12542852]

Canfield RE. The Amino Acid Sequence of Egg White Lysozyme. J Biol Chem. 1963; 238:2698–707. [PubMed: 14063294]

Chang CI, Pleguezuelos O, Zhang YA, Zou J, Secombes CJ. Identification of a novel cathelicidin gene in the rainbow trout, Oncorhynchus mykiss. Infect Immun. 2005; 73:5053–64. [PubMed: 16041021]

Chronnell CM, Ghali LR, Ali RS, Quinn AG, Holland DB, Bull JJ, et al. Human beta defensin-1 and -2 expression in human pilosebaceous units: upregulation in acne vulgaris lesions. J Invest Dermatol. 2001; 117:1120–5. [PubMed: 11710922]

Cogen AL, Yamasaki K, Muto J, Sanchez KM, Crotty Alexander L, Tanios J, et al. Staphylococcus epidermidis antimicrobial delta-toxin (phenol-soluble modulin-gamma) cooperates with host antimicrobial peptides to kill group A Streptococcus. PLoS One. 2010a; 5:e8557. [PubMed: 20052280]

Cogen AL, Yamasaki K, Sanchez KM, Dorschner RA, Lai Y, MacLeod DT, et al. Selective antimicrobial action is provided by phenol-soluble modulins derived from Staphylococcus

epidermidis, a normal resident of the skin. J Invest Dermatol. 2010b; 130:192–200. [PubMed: 19710683]

Cole AM, Hong T, Boo LM, Nguyen T, Zhao C, Bristol G, et al. Retrocyclin: a primate peptide that protects cells from infection by T- and M-tropic strains of HIV-1. Proc Natl Acad Sci U S A. 2002; 99:1813–8. [PubMed: 11854483]

Colilla FJ, Rocher A, Mendez E. gamma-Purothionins: amino acid sequence of two polypeptides of a new family of thionins from wheat endosperm. FEBS Lett. 1990; 270:191–4. [PubMed: 2226781]

Conner K, Nern K, Rudisill J, O'Grady T, Gallo RL. The antimicrobial peptide LL-37 is expressed by keratinocytes in condyloma acuminatum and verruca vulgaris. J Am Acad Dermatol. 2002; 47:347–50. [PubMed: 12196742]

Cowland JB, Johnsen AH, Borregaard N. hCAP-18, a cathelin/pro-bactenecin-like protein of human neutrophil specific granules. FEBS Lett. 1995; 368:173–6. [PubMed: 7615076]

Cumberbatch M, Dearman RJ, Uribe-Luna S, Headon DR, Ward PP, Conneely OM, et al. Regulation of epidermal Langerhans cell migration by lactoferrin. Immunology. 2000; 100:21–8. [PubMed: 10809955]

Cutuli M, Cristiani S, Lipton JM, Catania A. Antimicrobial effects of alpha-MSH peptides. J Leukoc Biol. 2000; 67:233–9. [PubMed: 10670585]

De Coninck BM, Sels J, Venmans E, Thys W, Goderis IJ, Carron D, et al. Arabidopsis thaliana plant defensin AtPDF1.1 is involved in the plant response to biotic stress. New Phytol. 2010; 187:1075–88. [PubMed: 20561213]

De Y, Chen Q, Schmidt AP, Anderson GM, Wang JM, Wooters J, et al. LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. J Exp Med. 2000; 192:1069–74. [PubMed: 11015447]

Di Nardo A, Vitiello A, Gallo RL. Cutting edge: mast cell antimicrobial activity is mediated by expression of cathelicidin antimicrobial peptide. J Immunol. 2003; 170:2274–8. [PubMed: 12594247]

Dorschner RA, Pestonjamasp VK, Tamakuwala S, Ohtake T, Rudisill J, Nizet V, et al. Cutaneous injury induces the release of cathelicidin anti-microbial peptides active against group A Streptococcus. J Invest Dermatol. 2001; 117:91–7. [PubMed: 11442754]

Doss M, White MR, Tecle T, Gantz D, Crouch EC, Jung G, et al. Interactions of alpha-, beta-, and theta-defensins with influenza A virus and surfactant protein D. J Immunol. 2009; 182:7878–87. [PubMed: 19494312]

Doss M, White MR, Tecle T, Hartshorn KL. Human defensins and LL-37 in mucosal immunity. J Leukoc Biol. 2010; 87:79–92. [PubMed: 19808939]

Dubos RJ. Studies on a bactericidal agent extracted from a soil bacillus: I. preparation of the agent. its activity *in vitro*. J Exp Med. 1939a; 70:1–10. [PubMed: 19870884]

Dubos RJ. Studies on a bactericidal agent extracted from a soil bacillus: II. protective effect of the bactericidal agent against experiemntal pneumococcus infection in mice. J Exp Med. 1939b; 70:11–7. [PubMed: 19870886]

Fleming A. On a remarkable bacteriolytic element found in tissues and secretions. Proceedings of the Royal Society of London, B. 1922; 93:306–17.

Fleming A. On the antibacterial action of cultures of a Penicillium, with special reference to thieir use in the isolation of *B. influenzae*. Br J Exp Pathol. 1929; 10:226–36.

Frank RW, Gennaro R, Schneider K, Przybylski M, Romeo D. Amino acid sequences of two proline-rich bactenecins. Antimicrobial peptides of bovine neutrophils. J Biol Chem. 1990; 265:18871–4. [PubMed: 2229048]

Frohm M, Agerberth B, Ahangari G, Stahle-Backdahl M, Liden S, Wigzell H, et al. The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders. J Biol Chem. 1997; 272:15258–63. [PubMed: 9182550]

Frohm M, Gunne H, Bergman AC, Agerberth B, Bergman T, Boman A, et al. Biochemical and antibacterial analysis of human wound and blister fluid. Eur J Biochem. 1996; 237:86–92. [PubMed: 8620898]

Froy O. Regulation of mammalian defensin expression by Toll-like receptor-dependent and independent signalling pathways. Cell Microbiol. 2005; 7:1387–97. [PubMed: 16153239]

Furci L, Sironi F, Tolazzi M, Vassena L, Lusso P. Alpha-defensins block the early steps of HIV-1 infection: interference with the binding of gp120 to CD4. Blood. 2007; 109:2928–35. [PubMed: 17132727]

Gallo RL, Kim KJ, Bernfield M, Kozak CA, Zanetti M, Merluzzi L, et al. Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse. J Biol Chem. 1997; 272:13088–93. [PubMed: 9148921]

Gallo RL, Murakami M, Ohtake T, Zaiou M. Biology and clinical relevance of naturally occurring antimicrobial peptides. J Allergy Clin Immunol. 2002; 110:823–31. [PubMed: 12464945]

Gallo RL, Nakatsuji T. Microbial Symbiosis with the Innate Immune Defense System of the Skin. J Invest Dermatol. 2011

Gallo RL, Ono M, Povsic T, Page C, Eriksson E, Klagsbrun M, et al. Syndecans, cell surface heparan sulfate proteoglycans, are induced by a proline-rich antimicrobial peptide from wounds. Proc Natl Acad Sci U S A. 1994; 91:11035–9. [PubMed: 7972004]

Ganguly D, Chamilos G, Lande R, Gregorio J, Meller S, Facchinetti V, et al. Self-RNA-antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8. J Exp Med. 2009; 206:1983–94. [PubMed: 19703986]

Ganz T. Defensins: antimicrobial peptides of innate immunity. Nat Rev Immunol. 2003; 3:710–20. [PubMed: 12949495]

Ganz T, Selsted ME, Szklarek D, Harwig SS, Daher K, Bainton DF, et al. Defensins. Natural peptide antibiotics of human neutrophils. J Clin Invest. 1985; 76:1427–35. [PubMed: 2997278]

Gause GF, Brazhnicova MG. Gramicidin S and its use in the treatment of infected wounds. Nature. 1944; 3918:703.

Glaser R, Harder J, Lange H, Bartels J, Christophers E, Schroder JM. Antimicrobial psoriasin (S100A7) protects human skin from Escherichia coli infection. Nat Immunol. 2005; 6:57–64. [PubMed: 15568027]

Gordon YJ, Huang LC, Romanowski EG, Yates KA, Proske RJ, McDermott AM. Human cathelicidin (LL-37), a multifunctional peptide, is expressed by ocular surface epithelia and has potent antibacterial and antiviral activity. Curr Eye Res. 2005; 30:385–94. [PubMed: 16020269]

Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, et al. Topographical and temporal diversity of the human skin microbiome. Science. 2009; 324:1190–2. [PubMed: 19478181]

Gutner M, Chaushu S, Balter D, Bachrach G. Saliva enables the antimicrobial activity of LL-37 in the presence of proteases of Porphyromonas gingivalis. Infect Immun. 2009; 77:5558–63. [PubMed: 19805540]

Harder J, Bartels J, Christophers E, Schroder JM. A peptide antibiotic from human skin. Nature. 1997; 387:861. [PubMed: 9202117]

Harder J, Bartels J, Christophers E, Schroder JM. Isolation and characterization of human beta -defensin-3, a novel human inducible peptide antibiotic. J Biol Chem. 2001; 276:5707–13. [PubMed: 11085990]

Harder J, Dressel S, Wittersheim M, Cordes J, Meyer-Hoffert U, Mrowietz U, et al. Enhanced expression and secretion of antimicrobial peptides in atopic dermatitis and after superficial skin injury. J Invest Dermatol. 2010; 130:1355–64. [PubMed: 20107483]

Harder J, Schroder JM. RNase 7, a novel innate immune defense antimicrobial protein of healthy human skin. J Biol Chem. 2002; 277:46779–84. [PubMed: 12244054]

Harder J, Schroder JM. Psoriatic scales: a promising source for the isolation of human skin-derived antimicrobial proteins. J Leukoc Biol. 2005; 77:476–86. [PubMed: 15629886]

Hata TR, Kotol P, Boguniewicz M, Taylor P, Paik A, Jackson M, et al. History of eczema herpeticum is associated with the inability to induce human beta-defensin (HBD)-2, HBD-3 and cathelicidin in the skin of patients with atopic dermatitis. Br J Dermatol. 2010; 163:659–61. [PubMed: 20545685]

Hata TR, Kotol P, Jackson M, Nguyen M, Paik A, Udall D, et al. Administration of oral vitamin D induces cathelicidin production in atopic individuals. J Allergy Clin Immunol. 2008; 122:829–31. [PubMed: 19014773]

Hazrati E, Galen B, Lu W, Wang W, Ouyang Y, Keller MJ, et al. Human alpha- and beta-defensins block multiple steps in herpes simplex virus infection. J Immunol. 2006; 177:8658–66. [PubMed: 17142766]

Heilborn JD, Nilsson MF, Kratz G, Weber G, Sorensen O, Borregaard N, et al. The cathelicidin antimicrobial peptide LL-37 is involved in re-epithelialization of human skin wounds and is lacking in chronic ulcer epithelium. J Invest Dermatol. 2003; 120:379–89. [PubMed: 12603850]

Huang YC, Lin YM, Chang TW, Wu SJ, Lee YS, Chang MD, et al. The flexible and clustered lysine residues of human ribonuclease 7 are critical for membrane permeability and antimicrobial activity. J Biol Chem. 2007; 282:4626–33. [PubMed: 17150966]

Jha S, Tank HG, Prasad BD, Chattoo BB. Expression of Dm-AMP1 in rice confers resistance to Magnaporthe oryzae and Rhizoctonia solani. Transgenic Res. 2009; 18:59–69. [PubMed: 18618285]

Johnston A, Gudjonsson JE, Aphale A, Guzman AM, Stoll SW, Elder JT. EGFR and IL-1 signaling synergistically promote keratinocyte antimicrobial defenses in a differentiation-dependent manner. J Invest Dermatol. 2011; 131:329–37. [PubMed: 20962853]

Jugeau S, Tenaud I, Knol AC, Jarrousse V, Quereux G, Khammari A, et al. Induction of toll-like receptors by Propionibacterium acnes. Br J Dermatol. 2005; 153:1105–13. [PubMed: 16307644]

Kloos WE, Musselwhite MS. Distribution and persistence of Staphylococcus and Micrococcus species and other aerobic bacteria on human skin. Appl Microbiol. 1975; 30:381–5. [PubMed: 810086]

Koczulla R, von Degenfeld G, Kupatt C, Krotz F, Zahler S, Gloe T, et al. An angiogenic role for the human peptide antibiotic LL-37/hCAP-18. J Clin Invest. 2003; 111:1665–72. [PubMed: 12782669]

Kopitar M, Ritonja A, Popovic T, Gabrijelcic D, Krizaj I, Turk V. A new type of low-molecular mass cysteine proteinase inhibitor from pig leukocytes. Biol Chem Hoppe Seyler. 1989; 370:1145–51. [PubMed: 2610932]

Kreuter A, Jaouhar M, Skrygan M, Tigges C, Stucker M, Altmeyer P, et al. Expression of antimicrobial peptides in different subtypes of cutaneous lupus erythematosus. J Am Acad Dermatol. 2011; 65:125–33. [PubMed: 21353331]

Kulkarni MM, Barbi J, McMaster WR, Gallo RL, Satoskar AR, McGwire BS. Mammalian antimicrobial peptide influences control of cutaneous Leishmania infection. Cell Microbiol. 2011; 13:913–23. [PubMed: 21501359]

Lai Y, Cogen AL, Radek KA, Park HJ, Macleod DT, Leichtle A, et al. Activation of TLR2 by a small molecule produced by Staphylococcus epidermidis increases antimicrobial defense against bacterial skin infections. J Invest Dermatol. 2010; 130:2211–21. [PubMed: 20463690]

Lai Y, Gallo RL. AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. Trends Immunol. 2009; 30:131–41. [PubMed: 19217824]

Lande R, Ganguly D, Facchinetti V, Frasca L, Conrad C, Gregorio J, et al. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. Sci Transl Med. 2011; 3:73ra19.

Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang YH, Homey B, et al. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature. 2007; 449:564–9. [PubMed: 17873860]

Lee DY, Huang CM, Nakatsuji T, Thiboutot D, Kang SA, Monestier M, et al. Histone H4 Is a Major Component of the Antimicrobial Action of Human Sebocytes. J Invest Dermatol. 2009a

Lee DY, Huang CM, Nakatsuji T, Thiboutot D, Kang SA, Monestier M, et al. Histone H4 is a major component of the antimicrobial action of human sebocytes. J Invest Dermatol. 2009b; 129:2489–96. [PubMed: 19536143]

Lee DY, Yamasaki K, Rudsil J, Zouboulis CC, Park GT, Yang JM, et al. Sebocytes express functional cathelicidin antimicrobial peptides and can act to kill propionibacterium acnes. J Invest Dermatol. 2008; 128:1863–6. [PubMed: 18200058]

Lee JY, Boman A, Sun CX, Andersson M, Jornvall H, Mutt V, et al. Antibacterial peptides from pig intestine: isolation of a mammalian cecropin. Proc Natl Acad Sci U S A. 1989; 86:9159–62. [PubMed: 2512577]

Lee PH, Ohtake T, Zaiou M, Murakami M, Rudisill JA, Lin KH, et al. Expression of an additional cathelicidin antimicrobial peptide protects against bacterial skin infection. Proc Natl Acad Sci U S A. 2005; 102:3750–5. [PubMed: 15728389]

Lehrer RI, Ganz T. Cathelicidins: a family of endogenous antimicrobial peptides. Curr Opin Hematol. 2002; 9:18–22. [PubMed: 11753073]

Lehrer RI, Szklarek D, Ganz T, Selsted ME. Correlation of binding of rabbit granulocyte peptides to Candida albicans with candidacidal activity. Infect Immun. 1985; 49:207–11. [PubMed: 3891625]

Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science. 2006; 311:1770–3. [PubMed: 16497887]

Maier VH, Schmitt CN, Gudmundsdottir S, Gudmundsson GH. Bacterial DNA indicated as an important inducer of fish cathelicidins. Mol Immunol. 2008; 45:2352–8. [PubMed: 18164061]

Mallbris L, Carlen L, Wei T, Heilborn J, Nilsson MF, Granath F, et al. Injury downregulates the expression of the human cathelicidin protein hCAP18/LL-37 in atopic dermatitis. Exp Dermatol. 2010; 19:442–9. [PubMed: 19645825]

Marchini G, Lindow S, Brismar H, Stabi B, Berggren V, Ulfgren AK, et al. The newborn infant is protected by an innate antimicrobial barrier: peptide antibiotics are present in the skin and vernix caseosa. Br J Dermatol. 2002; 147:1127–34. [PubMed: 12452861]

Meyer-Hoffert U, Schwarz T, Schroder JM, Glaser R. Increased expression of human beta-defensin 3 in mollusca contagiosum. Clin Exp Dermatol. 2010; 35:190–2. [PubMed: 19778306]

Meyer-Hoffert U, Wichmann N, Schwichtenberg L, White PC, Wiedow O. Supernatants of Pseudomonas aeruginosa induce the Pseudomonas-specific antibiotic elafin in human keratinocytes. Exp Dermatol. 2003; 12:418–25. [PubMed: 12930298]

Miller LS, Sorensen OE, Liu PT, Jalian HR, Eshtiaghpour D, Behmanesh BE, et al. TGF-alpha regulates TLR expression and function on epidermal keratinocytes. J Immunol. 2005; 174:6137–43. [PubMed: 15879109]

Morizane S, Yamasaki K, Mühleisen B, Kotol PF, Murakami M, Aoyama Y, et al. Cathelicidin antimicrobial peptide LL-37 in psoriasis enables keratinocyte reactivity against TLR9 ligands. J Invest Dermatol. 2011 In press.

Murakami M, Lopez-Garcia B, Braff M, Dorschner RA, Gallo RL. Postsecretory processing generates multiple cathelicidins for enhanced topical antimicrobial defense. J Immunol. 2004; 172:3070–7. [PubMed: 14978112]

Nagy I, Pivarcsi A, Kis K, Koreck A, Bodai L, McDowell A, et al. Propionibacterium acnes and lipopolysaccharide induce the expression of antimicrobial peptides and proinflammatory cytokines/chemokines in human sebocytes. Microbes Infect. 2006; 8:2195–205. [PubMed: 16797202]

Nakatsuji T, Kao MC, Zhang L, Zouboulis CC, Gallo RL, Huang CM. Sebum free fatty acids enhance the innate immune defense of human sebocytes by upregulating beta-defensin-2 expression. J Invest Dermatol. 2010; 130:985–94. [PubMed: 20032992]

Nguyen TX, Cole AM, Lehrer RI. Evolution of primate theta-defensins: a serpentine path to a sweet tooth. Peptides. 2003; 24:1647–54. [PubMed: 15019196]

Niyonsaba F, Iwabuchi K, Someya A, Hirata M, Matsuda H, Ogawa H, et al. A cathelicidin family of human antibacterial peptide LL-37 induces mast cell chemotaxis. Immunology. 2002; 106:20–6. [PubMed: 11972628]

Nizet V, Ohtake T, Lauth X, Trowbridge J, Rudisill J, Dorschner RA, et al. Innate antimicrobial peptide protects the skin from invasive bacterial infection. Nature. 2001; 414:454–7. [PubMed: 11719807]

Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, et al. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. N Engl J Med. 2002; 347:1151–60. [PubMed: 12374875]

Oono T, Matsuura H, Morizane S, Yamasaki O, Iwatsuki K. A case of infectious eccrine hidradenitis. J Dermatol. 2006; 33:142–5. [PubMed: 16556286]

Patterson-Delafield J, Martinez RJ, Lehrer RI. Microbicidal cationic proteins in rabbit alveolar macrophages: a potential host defense mechanism. Infect Immun. 1980; 30:180–92. [PubMed: 7439972]

Peric M, Koglin S, Dombrowski Y, Gross K, Bradac E, Buchau A, et al. Vitamin D analogs differentially control antimicrobial peptide/"alarmin" expression in psoriasis. PLoS One. 2009; 4:e6340. [PubMed: 19623255]

Radek KA, Lopez-Garcia B, Hupe M, Niesman IR, Elias PM, Taupenot L, et al. The neuroendocrine peptide catestatin is a cutaneous antimicrobial and induced in the skin after injury. J Invest Dermatol. 2008; 128:1525–34. [PubMed: 18185531]

Ramos R, Silva JP, Rodrigues AC, Costa R, Guardao L, Schmitt F, et al. Wound healing activity of the human antimicrobial peptide LL37. Peptides. 2011

Ryan LK, Dai J, Yin Z, Megjugorac N, Uhlhorn V, Yim S, et al. Modulation of human {beta}-defensin-1 (hBD-1) in plasmacytoid dendritic cells (PDC), monocytes, and epithelial cells by influenza virus, Herpes simplex virus, and Sendai virus and its possible role in innate immunity. J Leukoc Biol. 2011

Salzman NH, Ghosh D, Huttner KM, Paterson Y, Bevins CL. Protection against enteric salmonellosis in transgenic mice expressing a human intestinal defensin. Nature. 2003; 422:522–6. [PubMed: 12660734]

Sarges R, Witkop B. Gramicidin A. V. The Structure of Valine- and Isoleucine-Gramicidin A. J Am Chem Soc. 1965a; 87:2011–20. [PubMed: 14290276]

Sarges R, Witkop B. Gramicidin. Vii. The Structure of Valine- and Isoleucine-Gramicidin B. J Am Chem Soc. 1965b; 87:2027–30. [PubMed: 14290278]

Sarges R, Witkop B. Gramicidin. Viii. The Structure of Valine- and Isoleucine-Gramicidin C. Biochemistry. 1965c; 4:2491–4.

Schauber J, Oda Y, Buchau AS, Yun QC, Steinmeyer A, Zugel U, et al. Histone acetylation in keratinocytes enables control of the expression of cathelicidin and CD14 by 1,25-dihydroxyvitamin D3. J Invest Dermatol. 2008; 128:816–24. [PubMed: 17943182]

Schauer E, Trautinger F, Kock A, Schwarz A, Bhardwaj R, Simon M, et al. Proopiomelanocortin-derived peptides are synthesized and released by human keratinocytes. J Clin Invest. 1994; 93:2258–62. [PubMed: 8182158]

Schittek B, Hipfel R, Sauer B, Bauer J, Kalbacher H, Stevanovic S, et al. Dermcidin: a novel human antibiotic peptide secreted by sweat glands. Nat Immunol. 2001; 2:1133–7. [PubMed: 11694882]

Schroeder BO, Wu Z, Nuding S, Groscurth S, Marcinowski M, Beisner J, et al. Reduction of disulphide bonds unmasks potent antimicrobial activity of human beta-defensin 1. Nature. 2011; 469:419–23. [PubMed: 21248850]

Schutte BC, Mitros JP, Bartlett JA, Walters JD, Jia HP, Welsh MJ, et al. Discovery of five conserved beta -defensin gene clusters using a computational search strategy. Proc Natl Acad Sci U S A. 2002; 99:2129–33. [PubMed: 11854508]

Selsted ME, Brown DM, DeLange RJ, Harwig SS, Lehrer RI. Primary structures of six antimicrobial peptides of rabbit peritoneal neutrophils. J Biol Chem. 1985; 260:4579–84. [PubMed: 3988726]

Selsted ME, Szklarek D, Lehrer RI. Purification and antibacterial activity of antimicrobial peptides of rabbit granulocytes. Infect Immun. 1984; 45:150–4. [PubMed: 6735465]

Selsted ME, Tang YQ, Morris WL, McGuire PA, Novotny MJ, Smith W, et al. Purification, primary structures, and antibacterial activities of beta-defensins, a new family of antimicrobial peptides from bovine neutrophils. J Biol Chem. 1993; 268:6641–8. [PubMed: 8454635]

Sorensen OE, Follin P, Johnsen AH, Calafat J, Tjabringa GS, Hiemstra PS, et al. Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. Blood. 2001; 97:3951–9. [PubMed: 11389039]

Steiner H, Hultmark D, Engstrom A, Bennich H, Boman HG. Sequence and specificity of two antibacterial proteins involved in insect immunity. Nature. 1981; 292:246–8. [PubMed: 7019715]

Stenger S, Hanson DA, Teitelbaum R, Dewan P, Niazi KR, Froelich CJ, et al. An antimicrobial activity of cytolytic T cells mediated by granulysin. Science. 1998; 282:121–5. [PubMed: 9756476]

Stephens JM, Marshall JH. Some properties of an immune factor isolated from the blood of actively immunized wax moth larvae. Can J Microbiol. 1962; 8:719–25.

Stuart LS, Harris TH. Bactericidal and fungicidal properies of a crystalline protein isolated from unbleached wheat flour. Cereal Chem. 1942; 19:288–300.

Takeuchi O, Hoshino K, Akira S. Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to Staphylococcus aureus infection. J Immunol. 2000; 165:5392–6. [PubMed: 11067888]

Tang YQ, Yuan J, Osapay G, Osapay K, Tran D, Miller CJ, et al. A cyclic antimicrobial peptide produced in primate leukocytes by the ligation of two truncated alpha-defensins. Science. 1999; 286:498–502. [PubMed: 10521339]

Uzzell T, Stolzenberg ED, Shinnar AE, Zasloff M. Hagfish intestinal antimicrobial peptides are ancient cathelicidins. Peptides. 2003; 24:1655–67. [PubMed: 15019197]

van Dijk A, Veldhuizen EJ, van Asten AJ, Haagsman HP. CMAP27, a novel chicken cathelicidin-like antimicrobial protein. Vet Immunol Immunopathol. 2005; 106:321–7. [PubMed: 15963828]

Wang TT, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol. 2004; 173:2909–12. [PubMed: 15322146]

Wang Y, Hong J, Liu X, Yang H, Liu R, Wu J, et al. Snake cathelicidin from Bungarus fasciatus is a potent peptide antibiotics. PLoS One. 2008; 3:e3217. [PubMed: 18795096]

Wanke I, Steffen H, Christ C, Krismer B, Gotz F, Peschel A, et al. Skin commensals amplify the innate immune response to pathogens by activation of distinct signaling pathways. J Invest Dermatol. 2011; 131:382–90. [PubMed: 21048787]

Wiesner J, Vilcinskas A. Antimicrobial peptides: the ancient arm of the human immune system. Virulence. 2010; 1:440–64. [PubMed: 21178486]

Wilson CL, Ouellette AJ, Satchell DP, Ayabe T, Lopez-Boado YS, Stratman JL, et al. Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense. Science. 1999; 286:113–7. [PubMed: 10506557]

Wimley WC. Describing the mechanism of antimicrobial Peptide action with the interfacial activity model. ACS Chem Biol. 2010; 5:905–17. [PubMed: 20698568]

Wingens M, van Bergen BH, Hiemstra PS, Meis JF, van Vlijmen-Willems IM, Zeeuwen PL, et al. Induction of SLPI (ALP/HUSI-I) in epidermal keratinocytes. J Invest Dermatol. 1998; 111:996–1002. [PubMed: 9856807]

Yadava P, Zhang C, Sun J, Hughes JA. Antimicrobial activities of human beta-defensins against Bacillus species. Int J Antimicrob Agents. 2006; 28:132–7. [PubMed: 16797165]

Yamasaki K, Di Nardo A, Bardan A, Murakami M, Ohtake T, Coda A, et al. Increased serine protease activity and cathelicidin promotes skin inflammation in rosacea. Nat Med. 2007; 13:975–80. [PubMed: 17676051]

Yamasaki K, Gallo RL. Antimicrobial peptides in human skin disease. Eur J Dermatol. 2008; 18:11–21. [PubMed: 18086583]

Yamasaki K, Kanada K, Macleod DT, Borkowski AW, Morizane S, Nakatsuji T, et al. TLR2 Expression Is Increased in Rosacea and Stimulates Enhanced Serine Protease Production by Keratinocytes. J Invest Dermatol. 2010

Yamasaki K, Schauber J, Coda A, Lin H, Dorschner RA, Schechter NM, et al. Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin. FASEB J. 2006; 20:2068–80. [PubMed: 17012259]

Yang D, Biragyn A, Hoover DM, Lubkowski J, Oppenheim JJ. Multiple roles of antimicrobial defensins, cathelicidins, and eosinophil-derived neurotoxin in host defense. Annu Rev Immunol. 2004; 22:181–215. [PubMed: 15032578]

Zaalouk TK, Bajaj-Elliott M, George JT, McDonald V. Differential regulation of beta-defensin gene expression during Cryptosporidium parvum infection. Infect Immun. 2004; 72:2772–9. [PubMed: 15102787]

Zaiou M, Nizet V, Gallo RL. Antimicrobial and protease inhibitory functions of the human cathelicidin (hCAP18/LL-37) prosequence. J Invest Dermatol. 2003; 120:810–6. [PubMed: 12713586]

Zanetti M. Cathelicidins, multifunctional peptides of the innate immunity. J Leukoc Biol. 2004; 75:39–48. [PubMed: 12960280]

Zanetti M, Del Sal G, Storici P, Schneider C, Romeo D. The cDNA of the neutrophil antibiotic Bac5 predicts a pro-sequence homologous to a cysteine proteinase inhibitor that is common to other neutrophil antibiotics. J Biol Chem. 1993; 268:522–6. [PubMed: 8416958]

Zanetti M, Gennaro R, Romeo D. Cathelicidins: a novel protein family with a common proregion and a variable C-terminal antimicrobial domain. FEBS Lett. 1995; 374:1–5. [PubMed: 7589491]

Zanger P, Holzer J, Schleucher R, Steffen H, Schittek B, Gabrysch S. Constitutive expression of the antimicrobial peptide RNase 7 is associated with Staphylococcus aureus infection of the skin. J Infect Dis. 2009; 200:1907–15. [PubMed: 19919305]

Zeya HI, Spitznagel JK. Antibacterial and Enzymic Basic Proteins from Leukocyte Lysosomes: Separation and Identification. Science. 1963; 142:1085–7. [PubMed: 14068232]

Zhao H, Gan TX, Liu XD, Jin Y, Lee WH, Shen JH, et al. Identification and characterization of novel reptile cathelicidins from elapid snakes. Peptides. 2008; 29:1685–91. [PubMed: 18620012]

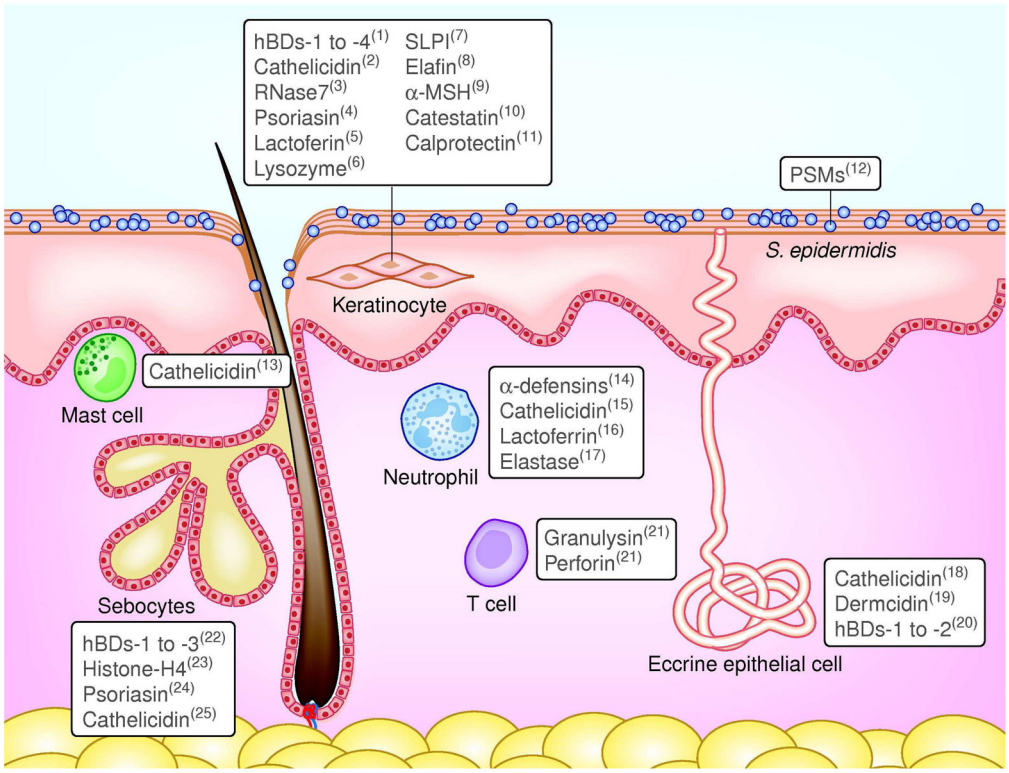

Figure 1. The layered antimicrobial peptides of human skin

A representative partial list of AMPs produced by skin-residing cells is shown. The composition, location, timing of expression and post-translational processing of AMPs in the skin are all important variables that enable them to serve a wide range of functions in defense of the skin. These functions are not only limited to action as a natural antibiotic shield but also include the capacity to trigger cell recruitment, growth and differentiation. Numbers in parentheses indicate references. [(1)], (Ganz *et al.*, 1985; Harder *et al.*, 2001; Johnston *et al.*, 2011); [(2)], (Frohm *et al.*, 1997); [(3)], (Harder and Schroder, 2002); [(4)], (Glaser *et al.*, 2005); [(5)], (Cumberbatch *et al.*, 2000); [(6)], (Marchini *et al.*, 2002); [(7)], (Wingens *et al.*, 1998); [(8)], (Meyer-Hoffert *et al.*, 2003); [(9)], (Cutuli *et al.*, 2000; Schauer *et al.*, 1994); [(10)], (Radek *et al.*, 2008); [(11)], (Abtin *et al.*, 2010); [(12)], (Cogen *et al.*, 2010b); [(13)], (Di Nardo *et al.*, 2003); [(14)], (Ganz, 2003); [(15)], (Agerberth *et al.*, 1995; Cowland *et al.*, 1995); [(16)], (Caccavo *et al.*, 2002); [(17)], (Belaaouaj *et al.*, 2000); [(18)], (Murakami *et al.*, 2004); [(19)], (Schittek *et al.*, 2001); [(20)], (Ali *et al.*, 2001; Oono *et al.*, 2006); [(21)], (Stenger *et al.*, 1998); [(22)], (Chronnell *et al.*, 2001; Nagy *et al.*, 2006; Nakatsuji *et al.*, 2010); [(23)], (Lee *et al.*, 2009b); [(24)], (Glaser *et al.*, 2005); [(25)], (Lee *et al.*, 2008).