

Protecting the boundary: the sentinel role of host defense peptides in the skin

Jamie J. Bernard · Richard L. Gallo

Received: 19 April 2011 / Revised: 26 April 2011 / Accepted: 26 April 2011 / Published online: 15 May 2011
© Springer Basel AG (outside the USA) 2011

Abstract The skin is our primary shield against microbial pathogens and has evolved innate and adaptive strategies to enhance immunity in response to injury or microbial insult. The study of antimicrobial peptide (AMP) production in mammalian skin has revealed several of the elegant strategies that AMPs use to prevent infection. AMPs are inducible by both infection and injury and protect the host by directly killing pathogens and/or acting as multifunctional effector molecules that trigger cellular responses to aid in the anti-infective and repair response. Depending on the specific AMP, these molecules can influence cytokine production, cell migration, cell proliferation, differentiation, angiogenesis and wound healing. Abnormal production of AMPs has been associated with the pathogenesis of several cutaneous diseases and plays a role in determining a patient's susceptibility to pathogens. This review will discuss current research on the regulation and function of AMPs in the skin and in skin disorders.

Keywords Antimicrobial peptides · Defensin · Cathelicidin · Innate immunity · Skin diseases · Keratinocytes

Abbreviations

AMPs Antimicrobial peptides
HBDs Human beta-defensins
LPS Lipopolysaccharide
LTA Lipoteichoic acid
MapK Mitogen-activated protein kinase

PSMs Phenol-soluble modulins
TLR Toll-like receptor
VDR Vitamin D receptor

Introduction

Skin is the largest organ of the body and the interface between the environment and our internal biology. It is comprised of three major layers (innermost to outermost): the hypodermis, which is mainly comprised of adipose tissue; the dermis, which consists of connective tissue and contains hair follicles, sebaceous glands and apocrine glands; and the epidermis, which contains squamous cells and basal cells. The upper layer of the epidermis, the stratum corneum, contains keratinocytes that are tightly linked by desmosomes in a hydrophobic cellular matrix and provides a physical barrier against physical, chemical and biological factors [1, 2]. While the dermis is traversed by a network of lymphatic and blood vessels, the epidermis contains antigen-presenting langerhans cells, dendritic epidermal T-lymphocytes, melanocytes, and keratinocytes, the major constituent of the epidermis. All these cells work in concert to provide immune protection against environmental toxic insults, infections and cutaneous neoplasms.

While immunologists in general have tended to focus on leukocytes as the central cell of the immune response, in skin the keratinocyte is an essential and underappreciated part of immunological function. They are the “first responders” to external pathogens and serve a critical protective role by forming a physical barrier as well as an immune shield that acts to directly kill microbes. Importantly, however, keratinocytes also play an active and dynamic role to alert the circulating immune system that

J. J. Bernard · R. L. Gallo (✉)
Division of Dermatology, Department of Medicine,
University of California, San Diego, Mail code 0869,
San Diego, CA 92126, USA
e-mail: rgallo@ucsd.edu

inflammatory recruitment is required. To accomplish this, the skin produces proinflammatory mediators such as cytokines, chemokines and host defense peptides (antimicrobial peptides; AMPs). In particular, the AMPs are a vital element in skin defense as they are present constitutively, or can be triggered by activation of pattern recognition receptors such as toll-like receptors (TLRs), mannose receptors, helicases, etc. These receptors are responsible for detecting danger signals such as the microbial products lipopolysaccharides (LPS) from Gram-negative bacteria, lipoteichoic acid (LTA) and peptidoglycans from Gram-positive bacteria, mannans of yeast and fungi, and nucleic acids from pathogens and self [3].

As in other tissues, AMPs of the skin are extremely diverse, abundantly expressed and have a broad spectrum of antimicrobial activity as evidenced by their ability to exhibit multifunctional roles in defending against pathogenic insult [4]. AMPs not only directly interact with pathogens but also modulate host immune responses [5]. In keratinocytes, AMPs are mainly synthesized in the stratum granulosum, packaged into lamellar bodies, and then transported to the stratum corneum [6]. In addition to keratinocytes, AMPs are found in the mucosal epithelial tissue and in skin secretions such as saliva and sweat. Furthermore, in neonatal mice and humans, the skin compensates for the lack of a fully developed adaptive immune system by expressing high levels of AMPs that kill Group B *Streptococcus* [7]. Moreover, during normal skin differentiation, AMPs are induced [8]. The differentiated skin forms the outer permeability barrier that is exposed to the highest number of microbial insults. Collectively, these data highlight the importance of AMPs in regulating our immune biology. This review will discuss the seminal role of AMPs in the immune response to cutaneous infection and in the pathologies of dermatological disease.

Host defense (antimicrobial) peptides

Over 12,000 naturally occurring AMPs have been described or predicted and are present in a wide range of species including plants, insects, animals, and humans [9–11]. Individually, AMPs function with modest potency; however, in most biological settings, they are co-expressed and act together to directly kill or stop the growth of a wide-range of microbial pathogens which include both Gram-positive and Gram-negative bacteria, fungi, viruses, and parasites. Most AMPs have an overall net positive charge which ensures their accumulation on, interaction with, and subsequent penetration of the negatively charged phospholipids in the cell walls of microbes [12, 13]. These actions facilitate the killing of microbes. Importantly, microbial resistance to AMPs is low because of difficulties

in altering the membrane phospholipid organization. In addition to their direct microbicidal activity, AMPs also upregulate inflammatory cytokines and chemokines to stimulate and recruit other immune mediators, bridging the gap between the innate and adaptive immune system. Despite the evolution of antibiotic resistance, AMPs remain effective natural antibiotics which may be due to their low potency and the absence of one defined, high affinity molecular target [4, 14]. In mammalian skin, a unique set of diverse antimicrobials have been identified that range from these classical pore-forming peptides to larger proteins with alternative functions described before their antimicrobial action was discovered. Table 1 provides a list of these skin AMPs. This review will discuss the actions of cathelicidins and defensins, the two major families of AMPs in the skin, as well as other cutaneous peptides and proteins such as RNase 7, psoriasin, cathelicidin, and phenol-soluble modulins (PSMs).

Cathelicidins

The first antimicrobial peptide family discovered in mammalian skin was the cathelicidins. Cathelicidins are found in most mammalian species, as well as fish, birds, and snakes [15]. The human cathelicidin gene (*CAMP*) encodes an 18-kDa α -helical structured precursor protein, known as hCAP-18 that consists of a conserved N-terminal region, cathelin, and a variable carboxy (C-) terminal domain. hCAP-18 is most commonly cleaved to release LL-37, a 37 amino acid peptide that begins with two leucine residues and is the only member of the cathelicidin family of host defense peptides expressed in humans [16, 17]. This processing is carried out by serine proteases, such as kallikrein 5 and 7 in keratinocytes, and by neutrophil proteases, such as serine protease 3, and is required for the diverse biological activity of cathelicidin [18, 19]. LL-37 can be further cleaved in the skin to form different peptides (RK-31, KS-30 and K20) with biological activities that differ from LL-37 [19]. LL-37 is found in neutrophils, natural killer cells, mast cells, and epithelial cells. In addition, it is ubiquitously released in many different tissues such as skin, lung, gut, mammary gland and epididymis.

Mechanisms regulating cathelicidin expression are not completely understood; however, recent advances establish a critical role for 1,25-dihydroxyvitamin D(3) (1,25 D3), the active form of vitamin D3 in epidermal keratinocytes. Stimulation with 1,25 D3 enhances hCAP18 expression in keratinocytes [20]. This form is produced when 1 α -hydroxylase (CYP27B1) catalyzes the hydroxylation of 25-hydroxyvitamin D(3) to 1,25 D3. The promoter of the hCAP18 gene contains vitamin D receptor (VDR)-response elements [20]. Upon skin infection and wounding, 1,25 D3

Table 1 Antimicrobial peptides and proteins identified in human skin (partial list)

Skin components/cells	Antimicrobial molecules	References
Epidermis, hair follicle/keratinocyte	hBD-1 to -4	[118, 119]
	Cathelicidin (LL-37, RK-31, KS-30, KR-20)	[8]
	RNase 7	[37]
	S100A7/psoriasin	[38]
	Lactoferrin/lactoferricin	[120]
	Lysozyme	[121]
	SLPI/ALP	[122]
	Elafin/ESI/SKALP	[123, 124]
	Alpha-MSH (melanocyte stimulating hormone)	[125, 126]
	Catestatin	[39]
Sweat gland/eccrine cells	Cathelicidin (RK-31, KS-30, KR-20)	[19]
	Dermcidin	[46]
Sebaceous gland/sebocyte	hBD-1, 2	[127, 128]
	hBD-1, 2	[129]
	Histone H4	[45]
Neutrophils	Alpha-defensins	[130]
	Cathelicidin	[110, 121]
	Lactoferrin/lactoferricin	[131]
	Elastase	[132]
Mast cell	Cathelicidin	[72]
	Elastase	[132]
Eosinophil	ECP/RNase 3	[36]
	EDN/RNase 2	[36]
Platelets	Regulated upon activation, normal T cells expressed and secreted (RANTES)	[133]
	Platelet factor 4 (PF-4)	[133]
	Connective tissue activating peptide 3 (CTAP-3)	[133]
	Platelet basic protein	[133]
	Thymosin beta-4 (Tbeta-4)	[133]
	Fibrinopeptide B (FP-B)	[133]
	Fibrinopeptide A (FP-A)	[133]
T cells	Granulysin	[134]
	Perforin	[134]
<i>S. epidermidis</i>	Phenol-soluble modulins	[48]

is increased by the local increased expression and activity of CYP27B1 [21–23] and binds to VDR to stimulate *CAMP* gene transcription. The precursor of 1,25 D3, vitamin D3 is naturally produced in the skin upon exposure to UV light or deposited after oral ingestion. Cathelicidin expression is also influenced by other factors such as hypoxia inducible factor 1 alpha (HIF-1alpha), the key mediator of the hypoxia response pathway in mammalian cells [24, 25]. Reducing HIF-1alpha protein in human keratinocytes was associated with reduced cathelicidin levels and suppressed immunity to group A *Streptococcus pyogenes* (GAS) infections [26]. These alternative pathways of regulation appear particularly important in non-human species such as mice, as in these normally nocturnal animals the cathelicidin gene does not contain a functional

vitamin D response element and is not directly inducible by 1,25 D3.

Defensins

Defensins are a family of low molecular weight (3–5 kDa) AMPs [27, 28] that lack acyl- and glycosyl-side chain modifications, contain arginine residues and have 6 conserved cysteine residues that form intramolecular disulfide bridges to stabilize its triple-stranded beta-sheet structure. The two main defensin families produced in the skin are alpha defensins and beta defensins, differing in the length of peptide segments between the cysteines and the disulfide bond pairs. In the skin, alpha defensins are produced by neutrophils and beta defensins are produced by

keratinocytes, sebocytes, and sweat glands [29]. Defensins exhibit antimicrobial activity against bacteria, fungi, and viruses [30–32]. Many mechanisms for human beta defensin regulation have been studied. Human beta defensin (hBD) expression is less abundantly induced by 1,25 D3 compared with cathelicidin and more so induced by TLR-ligand activation and inflammatory stimuli. Keratinocytes constitutively express hBD1 and express hBD2–4 upon stimulation with bacteria, IL-1 α , IL-1 β , TNF- α , calcium, IFN- γ , or phorbol 12 myristate 13 acetate or during differentiation. hBD2 and hBD3 are inhibited by Th2 cytokines (IL-4, IL-13) in human keratinocytes [33, 34]. A recent finding by our laboratory has also found that a skin commensal bacteria, *Staphylococcus epidermidis*, upregulates hBD2 and hBD3 expression through TLR2-induced p38 mitogen-activated protein kinase (MapK) signaling [35], suggesting that normal skin microflora bacteria can enhance the innate immune response. Collectively, these studies demonstrate the diversity in and the multiple mechanisms for hBD regulation.

Other antimicrobial peptides and proteins

While the cathelicidin and defensin families are the most well-studied AMPs, many other peptides, some of which have alternate functions in the skin, exhibit antimicrobial activity. RNases, which include ECP/RNase 3, EDN/RNase 2 and RNase 7, have recently been identified as a novel class of antimicrobials. ECP/RNase 3 and EDN/RNase 2, found in skin eosinophils, have antimicrobial activity that functions independently from their ribonuclease activity [36]. A 14.5-kDa ribonuclease, termed RNase7, was isolated from skin-derived stratum corneum, and demonstrates broad-spectrum antimicrobial activity against several microorganisms [37]. Further, its expression is induced in skin with bacterial contact [37]. Human keratinocytes and sebocytes contain the 11-kDa metal ion-binding S100 protein, psoriasin (S100A7), which is induced upon bacterial challenge and may participate in preventing *Escherichia coli* colonization of the skin [38]. Catestatins which are derived from the neuroendocrine protein chromogranin A, were recently identified in skin and exhibit antimicrobial activity against Gram-positive and Gram-negative bacteria, yeast and fungi [39]. Lactoferrin, a multifunction iron-binding glycoprotein, exhibits antimicrobial activity against dermatophytic fungi such as *Trichophyton mentagrophytes* and *Trichophyton rubrum* [40, 41]. Secretory leukocyte proteinase inhibitor (SLPI), or antileukoprotease, has serine protease inhibitor activity and antimicrobial activity [42, 43]. Moreover, SLPI-deficient mice demonstrate impaired cutaneous wound healing and increased inflammation [44]. An AMP with hydrolase activity in human skin is lysozyme, a cationic 148 amino

acid peptide that cleaves glycosidic bonds of *N*-acetylmuramic acid. This action damages bacterial cell walls and ultimately kills by lysis. Sebocytes, an abundant secretory cell in this skin, release their contents by the process of holocrine secretion, and with this event release histone H4, which has a major antimicrobial role for this cell type [45]. Dermcidin, an anionic peptide, is produced exclusively by human sweat glands, and in some experimental systems has been found to have antimicrobial activity [46]. Unlike many other AMPs, dermcidin is not effected by the low pH value and the high salt concentrations of human sweat [47]. Most recently, the PSMs gamma and delta produced in *Staphylococcus epidermidis* were found to have potent antimicrobial action, demonstrating that commensal bacteria contribute to the antimicrobial milieu on the skin [48]. All these classes of AMPs described in this section can act additively or synergistically to exert their effects. For example, LL-37 can act with hBD2, lysozyme and lactoferrin to kill bacteria. This diversity and selective activity of AMPs is important for normal antimicrobial defense strategy of the skin.

AMPs orchestrate the host inflammatory response

The skin is one of the most widely studied systems for how AMPs confer protection and thwart unwanted infection. Mice deficient in the murine cathelicidin, cathelicidin-related antimicrobial peptide (CRAMP), have normal growth, development and numbers of circulating lymphocytes, but have an enhanced susceptibility to necrotizing skin infections [49]. Supporting this, the overexpression of cathelicidin in lungs of mice attenuates bacterial load and the inflammatory response [50]. Mice deficient in mBD1 have slightly enhanced susceptibility to *S. aureus* infection in the lung [51]. hBD3 is upregulated during *S. aureus* infection and has potent antistaphylococcal activity [52, 53]. These studies suggest a correlation between the levels of AMPs and disease severity. However, direct antimicrobial activity may not be the primary mechanism for AMP protection against infection [54]. AMPs exhibit multifunctional roles as effector cells of the immune system. AMPs modulate the skin immune system by at least three mechanisms: (1) stimulating chemotactic activity, (2) inducing cytokine release, and (3) modulating the TLR response.

Defensins act as chemotactic agents for neutrophils, mast cells, T cells and dendritic cells [55–58]. It has been postulated hBD2 acts as a chemoattractant for memory T cells and immature dendritic cells by binding to the chemokine receptor CCR6 [58]. However, defensins attract mast cells and macrophages most likely independently of CCR6 [59]. In keratinocytes, defensins have the ability to

induce cytokines such as IL-18, IL-6, IL-10 and chemokines such as interferon inducible protein 10, monocyte chemoattractant protein 1, macrophage inflammatory protein 3 alpha and RANTES [60, 61]. In addition, mouse beta-defensin 2 was shown to exhibit adjuvant activity dependent on TLR4 [62].

Cathelicidins also exhibit chemoattractant activity. In part, LL-37 attracts neutrophils, monocytes, and T cells through binding the formyl peptide receptor-like 1 (FPRL-1) [63]. Similar activity was observed with CRAMP for human monocytes, neutrophils, macrophages, and mouse peripheral blood leukocytes [64]. Furthermore, LL-37 is a chemoattractant for and is produced by mast cells, although the mechanism responsible for this appeared to be independent of FPRL-1 [65]. LL-37 enhanced the expression of costimulatory molecules and Th1 cytokines by monocyte-derived dendritic cells [66]. Moreover, LL-37 has been shown to promote the release of alpha-defensins from neutrophils [67], enhance the vascular permeability of mast cells [68], and stimulate keratinocytes [61] and dendritic cells [60].

Adding complexity to function, LL-37 has potent effects on TLR responses. LL-37 suppressed LPS-, flagellin- and LTA-induced dendritic cell activation [69] and suppressed LPS- and LTA-induced monocyte and macrophage activation [70, 71]. Furthermore, cathelicidin can act to inhibit TLR4 stimulation. In a model of contact dermatitis, LL-37 inhibited TLR4- and CD44-mediated induction of cytokine release in dendritic cells and macrophages [72]. Hyaluronan, a glycosaminoglycan and alternate TLR4 ligand, activity was attenuated with cathelicidin treatment in a model of chronic allergic dermatitis, and exacerbated in the cathelicidin-deficient mouse *Camp*^{-/-} [73]. While immunomodulatory functions of AMPs remain unclear, the importance of epithelial AMP function is highlighted by differential AMP levels in dermatological disease states and during wound healing.

AMP production in disease and wound healing

Atopic dermatitis

Atopic dermatitis is an inflammatory pruritic skin disease. A significant portion of patients have abnormalities in the epidermal barrier function and a deficiency in the production of LL-37, hBD2 and hBD3 in lesional skin [33, 74]. In addition to the lower levels of LL-37 and defensins in lesional skin of atopics compared to lesional skin of other inflammatory skin disease such as psoriasis or in wounds, levels of dermcidin are decreased in atopics and in patients with previous bacterial or viral infections [75]. These observations may account for why over 90% of atopics

have *S. aureus* skin colonization which directly correlates with their clinical severity and activates eczema [76, 77]. The reduction in AMPs increases the risk of not only bacterial colonization but also viral infections such as vaccinia and herpes simplex virus [78–80].

The reduced levels of AMPs are thought to be caused in part by the heightened Th2 response and overproduction of IL-4, IL-10, and IL-13 [81, 82]. IL-4 and IL-13 directly inhibit hBD2 and hBD3 production in keratinocytes [83–85]. They can also indirectly inhibit hBD3 and LL-37 by inhibiting STAT6 activation and causing the subsequent inhibition of TNF alpha/NF- κ B and IFN-gamma [81–83, 86].

UVB therapy is frequently used to treat atopic dermatitis. Its therapeutic action is mainly thought to occur by targeting T cell-mediated immune responses [87]. However, UVB also causes the cutaneous activation of vitamin D3 [88] and subsequent upregulation of hCAP18 [89]. Influencing cathelicidin expression may help ameliorate symptoms of atopic dermatitis.

Interestingly, Harder et al. [37] recently demonstrated enhanced expression of hBD2, hBD3, psoriasin, and RNase 7 in atopic lesional skin when compared with non-lesional skin and controls, suggesting that a disturbed skin barrier may trigger AMP induction in disease. This was also observed in psoriatic patients. Such an increase in AMPs in atopic dermatitis was predicted by earlier studies [74], as the capacity to increase AMPs is not completely abolished in all patients with atopic dermatitis. In fact, a recent study by Hata et al. [90] has demonstrated that the magnitude of suppressed AMP expression in atopics correlates with disease severity and history of infection. Thus, patients with a history of skin infection were the population with the lowest AMP expression in lesional skin.

Psoriasis

Psoriasis affects up to 3% of the population and is characterized by the formation of typical scaly plaques and hypertrophic skin lesions [91]. In contrast to atopic dermatitis, psoriasis is an inflammatory skin disease with elevated levels of AMPs and a heightened Th1 response [92]. The induction of Th1 cytokines and reduction of Th2 cytokines accounts for the high expression of hBDs in psoriatic patients. In addition, higher genetic copy numbers of beta defensins are associated with psoriasis risk [92]. hBD2 and hBD3 are abundant in psoriatic scales [93, 94]. These plaques are also rich in lysozyme, RNase 7, psoriasin and human neutrophil defensins 1–3 [94]. Cathelicidin levels are also elevated and are thought to exacerbate the disease [95]. The abundance of LL-37 has been proposed to enable patients to react to self-DNA and drive an autoimmune reaction. In this model, human self-DNA, only when

complexed with LL-37, activates plasmacytoid dendritic cells (pDCs) through TLR9 [96]. Activation of pDCs initiates the release of IFN- α and serves to augment a Th17 inflammatory response. Unlike atopic dermatitis which is frequently accompanied by bacterial infections, in psoriasis, secondary bacterial infections are rare which may be due to the protective effects of elevated AMPs [74].

Rosacea

Rosacea is common chronic inflammatory skin disease that is characterized primarily by flushing, non-transient erythema, papules, pustules, inflammatory nodules and telangiectasia. Symptoms are often exacerbated by exercise, emotional stress and alcohol [97]. Rosacea is characterized by abnormal AMP expression and processing. Yamasaki et al. [98] showed that individuals with rosacea express elevated levels of cathelicidin. The cathelicidin peptide forms that characterize the disease were found to promote vasoactivation [99] and leukocyte infiltration [63]. Furthermore, mice exhibit an inflammatory response similar to rosacea when administered these cathelicidin peptides [98].

Mechanisms which control cathelicidin production are abnormal in rosacea. Increased activity of kallikrein 5, the protease which controls cathelicidin production, is observed [98]. In addition, some patients with severe rosacea have polymorphisms in their VDR gene [100], suggesting an increased sensitivity to vitamin D processing of cathelicidin.

Other skin infections

Evidence suggests that the pathogenesis of acne vulgaris and superficial folliculitis may be linked with AMP expression. Inflammatory acne vulgaris affects up to 80% of people and is caused when the overgrowth of *Propionibacterium acnes* (*P. acnes*), a Gram-positive anaerobic bacterium residing in pilosebaceous follicles, disrupts the epidermal barrier allowing other bacteria to spread from the microcomedone to the dermis [54]. This translocation of bacteria triggers the production of pro-inflammatory cytokines and granulomatous reactions [101, 102]. In addition, hBD1 and hBD2 are upregulated in the suprabasal layers and in the hair follicle [103]. Similarly, in superficial folliculitis, a skin disease often caused by *S. aureus* infection and inflammation in the hair follicle, hBD2 is upregulated in lesional skin [104]. Other skin disorders have also been observed to be accompanied by changes in AMP expression. These include higher amounts of hBD2 and lower levels of hBD1 in basal cell carcinoma tumors compared to normal skin [105], and increased cathelicidin expression in systemic lupus

erythematosus [8]. The precise role of AMPs in these skin diseases remains unclear.

Wound healing

The role of defensins and cathelicidin in wound healing has been extensively studied. Low levels of AMPs are present in healthy intact skin whereas cathelicidin and defensin expression are abundant post-wounding and in healing skin epithelia [106–109]. Our laboratory first discovered that AMPs were present in mammalian skin when we demonstrated that a cathelicidin peptide in wounded pig skin (PR-39) induced syndecans, a cell surface heparin sulfate proteoglycans that regulates cell proliferation and migration [110]. Since that time, several studies have uncovered a wide range of roles for AMPs in addition to their antimicrobial function. For example, hBD2 accelerates wound closure by promoting proliferation, migration, and tube formation of endothelial cells [109]. Similarly, LL-37 accelerates wound closure by promoting proliferation, migration and neovascularization [63, 99]. These findings are consistent with data demonstrating that blocking LL-37 with an antibody inhibits re-epithelialization [107] and that CRAMP^{-/-} mice exhibit decreased vascularization during wound repair [99]. Keratinocyte migration has been shown to be influenced by LL-37-induced transactivation of the epidermal growth factor receptor (EGFR) and activation of STAT1 and STAT3 [111]. This occurs when LL-37 initiates matrix metalloproteinases to cleave the heparin-binding-EGF (HB-EGF) subsequently phosphorylating EGFR [111]. Similarly, LL-37 stimulates EGFR transactivation in corneal epithelial cells under conditions of normal and high glucose [112]. Furthermore, LL-37 induces angiogenesis by activating FPRL-1 expressed on endothelial cells [99] and increases wound closure by activating signaling pathways involved in endothelial proliferation such as MAPK, PI3K and Akt [99, 113]. Interestingly, LL-37 may also accelerate wound healing by attenuating scar tissue formation in dermal fibroblasts by inhibiting transforming growth factor- β -induced collagen expression in these cells [114].

Skin ulcers are often due to venous insufficiency and in association with immune diseases such as pyoderma gangrenosum and vasculitis. In patients with non-healing chronic ulcers of the skin, hCAP18 is present at low levels, and LL-37 is absent from the wound edge [107]. hBD3 was found to promote wound closure in a preclinical, large-animal model of diabetes [115]. These data suggest a therapeutic role for AMPs in successful wound closure. Pexiganan, the frog magainin derivative MSI-78, is a cationic antimicrobial peptide topically administered to patients with diabetic foot ulcers and has had significant therapeutic efficacy in 90% of patients [116]. The use of

novel clinically useable antimicrobial peptides is currently being investigated in several skin conditions.

Conclusion

Much of our knowledge of how AMPs are regulated and how they function to dampen effects of harmful microbial compounds comes from our study of them in the skin. AMPs have the capacity to directly kill microbes and modulate the immune response. These activities are integral to their promise as novel anti-infective and wound healing agents. Several synthetic AMPs are currently in commercial development [117]. However, the clinical use of these molecules has therapeutic benefits that extend beyond the treatment of skin diseases. Infectious disease remains a leading cause of morbidity and mortality worldwide and presents a major economic burden. While we continue to be faced with pandemics such as HIV/AIDS, new pathogens (such as H1N1) emerge and threaten our survival. The greatest problem facing modern drug discovery is the ability of microorganisms to withstand the effects of antibiotics. Reasons such as these create an urgent need for the development of new antimicrobial drugs that are cheap and effective, and augment defense systems that have naturally evolved in our environment. Understanding the function of AMPs and ways to regulate AMP production will provide insight for alternative therapies to prevent and lessen disease.

References

- Cork MJ, Danby SG, Vasilopoulos Y, Hadgraft J, Lane ME, Moustafa M, Guy RH, Macgowan AL, Tazi-Ahnini R, Ward SJ (2009) Epidermal barrier dysfunction in atopic dermatitis. *J Invest Dermatol* 129:1892–1908
- Proksch E, Brandner JM, Jensen JM (2008) The skin: an indispensable barrier. *Exp Dermatol* 17:1063–1072
- Janeway CA Jr, Medzhitov R (2002) Innate immune recognition. *Annu Rev Immunol* 20:197–216
- Zasloff M (2002) Antimicrobial peptides of multicellular organisms. *Nature* 415:389–395
- Gallo RL, Huttner KM (1998) Antimicrobial peptides: an emerging concept in cutaneous biology. *J Invest Dermatol* 111:739–743
- Aberg KM, Man MQ, Gallo RL, Ganz T, Crumrine D, Brown BE, Choi EH, Kim DK, Schroder JM, Feingold KR, Elias PM (2008) Co-regulation and interdependence of the mammalian epidermal permeability and antimicrobial barriers. *J Invest Dermatol* 128:917–925
- Dorschner RA, Lin KH, Murakami M, Gallo RL (2003) Neonatal skin in mice and humans expresses increased levels of antimicrobial peptides: innate immunity during development of the adaptive response. *Pediatr Res* 53:566–572
- Frohm M, Agerberth B, Ahangari G, Stahle-Backdahl M, Liden S, Wigzell H, Gudmundsson GH (1997) The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders. *J Biol Chem* 272:15258–15263
- Brahmachary M, Krishnan SP, Koh JL, Khan AM, Seah SH, Tan TW, Brusic V, Bajic VB (2004) ANTIMIC: a database of antimicrobial sequences. *Nucleic Acids Res* 32:D586–D589
- Fjell CD, Hancock RE, Cherkasov A (2007) AMPer: a database and an automated discovery tool for antimicrobial peptides. *Bioinformatics* 23:1148–1155
- Wang G, Li X, Wang Z (2009) APD2: the updated antimicrobial peptide database and its application in peptide design. *Nucleic Acids Res* 37:D933–D937
- Jenssen H, Hamill P, Hancock RE (2006) Peptide antimicrobial agents. *Clin Microbiol Rev* 19:491–511
- Yeaman MR, Yount NY (2003) Mechanisms of antimicrobial peptide action and resistance. *Pharmacol Rev* 55:27–55
- Hancock RE, Lehrer R (1998) Cationic peptides: a new source of antibiotics. *Trends Biotechnol* 16:82–88
- Gennaro R, Zanetti M (2000) Structural features and biological activities of the cathelicidin-derived antimicrobial peptides. *Biopolymers* 55:31–49
- Durr UH, Sudheendra US, Ramamoorthy A (2006) LL-37, the only human member of the cathelicidin family of antimicrobial peptides. *Biochim Biophys Acta* 1758:1408–1425
- Zanetti M (2004) Cathelicidins, multifunctional peptides of the innate immunity. *J Leukoc Biol* 75:39–48
- Yamasaki K, Schaubert J, Coda A, Lin H, Dorschner RA, Schechter NM, Bonnart C, Descargues P, Hovnanian A, Gallo RL (2006) Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin. *FASEB J* 20:2068–2080
- Murakami M, Lopez-Garcia B, Braff M, Dorschner RA, Gallo RL (2004) Postsecretory processing generates multiple cathelicidins for enhanced topical antimicrobial defense. *J Immunol* 172:3070–3077
- Wang TT, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, Tavera-Mendoza L, Lin R, Hanrahan JW, Mader S, White JH (2004) Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J Immunol* 173:2909–2912
- Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schaubert J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zugel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin RL (2006) Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 311:1770–1773
- Schaubert J, Dorschner RA, Coda AB, Buchau AS, Liu PT, Kiken D, Helfrich YR, Kang S, Elalieh HZ, Steinmeyer A, Zugel U, Bickle DD, Modlin RL, Gallo RL (2007) Injury enhances TLR2 function and antimicrobial peptide expression through a vitamin D-dependent mechanism. *J Clin Invest* 117:803–811
- Schaubert J, Dorschner RA, Yamasaki K, Brouha B, Gallo RL (2006) Control of the innate epithelial antimicrobial response is cell-type specific and dependent on relevant microenvironmental stimuli. *Immunology* 118:509–519
- Gallo RL, Kim KJ, Bernfield M, Kozak CA, Zanetti M, Merluzzi L, Gennaro R (1997) Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse. *J Biol Chem* 272:13088–13093
- Peyssonnaud C, Johnson RS (2004) An unexpected role for hypoxic response: oxygenation and inflammation. *Cell Cycle* 3:168–171
- Peyssonnaud C, Boutin AT, Zinkernagel AS, Datta V, Nizet V, Johnson RS (2008) Critical role of HIF-1 α in keratinocyte defense against bacterial infection. *J Invest Dermatol* 128:1964–1968

27. Ganz T, Selsted ME, Szklarek D, Harwig SS, Daher K, Bainton DF, Lehrer RI (1985) Defensins. Natural peptide antibiotics of human neutrophils. *J Clin Invest* 76:1427–1435
28. Selsted ME, Harwig SS, Ganz T, Schilling JW, Lehrer RI (1985) Primary structures of three human neutrophil defensins. *J Clin Invest* 76:1436–1439
29. Koczulla AR, Bals R (2003) Antimicrobial peptides: current status and therapeutic potential. *Drugs* 63:389–406
30. Selsted ME, Szklarek D, Ganz T, Lehrer RI (1985) Activity of rabbit leukocyte peptides against *Candida albicans*. *Infect Immun* 49:202–206
31. Selsted ME, Szklarek D, Lehrer RI (1984) Purification and antibacterial activity of antimicrobial peptides of rabbit granulocytes. *Infect Immun* 45:150–154
32. Daher KA, Selsted ME, Lehrer RI (1986) Direct inactivation of viruses by human granulocyte defensins. *J Virol* 60:1068–1074
33. Nomura I, Goleva E, Howell MD, Hamid QA, Ong PY, Hall CF, Darst MA, Gao B, Boguniewicz M, Travers JB, Leung DY (2003) Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes. *J Immunol* 171:3262–3269
34. Harder J, Bartels J, Christophers E, Schroder JM (1997) A peptide antibiotic from human skin. *Nature* 387:861
35. Lai Y, Cogen AL, Radek KA, Park HJ, Macleod DT, Leichte A, Ryan AF, Di Nardo A, Gallo RL (2010) Activation of TLR2 by a small molecule produced by *Staphylococcus epidermidis* Increases antimicrobial defense against bacterial skin infections. *J Invest Dermatol* 130:2211–2221
36. Domachowske JB, Dyer KD, Adams AG, Leto TL, Rosenberg HF (1998) Eosinophil cationic protein/RNase 3 is another RNase A-family ribonuclease with direct antiviral activity. *Nucleic Acids Res* 26:3358–3363
37. Harder J, Schroder JM (2002) RNase 7, a novel innate immune defense antimicrobial protein of healthy human skin. *J Biol Chem* 277:46779–46784
38. Glaser R, Harder J, Lange H, Bartels J, Christophers E, Schroder JM (2005) Antimicrobial psoriasin (S100A7) protects human skin from *Escherichia coli* infection. *Nat Immunol* 6:57–64
39. Radek KA, Lopez-Garcia B, Hupe M, Niesman IR, Elias PM, Taupenot L, Mahata SK, O'Connor DT, Gallo RL (2008) The neuroendocrine peptide catestatin is a cutaneous antimicrobial and induced in the skin after injury. *J Invest Dermatol* 128:1525–1534
40. Weitzman I, Summerbell RC (1995) The dermatophytes. *Clin Microbiol Rev* 8:240–259
41. Wakabayashi H, Uchida K, Yamauchi K, Teraguchi S, Hayasawa H, Yamaguchi H (2000) Lactoferrin given in food facilitates dermatophytosis cure in guinea pig models. *J Antimicrob Chemother* 46:595–602
42. Franzke CW, Baici A, Bartels J, Christophers E, Wiedow O (1996) Antileukoprotease inhibits stratum corneum chymotryptic enzyme. Evidence for a regulative function in desquamation. *J Biol Chem* 271:21886–21890
43. Wiedow O, Harder J, Bartels J, Streit V, Christophers E (1998) Antileukoprotease in human skin: an antibiotic peptide constitutively produced by keratinocytes. *Biochem Biophys Res Commun* 248:904–909
44. Ashcroft GS, Lei K, Jin W, Longenecker G, Kulkarni AB, Greenwell-Wild T, Hale-Donze H, McGrady G, Song XY, Wahl SM (2000) Secretory leukocyte protease inhibitor mediates non-redundant functions necessary for normal wound healing. *Nat Med* 6:1147–1153
45. Lee DY, Huang CM, Nakatsuji T, Thiboutot D, Kang SA, Monestier M, Gallo RL (2009) Histone H4 is a major component of the antimicrobial action of human sebocytes. *J Invest Dermatol* 129:2489–2496
46. Schitteck B, Hipfel R, Sauer B, Bauer J, Kalbacher H, Stevanovic S, Schirle M, Schroeder K, Blin N, Meier F, Rassner G, Garbe C (2001) Dermcidin: a novel human antibiotic peptide secreted by sweat glands. *Nat Immunol* 2:1133–1137
47. Flad T, Bogumil R, Tolson J, Schitteck B, Garbe C, Deeg M, Mueller CA, Kalbacher H (2002) Detection of dermcidin-derived peptides in sweat by ProteinChip technology. *J Immunol Methods* 270:53–62
48. Cogen AL, Yamasaki K, Sanchez KM, Dorschner RA, Lai Y, MacLeod DT, Torpey JW, Otto M, Nizet V, Kim JE, Gallo RL (2010) Selective antimicrobial action is provided by phenol-soluble modulins derived from *Staphylococcus epidermidis*, a normal resident of the skin. *J Invest Dermatol* 130:192–200
49. Nizet V, Ohtake T, Lauth X, Trowbridge J, Rudisill J, Dorschner RA, Pestonjamas V, Piraino J, Huttner K, Gallo RL (2001) Innate antimicrobial peptide protects the skin from invasive bacterial infection. *Nature* 414:454–457
50. Bals R, Weiner DJ, Moscioni AD, Meegalla RL, Wilson JM (1999) Augmentation of innate host defense by expression of a cathelicidin antimicrobial peptide. *Infect Immun* 67:6084–6089
51. Morrison G, Kilanowski F, Davidson D, Dorin J (2002) Characterization of the mouse beta defensin 1, Defb1, mutant mouse model. *Infect Immun* 70:3053–3060
52. Garcia JR, Jaumann F, Schulz S, Krause A, Rodriguez-Jimenez J, Forssmann U, Adermann K, Kluver E, Vogelmeier C, Becker D, Hedrich R, Forssmann WG, Bals R (2001) Identification of a novel, multifunctional beta-defensin (human beta-defensin 3) with specific antimicrobial activity. Its interaction with plasma membranes of *Xenopus* oocytes and the induction of macrophage chemoattraction. *Cell Tissue Res* 306:257–264
53. Menzies BE, Kenoyer A (2006) Signal transduction and nuclear responses in *Staphylococcus aureus*-induced expression of human beta-defensin 3 in skin keratinocytes. *Infect Immun* 74:6847–6854
54. Bowdish DM, Davidson DJ, Lau YE, Lee K, Scott MG, Hancock RE (2005) Impact of LL-37 on anti-infective immunity. *J Leukoc Biol* 77:451–459
55. Gallo RL, Murakami M, Ohtake T, Zaiou M (2002) Biology and clinical relevance of naturally occurring antimicrobial peptides. *J Allergy Clin Immunol* 110:823–831
56. Niyonsaba F, Iwabuchi K, Matsuda H, Ogawa H, Nagaoka I (2002) Epithelial cell-derived human beta-defensin-2 acts as a chemotaxin for mast cells through a pertussis toxin-sensitive and phospholipase C-dependent pathway. *Int Immunol* 14:421–426
57. Niyonsaba F, Ogawa H, Nagaoka I (2004) Human beta-defensin-2 functions as a chemotactic agent for tumour necrosis factor- α -treated human neutrophils. *Immunology* 111:273–281
58. Yang D, Chertov O, Bykovskaia SN, Chen Q, Buffo MJ, Shogan J, Anderson M, Schroder JM, Wang JM, Howard OM, Oppenheim JJ (1999) Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. *Science* 286:525–528
59. Soruri A, Grigat J, Forssmann U, Riggert J, Zwirner J (2007) beta-Defensins chemoattract macrophages and mast cells but not lymphocytes and dendritic cells: CCR6 is not involved. *Eur J Immunol* 37:2474–2486
60. Niyonsaba F, Ushio H, Nagaoka I, Okumura K, Ogawa H (2005) The human beta-defensins (-1, -2, -3, -4) and cathelicidin LL-37 induce IL-18 secretion through p38 and ERK MAPK activation in primary human keratinocytes. *J Immunol* 175:1776–1784
61. Niyonsaba F, Ushio H, Nakano N, Ng W, Sayama K, Hashimoto K, Nagaoka I, Okumura K, Ogawa H (2007) Antimicrobial peptides human beta-defensins stimulate epidermal keratinocyte migration, proliferation and production of proinflammatory cytokines and chemokines. *J Invest Dermatol* 127:594–604

62. Biragyn A, Ruffini PA, Leifer CA, Klyushnenkova E, Shakhov A, Chertov O, Shirakawa AK, Farber JM, Segal DM, Oppenheim JJ, Kwak LW (2002) Toll-like receptor 4-dependent activation of dendritic cells by beta-defensin 2. *Science* 298:1025–1029
63. De Y, Chen Q, Schmidt AP, Anderson GM, Wang JM, Wooters J, Oppenheim JJ, Chertov O (2000) 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* 192:1069–1074
64. Kurosaka K, Chen Q, Yarovsky F, Oppenheim JJ, Yang D (2005) Mouse cathelin-related antimicrobial peptide chemoattracts leukocytes using formyl peptide receptor-like 1/mouse formyl peptide receptor-like 2 as the receptor and acts as an immune adjuvant. *J Immunol* 174:6257–6265
65. Di Nardo A, Vitiello A, Gallo RL (2003) Cutting edge: mast cell antimicrobial activity is mediated by expression of cathelicidin antimicrobial peptide. *J Immunol* 170:2274–2278
66. Davidson DJ, Currie AJ, Reid GS, Bowdish DM, MacDonald KL, Ma RC, Hancock RE, Speert DP (2004) The cationic antimicrobial peptide LL-37 modulates dendritic cell differentiation and dendritic cell-induced T cell polarization. *J Immunol* 172:1146–1156
67. Zheng Y, Niyonsaba F, Ushio H, Nagaoka I, Ikeda S, Okumura K, Ogawa H (2007) Cathelicidin LL-37 induces the generation of reactive oxygen species and release of human alpha-defensins from neutrophils. *Br J Dermatol* 157:1124–1131
68. Niyonsaba F, Iwabuchi K, Someya A, Hirata M, Matsuda H, Ogawa H, Nagaoka I (2002) A cathelicidin family of human antibacterial peptide LL-37 induces mast cell chemotaxis. *Immunology* 106:20–26
69. Kandler K, Shaykhiev R, Kleemann P, Kleszcz F, Lohoff M, Vogelmeier C, Bals R (2006) The anti-microbial peptide LL-37 inhibits the activation of dendritic cells by TLR ligands. *Int Immunol* 18:1729–1736
70. Mookherjee N, Brown KL, Bowdish DM, Doria S, Falsafi R, Hokamp K, Roche FM, Mu R, Doho GH, Pistolic J, Powers JP, Bryan J, Brinkman FS, Hancock RE (2006) Modulation of the TLR-mediated inflammatory response by the endogenous human host defense peptide LL-37. *J Immunol* 176:2455–2464
71. Mookherjee N, Wilson HL, Doria S, Popowych Y, Falsafi R, Yu JJ, Li Y, Veatch S, Roche FM, Brown KL, Brinkman FS, Hokamp K, Potter A, Babiuk LA, Griebel PJ, Hancock RE (2006) Bovine and human cathelicidin cationic host defense peptides similarly suppress transcriptional responses to bacterial lipopolysaccharide. *J Leukoc Biol* 80:1563–1574
72. Di Nardo A, Braff MH, Taylor KR, Na C, Granstein RD, McInturff JE, Krutzik S, Modlin RL, Gallo RL (2007) Cathelicidin antimicrobial peptides block dendritic cell TLR4 activation and allergic contact sensitization. *J Immunol* 178:1829–1834
73. Morioka Y, Yamasaki K, Leung D, Gallo RL (2008) Cathelicidin antimicrobial peptides inhibit hyaluronan-induced cytokine release and modulate chronic allergic dermatitis. *J Immunol* 181:3915–3922
74. Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, Gallo RL, Leung DY (2002) Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Engl J Med* 347:1151–1160
75. Rieg S, Steffen H, Seeber S, Humeny A, Kalbacher H, Dietz K, Garbe C, Schitteck B (2005) Deficiency of dermcidin-derived antimicrobial peptides in sweat of patients with atopic dermatitis correlates with an impaired innate defense of human skin in vivo. *J Immunol* 174:8003–8010
76. Leung DY (2003) Infection in atopic dermatitis. *Curr Opin Pediatr* 15:399–404
77. Machura E, Mazur B, Golemić E, Pindel M, Halkiewicz F (2008) *Staphylococcus aureus* skin colonization in atopic dermatitis children is associated with decreased IFN-gamma production by peripheral blood CD4+ and CD8+ T cells. *Pediatr Allergy Immunol* 19:37–45
78. Howell MD, Gallo RL, Boguniewicz M, Jones JF, Wong C, Streib JE, Leung DY (2006) Cytokine milieu of atopic dermatitis skin subverts the innate immune response to vaccinia virus. *Immunity* 24:341–348
79. Howell MD, Wollenberg A, Gallo RL, Flaig M, Streib JE, Wong C, Pavicic T, Boguniewicz M, Leung DY (2006) Cathelicidin deficiency predisposes to eczema herpeticum. *J Allergy Clin Immunol* 117:836–841
80. Wollenberg A, Wetzel S, Burgdorf WH, Haas J (2003) Viral infections in atopic dermatitis: pathogenic aspects and clinical management. *J Allergy Clin Immunol* 112:667–674
81. Howell MD, Novak N, Bieber T, Pastore S, Girolomoni G, Boguniewicz M, Streib J, Wong C, Gallo RL, Leung DY (2005) Interleukin-10 downregulates anti-microbial peptide expression in atopic dermatitis. *J Invest Dermatol* 125:738–745
82. Homey B, Steinhoff M, Ruzicka T, Leung DY (2006) Cytokines and chemokines orchestrate atopic skin inflammation. *J Allergy Clin Immunol* 118:178–189
83. Howell MD, Boguniewicz M, Pastore S, Novak N, Bieber T, Girolomoni G, Leung DY (2006) Mechanism of HBD-3 deficiency in atopic dermatitis. *Clin Immunol* 121:332–338
84. Kaburagi Y, Shimada Y, Nagaoka T, Hasegawa M, Takehara K, Sato S (2001) Enhanced production of CC-chemokines (RANTES, MCP-1, MIP-1alpha, MIP-1beta, and eotaxin) in patients with atopic dermatitis. *Arch Dermatol Res* 293:350–355
85. Campbell JJ, Butcher EC (2000) Chemokines in tissue-specific and microenvironment-specific lymphocyte homing. *Curr Opin Immunol* 12:336–341
86. Jeong CW, Ahn KS, Rho NK, Park YD, Lee DY, Lee JH, Lee ES, Yang JM (2003) Differential in vivo cytokine mRNA expression in lesional skin of intrinsic vs. extrinsic atopic dermatitis patients using semiquantitative RT-PCR. *Clin Exp Allergy* 33:1717–1724
87. Schwarz T (2008) 25 years of UV-induced immunosuppression mediated by T cells—from disregarded T suppressor cells to highly respected regulatory T cells. *Photochem Photobiol* 84:10–18
88. Lehmann B, Genehr T, Knuschke P, Pietzsch J, Meurer M (2001) UVB-induced conversion of 7-dehydrocholesterol to 1alpha, 25-dihydroxyvitamin D3 in an in vitro human skin equivalent model. *J Invest Dermatol* 117:1179–1185
89. Mallbris L, Edstrom DW, Sundblad L, Granath F, Stahle M (2005) UVB upregulates the antimicrobial protein hCAP18 mRNA in human skin. *J Invest Dermatol* 125:1072–1074
90. Hata T (2010) History of eczema herpeticum is associated with the inability to induced HBD-2, HBD-3 and cathelicidin in the skin of patients with atopic dermatitis. *Br J Dermatol* 163:659–661
91. Nickoloff BJ, Nestle FO (2004) Recent insights into the immunopathogenesis of psoriasis provide new therapeutic opportunities. *J Clin Invest* 113:1664–1675
92. Hollox EJ, Huffmeier U, Zeeuwen PL, Palla R, Lascorz J, Rodijk-Olthuis D, van de Kerkhof PC, Traupe H, de Jongh G, den Heijer M, Reis A, Armour JA, Schalkwijk J (2008) Psoriasis is associated with increased beta-defensin genomic copy number. *Nat Genet* 40:23–25
93. Huh WK, Oono T, Shirafuji Y, Akiyama H, Arata J, Sakaguchi M, Huh NH, Iwatsuki K (2002) Dynamic alteration of human

- beta-defensin 2 localization from cytoplasm to intercellular space in psoriatic skin. *J Mol Med* 80:678–684
94. Harder J, Schroder JM (2005) Psoriatic scales: a promising source for the isolation of human skin-derived antimicrobial proteins. *J Leukoc Biol* 77:476–486
 95. Schaubert J, Gallo RL (2009) Antimicrobial peptides and the skin immune defense system. *J Allergy Clin Immunol* 124:R13–R18
 96. Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang YH, Homey B, Cao W, Wang YH, Su B, Nestle FO, Zai T, Mellman I, Schroder JM, Liu YJ, Gilliet M (2007) Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature* 449:564–569
 97. Crawford GH, Pelle MT, James WD (2004) Rosacea: I. Etiology, pathogenesis, and subtype classification. *J Am Acad Dermatol* 51:327–341 (quiz 342–324)
 98. Yamasaki K, Di Nardo A, Bardan A, Murakami M, Ohtake T, Coda A, Dorschner RA, Bonnart C, Descargues P, Hovnanian A, Morhenn VB, Gallo RL (2007) Increased serine protease activity and cathelicidin promotes skin inflammation in rosacea. *Nat Med* 13:975–980
 99. Koczulla R, von Degenfeld G, Kupatt C, Krotz F, Zahler S, Gloe T, Issbrucker K, Unterberger P, Zaiou M, Lebherz C, Karl A, Raake P, Pfosser A, Boekstegers P, Welsch U, Hiemstra PS, Vogelmeier C, Gallo RL, Clauss M, Bals R (2003) An angiogenic role for the human peptide antibiotic LL-37/hCAP-18. *J Clin Invest* 111:1665–1672
 100. Jansen T, Krug S, Kind P, Plewig G, Messer G (2004) BsmI polymorphism of the vitamin D receptor gene in patients with the fulminant course of rosacea conglobata (*Rosacea fulminans*). *J Dermatol* 31:244–246
 101. Degitz K, Placzek M, Borelli C, Plewig G (2007) Pathophysiology of acne. *J Dtsch Dermatol Ges* 5:316–323
 102. Toyoda M, Morohashi M (2001) Pathogenesis of acne. *Med Electron Microsc* 34:29–40
 103. Philpott MP (2003) Defensins and acne. *Mol Immunol* 40:457–462
 104. Oono T, Huh WK, Shirafuji Y, Akiyama H, Iwatsuki K (2003) Localization of human beta-defensin-2 and human neutrophil peptides in superficial folliculitis. *Br J Dermatol* 148:188–191
 105. Gambichler T, Skrygan M, Huyn J, Bechara FG, Sand M, Altmeyer P, Kreuter A (2006) Pattern of mRNA expression of beta-defensins in basal cell carcinoma. *BMC Cancer* 6:163
 106. Dorschner RA, Pestonjamas VK, Tamakuwala S, Ohtake T, Rudisill J, Nizet V, Agerberth B, Gudmundsson GH, Gallo RL (2001) Cutaneous injury induces the release of cathelicidin antimicrobial peptides active against group A *Streptococcus*. *J Invest Dermatol* 117:91–97
 107. Heilborn JD, Nilsson MF, Kratz G, Weber G, Sorensen O, Borregaard N, Stahle-Backdahl M (2003) 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* 120:379–389
 108. Frohm M, Gunne H, Bergman AC, Agerberth B, Bergman T, Boman A, Liden S, Jornvall H, Boman HG (1996) Biochemical and antibacterial analysis of human wound and blister fluid. *Eur J Biochem* 237:86–92
 109. Baroni A, Donnarumma G, Paoletti I, Longanesi-Cattani I, Bifulco K, Tufano MA, Carriero MV (2009) Antimicrobial human beta-defensin-2 stimulates migration, proliferation and tube formation of human umbilical vein endothelial cells. *Peptides* 30:267–272
 110. Gallo RL, Ono M, Povsic T, Page C, Eriksson E, Klagsbrun M, Bernfield M (1994) Syndecans, cell surface heparan sulfate proteoglycans, are induced by a proline-rich antimicrobial peptide from wounds. *Proc Natl Acad Sci USA* 91:11035–11039
 111. Tokumaru S, Sayama K, Shirakata Y, Komatsuzawa H, Ouhara K, Hanakawa Y, Yahata Y, Dai X, Tohyama M, Nagai H, Yang L, Higashiyama S, Yoshimura A, Sugai M, Hashimoto K (2005) Induction of keratinocyte migration via transactivation of the epidermal growth factor receptor by the antimicrobial peptide LL-37. *J Immunol* 175:4662–4668
 112. Yin J, Yu FS (2010) LL-37 promotes high glucose-attenuated epithelial wound healing via EGFR transactivation in organ cultured corneas. *Invest Ophthalmol Vis Sci* 51:1891–1897
 113. Carretero M, Escamez MJ, Garcia M, Duarte B, Holguin A, Retamosa L, Jorcano JL, Rio MD, Larcher F (2008) In vitro and in vivo wound healing-promoting activities of human cathelicidin LL-37. *J Invest Dermatol* 128:223–236
 114. Park HJ, Cho DH, Kim HJ, Lee JY, Cho BK, Bang SI, Song SY, Yamasaki K, Di Nardo A, Gallo RL (2009) Collagen synthesis is suppressed in dermal fibroblasts by the human antimicrobial peptide LL-37. *J Invest Dermatol* 129:843–850
 115. Hirsch T, Spielmann M, Zuhaili B, Fossum M, Metzger M, Koehler T, Steinau HU, Yao F, Onderdonk AB, Steintraesser L, Eriksson E (2009) Human beta-defensin-3 promotes wound healing in infected diabetic wounds. *J Gene Med* 11:220–228
 116. Hancock RE, Sahl HG (2006) Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nat Biotechnol* 24:1551–1557
 117. Steintraesser L, Kraneburg UM, Hirsch T, Kesting M, Steinau HU, Jacobsen F, Al-Benna S (2009) Host defense peptides as effector molecules of the innate immune response: a sledgehammer for drug resistance? *Int J Mol Sci* 10:3951–3970
 118. Liu L, Roberts AA, Ganz T (2003) By IL-1 signaling, monocyte-derived cells dramatically enhance the epidermal antimicrobial response to lipopolysaccharide. *J Immunol* 170:575–580
 119. Harder J, Bartels J, Christophers E, Schroder JM (2001) Isolation and characterization of human beta-defensin-3, a novel human inducible peptide antibiotic. *J Biol Chem* 276:5707–5713
 120. Cumberbatch M, Dearman RJ, Uribe-Luna S, Headon DR, Ward PP, Conneely OM, Kimber I (2000) Regulation of epidermal Langerhans cell migration by lactoferrin. *Immunology* 100:21–28
 121. Marchini G, Lindow S, Brismar H, Stabi B, Berggren V, Ulfgren AK, Lonne-Rahm S, Agerberth B, Gudmundsson GH (2002) The newborn infant is protected by an innate antimicrobial barrier: peptide antibiotics are present in the skin and vernix caseosa. *Br J Dermatol* 147:1127–1134
 122. Wiggins M, van Bergen BH, Hiemstra PS, Meis JF, van Vlijmen-Willems IM, Zeeuwen PL, Mulder J, Kramps HA, van Ruisen F, Schalkwijk J (1998) Induction of SLPI (ALP/HUSI-I) in epidermal keratinocytes. *J Invest Dermatol* 111:996–1002
 123. Simpson AJ, Maxwell AI, Govan JR, Haslett C, Sallenave JM (1999) Elafin (elastase-specific inhibitor) has anti-microbial activity against gram-positive and gram-negative respiratory pathogens. *FEBS Lett* 452:309–313
 124. Meyer-Hoffert U, Wichmann N, Schwichtenberg L, White PC, Wiedow O (2003) Supernatants of *Pseudomonas aeruginosa* induce the *Pseudomonas*-specific antibiotic elafin in human keratinocytes. *Exp Dermatol* 12:418–425
 125. Cutuli M, Cristiani S, Lipton JM, Catania A (2000) Antimicrobial effects of alpha-MSH peptides. *J Leukoc Biol* 67:233–239
 126. Schauer E, Trautinger F, Kock A, Schwarz A, Bhardwaj R, Simon M, Ansel JC, Schwarz T, Luger TA (1994) Proopiomelanocortin-derived peptides are synthesized and released by human keratinocytes. *J Clin Invest* 93:2258–2262
 127. Ali RS, Falconer A, Ikram M, Bissett CE, Cerio R, Quinn AG (2001) Expression of the peptide antibiotics human beta

- defensin-1 and human beta defensin-2 in normal human skin. *J Invest Dermatol* 117:106–111
128. Oono T, Matsuura H, Morizane S, Yamasaki O, Iwatsuki K (2006) A case of infectious eccrine hidradenitis. *J Dermatol* 33:142–145
129. Chronnell CM, Ghali LR, Ali RS, Quinn AG, Holland DB, Bull JJ, Cunliffe WJ, McKay IA, Philpott MP, Muller-Rover S (2001) Human beta defensin-1 and -2 expression in human pilosebaceous units: upregulation in acne vulgaris lesions. *J Invest Dermatol* 117:1120–1125
130. Harwig SS, Ganz T, Lehrer RI (1994) Neutrophil defensins: purification, characterization, and antimicrobial testing. *Methods Enzymol* 236:160–172
131. Caccavo D, Pellegrino NM, Altamura M, Rigon A, Amati L, Amoroso A, Jirillo E (2002) Antimicrobial and immunoregulatory functions of lactoferrin and its potential therapeutic application. *J Endotoxin Res* 8:403–417
132. Belaouaj A, Kim KS, Shapiro SD (2000) Degradation of outer membrane protein A in *Escherichia coli* killing by neutrophil elastase. *Science* 289:1185–1188
133. Tang YQ, Yeaman MR, Selsted ME (2002) Antimicrobial peptides from human platelets. *Infect Immun* 70:6524–6533
134. Stenger S, Hanson DA, Teitelbaum R, Dewan P, Niazi KR, Froelich CJ, Ganz T, Thoma-Uszynski S, Melian A, Bogdan C, Porcelli SA, Bloom BR, Krensky AM, Modlin RL (1998) An antimicrobial activity of cytolytic T cells mediated by granulysin. *Science* 282:121–125