See related Commentary on page xiv

Cutaneous Defense Mechanisms by Antimicrobial Peptides

Marissa H. Braff,* Antoanella Bardan,* Victor Nizet,† and Richard L. Gallo*†
*Department of Medicine, University of California San Diego, and VA San Diego Healthcare System, San Diego, California, USA; †Department of Pediatrics, University of California San Diego, San Diego, California, USA

The skin actively contributes to host defense by mounting an innate immune response that includes the production of antimicrobial peptides. These peptides, which include but are not limited to the cathelicidin and defensin gene families, provide rapid, broad-spectrum defense against infection by acting as natural antibiotics and by participating in host cell processes involved in immune defense. This review discusses the biology and clinical relevance of antimicrobial peptides expressed in the skin. The importance of the epithelial contribution to host immunity is evident, as alterations in antimicrobial peptide expression have been associated with various pathologic processes.

Key words: defensins/cathelicidins/streptococcus/staphylococcus/atopic/dermatitis/psoriasis
J Invest Dermatol 125:9–13, 2005

Antimicrobial peptides are predominantly small cationic polypeptides that are classified together due to their capacity to inhibit the growth of microbes. As effectors of innate immunity, antimicrobial peptides directly kill a broad spectrum of bacteria, fungi, and viruses. In addition, these peptides modify the local inflammatory response and activate mechanisms of cellular and adaptive immunity. Cathelicidins and defensins comprise the major families of antimicrobial peptides in the skin, although other cutaneous peptides, such as proteinase inhibitors, chemokines, and neuropeptides, also demonstrate antimicrobial activity. Together, these multifunctional antimicrobial peptides play an important role in skin immune defense and disease pathogenesis.

Antimicrobial Peptides in the Skin: Biological Relevance

Antimicrobial peptides, which are synthesized in the skin at sites of potential microbial entry, provide a soluble barrier that acts as an impediment to infection. In the case of infection or injury, antimicrobial peptide expression in the skin is upregulated due to increased synthesis by keratinocytes and deposition from degranulation of recruited neutrophils. Constitutive and inducible expression of human cathelicidin (hCAP18/LL-37), as well as human β-defensins 1, 2, and 3 (hBD-1, hBD-2, hBD-3), have been observed in epidermal keratinocytes (Frohm *et al*, 1997; Dorschner *et al*, 2001; Harder *et al*, 2001).

Although antimicrobial peptides clearly demonstrate *in vitro* antimicrobial activity, studies have shown that many such peptides, including cathelicidins and defensins, are inactivated by physiological salt concentrations (Goldman *et al*, 1997). It is important to note that most antimicrobial activity assays have been performed under non-physiological conditions, using bacterial growth media rather than a culture environment that closely resembles mammalian skin. In fact, a recent study has shown that mammalian skin contains an essential antimicrobial-enhancing factor that renders bacteria susceptible to cathelicidin *in vitro*, despite the presence of physiological salt and serum (Dorschner *et al*, 2004). The *in vivo* importance of antimicrobial peptides in the physiological environment is further highlighted by the laboratory animal models and human skin diseases that are discussed below.

Abbreviation: hBD, human β-defensin

Cathelicidins Cathelicidins are characterized by an N-terminal signal peptide, a highly conserved cathelin domain and a structurally variable cationic antimicrobial peptide at the C-terminus. The cathelin domain functions as both a protease inhibitor and as an antimicrobial peptide in humans (Zaiou *et al*, 2003). Mature cathelicidin peptides show rapid, potent, and broad-spectrum antimicrobial activity and have been implicated in various immunomodulatory functions (Koczulla *et al*, 2003). Humans and mice have only one cathelicidin gene, whereas other mammals, like pigs and cattle, possess a variety of cathelicidin genes.

Human cathelicidin, LL-37, assumes an α-helical structure in solutions with ion compositions similar to human plasma, interstitial fluid, or intracellular fluid. Processing of LL-37 from the cathelicidin precursor is essential for activation of its antimicrobial activity and is accomplished by neutrophil proteases such as proteinase 3 (Sorensen *et al*, 2001). LL-37 expression in squamous epithelia is differentially regulated in several inflammatory conditions (Frohm *et al*, 1997; Dorschner *et al*, 2001). LL-37 is produced in eccrine structures, where it is secreted and processed in sweat, suggesting a further barrier function against topical skin infection (Murakami *et al*, 2004). In addition, LL-37 is produced by mast cells and recruits mast cells (Di Nardo *et al*, 2003), thereby participating in innate immunity both by direct antimicrobial activity and by recruitment of cellular defenses. LL-37 production is upregulated in neonatal skin,

where it may compensate for the developmental immaturity of adaptive immune responses (Dorschner *et al*, 2003). A true immunomodulatory effector molecule, LL-37 has direct antimicrobial activity, acts synergistically with other antimicrobial peptides, functions as a chemoattractant for neutrophils, monocytes and T cells, and stimulates endothelial cell proliferation by binding to formyl peptide receptor-like 1 (FPRL-1) (Koczulla *et al*, 2003). As illustrated in Fig 1, the multilayered expression and multifunctionality of cathelicidin in the skin present a formidable innate defense system against infection.

Studies of animal models have provided direct evidence that cathelicidins are important components of host innate immune defense (Nizet *et al*, 2001). For example, mice harboring a disruption of their single cathelicidin gene are unable to control Group A *Streptococcus* infection (Nizet *et al*, 2001). Pig wounds inhibited from processing porcine cathelicidins to their active peptide form also show increased susceptibility to infection (Cole *et al*, 2001b).

Defensins Defensins contain six cysteine residues that form characteristic disulfide bridges. Disulfide bridge alignment and molecular structure separate this major antimicrobial peptide family into α-, β-, and θ-defensins. Distantly related peptides are found in insects and plants. Mammalian defensins exhibit antimicrobial activity against bacteria, fungi, and enveloped viruses.

α-Defensins contain three disulfide bridges in a 1–6, 2–4, 3–5 alignment. Human neutrophils express four α-defensins, which are also referred to as human neutrophil peptides 1 through 4 (HNP-1 to -4) (Harwig *et al*, 1994). Human defensins 5 and 6 (HD-5 and -6) are abundantly expressed as propeptides in Paneth cells of small intestinal crypts and in epithelial cells of the female urogenital tract. In humans, defensins are stored in azurophil granules of neutrophils as fully processed, mature peptides. Like cathelicidins, α-defensins affect both microbes and the host. For example, HNP-1, -2, and -3 upregulate tumor necrosis factor alpha (TNF-α) and IL-1 in human monocytes that have been activated by bacteria; these peptides also reduce the expression of the adhesion molecule VCAM-1 in endothelial cells activated by TNF-α (Chaly *et al*, 2000).

β-Defensins contain three disulfide bridges that are spaced in a 1–5, 2–4, 3–6 pattern. The four best-known human β-defensins, hBD-1 to -4, have been identified in various cell types, including epithelial and peripheral blood mononuclear cells (Harder *et al*, 2001; Fang *et al*, 2003; Liu *et al*, 2003). hBD-1 is constitutively expressed in epithelia, whereas hBD-2 is highly upregulated in inflamed skin. hBD-3, which was purified from human psoriatic scales and calluses (Harder *et al*, 2001), is inducible in a variety of tissues. Recent genomic analysis suggests that many β-defensins have yet to be discovered, although several have recently been identified through novel computational gene discovery strategies (Schutte *et al*, 2002).

β-Defensins have broad-spectrum antimicrobial activity and additional immune-related cellular functions. For example, hBD-2 binds to CCR6 and is chemotactic for immature dendritic cells and memory T cells (Yang *et al*, 1999). hBD-2 also promotes histamine release and prostaglandin D2 production in mast cells, suggesting a potential immunotherapeutic role as a vaccine adjuvant to enhance antibody production (Befus *et al*, 1999). hBD-2 is virtually absent in normal skin and its expression in human keratinocytes requires stimulation by cytokines or bacteria (Liu *et al*, 2003). The upregulation of hBD-2 by keratinocytes illustrates the important role that defensins play in host defense against cutaneous pathogens.

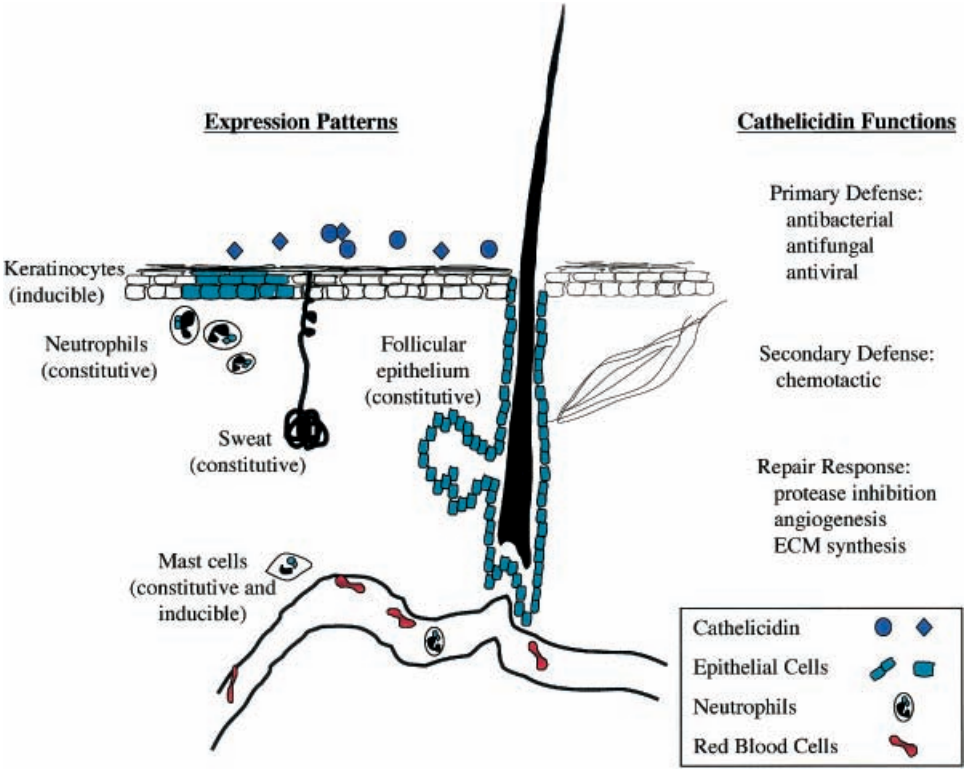

Figure 1
Cathelicidins are strategically expressed and contribute multiple functions to skin defense. The human cathelicidin precursor protein hCAP18 is expressed by several cell types in the skin including keratinocytes, neutrophils, eccrine ducts, and mast cells. Cathelicidins are processed to active peptides such as LL-37 in neutrophils and more potent peptides in sweat. These peptides have been best characterized as natural antibiotics, killing a variety of bacterial, fungal, and viral pathogens. Other functions include chemotactic and angiogenic behaviors, and an ability to modify fibroblast proteoglycan synthesis. The N-terminal cathelin-like domain of the hCAP18 precursor protein contains both antimicrobial and proteinase inhibitor activity.

Other antimicrobial peptides and proteins Whereas the cathelicidin and defensin families are best known for their antimicrobial properties, many peptides that have been ascribed alternate functions in the skin also demonstrate antimicrobial activity, including proteinase inhibitors, chemokines, and neuropeptides, to name a few (Table I). Interestingly, the dependence of the antimicrobial activity of these peptides on their originally described function varies, and no clear trend is observed. For example, the antimicrobial activity of ECP/RNase 3 does not require ribonuclease activity, which is essential for the antiviral activity of both ECP/RNase 3 and EDN/RNase 2 (Domachowske *et al*, 1998a, b). P-cystatin α inhibits bacterial proteinase activity as a mechanism of microbial growth inhibition (Takahashi *et al*, 1994), whereas cystatin C antimicrobial activity does not depend on its ability to inhibit bacterial proteinases (Blankenvoorde *et al*, 1998). The antiviral activity of cystatin C, however, appears to reside in the proteinase-binding domain. Calprotectin contains zinc-binding sites and inhibits microbial growth through competition for metals (Sohnle *et al*, 2000), whereas NGAL interferes with bacterial iron acquisition (Goetz *et al*, 2002). α-MSH contains a tripeptide that is important for antipyretic, anti-inflammatory and antimicrobial activity, while sequences involved in learning and memory are not necessary for antimicrobial activity (Cutuli

Table I. Mammalian peptides and proteins relevant to skin with antimicrobial activity (AMP)[a]

	References
AMP identified in resident cells	
Cathelicidins	Frohm *et al* (1997), Marchini *et al* (2002)
β-Defensins	Harder *et al* (2001), Liu *et al* (2003)
Bactericidal/permeability-increasing protein (BPI)	Takahashi *et al* (2004)
Lactoferrin	Cumberbatch *et al* (2000)
Lysozyme	Marchini *et al* (2002)
Dermcidin	Schittek *et al* (2001), Murakami *et al* (2004)
RNase 7	Harder and Schroder (2002)
AMP identified in infiltrating cells	
Cathelicidins	Gallo *et al* (1994), Marchini *et al* (2002)
α-Defensins	Harwig *et al* (1994)
Lactoferrin	Caccavo *et al* (2002)
Granulysin	Stenger *et al* (1998)
Perforin	Stenger *et al* (1998)
Eosinophil cationic protein (ECP)/RNase 3	Domachowske *et al* (1998a)
Eosinophil-derived neurotoxin (EDN)/RNase 2	Domachowske *et al* (1998b)
Regulated upon activation, normal T cells expressed and secreted (RANTES)	Tang *et al* (2002)
Platelet factor 4 (PF-4)	Tang *et al* (2002)
Connective tissue activating peptide 3 (CTAP-3)	Tang *et al* (2002)
Platelet basic protein	Tang *et al* (2002)
Thymosin β-4 (Tβ-4)	Tang *et al* (2002)
Fibrinopeptide B (FP-B)	Tang *et al* (2002)
Fibrinopeptide A (FP-A)	Tang *et al* (2002)
AMP identified as proteinase inhibitors	
hCAP18/LL-37 prosequence (cathelin-like domain)	Zaiou *et al* (2003)
Secretory leukocyte proteinase inhibitor (SLPI)/Antileukoprotease	Wingens *et al* (1998)
Elafin/skin-derived antileukoprotease (SKALP)	Simpson *et al* (1999), Meyer-Hoffert *et al* (2003)
P-cystatin α	Takahashi *et al* (1994)
Cystatin C	Blankenvoorde *et al* (1998)
AMP identified as chemokines	
Psoriasin	Glaser *et al* (2001)
Monokine induced by IFN-γ (MIG/CXCL9)	Cole *et al* (2001a)
IFN-γ-inducible protein of 10 kDa (IP-10/CXCL10)	Cole *et al* (2001a)
IFN-γ-inducible T cell α chemoattractant (I-TAC/CXCL11)	Cole *et al* (2001a)
Antimicrobial peptides identified as neuropeptides	
α-Melanocyte stimulating hormone (α-MSH)	Cutuli *et al* (2000)
Substance P	Kowalska *et al* (2002)
Bradykinin	Kowalska *et al* (2002)
Neurotensin	Kowalska *et al* (2002)
Vasostatin-1 and chromofungin (chromogranin A)	Tasiemski *et al* (2002)
Secretolytin (chromogranin B)	Tasiemski *et al* (2002)
Enkelytin and peptide B (proenkephalin A)	Tasiemski *et al* (2002)
Ubiquitin	Kieffer *et al* (2003)
Neuropeptide Y	Lambert *et al* (2002)
Polypeptide YY/skin-polypeptide YY	Lambert *et al* (2002)
Adrenomedullin	Allaker *et al* (1999)
AMP identified based on other functions	
Hemoglobin-derived peptides	Parish *et al* (2001)
Calprotectin (MRP8/MRP14)/calgranulin A/B	Sohnle *et al* (2000)
Neutrophil gelatinase-associated lipocalin (NGAL)	Goetz *et al* (2002)
Epidermal H1 histones	Kashima (1991)
Myeloperoxidase	Rosen and Michel (1997)

[a]References limited due to space restrictions.

et al, 2000). Lactoferrin antimicrobial activity does not depend on antitumor activity (Yang *et al*, 2004) and the antiproteinase and antimicrobial activity of elafin/SKALP reside in separate domains (Simpson *et al*, 1999). Chemotactic and antimicrobial activities are intertwined in MIG/CXCL9, IP-10/CXCL10, and I-TAC/CXCL11, which are structurally related to defensins in size, cationic charge, and disulfide bonding (Cole *et al*, 2001a). Similarly, the structure of substance P resembles that of Arg/Pro-rich bactericidal peptides, suggesting that substance P antimicrobial activity is linked to its ability to modulate the nervous and immune systems (Kowalska *et al*, 2002).

Antimicrobial Peptides in the Skin: Clinical Relevance

Differential expression of antimicrobial peptides appears to play a role in the susceptibility of patients with chronic inflammatory skin disorders to infectious complications. For example, LL-37 is induced in human keratinocytes during psoriasis, lupus erythematosus and contact dermatitis (Frohm *et al*, 1997). hBD-2 and hBD-3 are also upregulated in keratinocytes of inflamed psoriatic lesions (Harder *et al*, 2001; Nomura *et al*, 2003). The increased expression of antimicrobial peptides in psoriasis correlates with a low rate of secondary infection. In contrast, the expression of LL-37 and hBD-2 is not upregulated in individuals with atopic dermatitis, who are highly susceptible to bacterial and viral infections (Ong *et al*, 2002). The differences in antimicrobial peptide expression between these two disorders gain immunological relevance in light of the antimicrobial activity of LL-37 against *S. pyogenes* (Dorschner *et al*, 2001) and its synergistic activity with β-defensins against *S. aureus* (Ong *et al*, 2002), a leading agent of human skin infections.

LL-37 expression is upregulated in inflammatory skin lesions of erythema toxicum neonatorum and immunolocalizes within CD15-expressing neutrophils, EG-2-expressing eosinophils, and CD1a-expressing dendritic cells (Marchini *et al*, 2002). LL-37 is also induced within the epidermis during development of verruca vulgaris and condyloma accuminata, suggesting that it represents a component of the innate immune response to papillomavirus infection (Conner *et al*, 2002). Both hBD-1 and hBD-2 are upregulated in the lesions of acne vulgaris and may therefore be involved in the pathogenesis or resolution of this condition (Philpott, 2003). In addition, hBD-2 and the HNP are abundant in lesions of superficial folliculitis, a common skin disease characterized by inflammation of the hair follicle and infection with *S. aureus* (Oono *et al*, 2003). These studies indicate potential roles for antimicrobial peptides in host immune defense against skin infection.

Cathelicidin is produced at high levels in the skin after wounding (Dorschner *et al*, 2001) and is strongly expressed in healing skin epithelium (Heilborn *et al*, 2003). After cutaneous wounding, growth factors stimulate tissue regeneration until the physical barrier protecting the skin from microbial infections has been re-established. Growth factors important in skin wound healing, such as insulin-like growth factor-1 (IGF-1) and transforming growth factor-α (TGF-α, induce the expression of cathelicidins and defensins in human keratinocytes (Sorensen *et al*, 2003). LL-37 antibodies inhibit post-wounding re-epithelialization in a concentration-dependent manner and cathelicidin expression is low or absent in chronic ulcers (Heilborn *et al*, 2003). The ability of LL-37 to induce angiogenesis further highlights the importance of cathelicidin in wound healing and tissue repair (Koczulla *et al*, 2003). In addition, the expression of hBD-2 is dramatically decreased in burn wounds and blister fluid from partial thickness burns (Ortega *et al*, 2000), providing evidence that innate immune defects may contribute to the risk of burn wound infection and sepsis.

Conclusion

Skin innate immune defense function is greatly enhanced by a soluble antimicrobial peptide barrier that is activated when physical barriers fail to prevent microbial entry. Even under resting conditions, low levels of antimicrobial peptides are synthesized at sites of potential microbial entry into the skin and provide a further impediment to infection. After injury, antimicrobial peptide levels in the skin rise rapidly due to increased synthesis by keratinocytes and deposition from degranulation of recruited neutrophils. The chemoattractant properties of cathelicidins and defensins may further amplify this process through their functional interactions with leukocyte surface receptors. The growing number of multifunctional peptides found to inhibit microbial growth further expands the mammalian antimicrobial arsenal, demonstrating that the host antimicrobial peptide defense system acts both directly and indirectly to prevent cutaneous infection.

DOI: 10.1111/j.0022-202X.2004.23587.x

Manuscript received April 12, 2004; revised July 1, 2004; accepted for publication July 20, 2004

Address correspondence to: Richard L. Gallo, MD, PhD, Mail Code 111B, University of California San Diego, San Diego, CA 92161, USA. Email: rgallo@vapop.ucsd.edu

References

Allaker RP, Zihni C, Kapas S: An investigation into the antimicrobial effects of adrenomedullin on members of the skin, oral, respiratory tract and gut microflora. FEMS Immunol Med Microbiol 23:289–293, 1999

Befus AD, Mowat C, Gilchrist M, Hu J, Solomon S, Bateman A: Neutrophil defensins induce histamine secretion from mast cells: Mechanisms of action. J Immunol 163:947–953, 1999

Blankenvoorde MF, van't Hof W, Walgreen-Weterings E, van Steenbergen TJ, Brand HS, Veerman EC, Nieuw Amerongen AV: Cystatin and cystatin-derived peptides have antibacterial activity against the pathogen *Porphyromonas gingivalis*. Biol Chem 379:1371–1375, 1998

Caccavo D, Pellegrino NM, Altamura M, Rigon A, Amati L, Amoroso A, Jirillo E: Antimicrobial and immunoregulatory functions of lactoferrin and its potential therapeutic application. J Endotoxin Res 8:403–417, 2002

Chaly YV, Paleolog EM, Kolesnikova TS, Tikhonov II, Petratchenko EV, Voitenok NN: Neutrophil alpha-defensin human neutrophil peptide modulates cytokine production in human monocytes and adhesion molecule expression in endothelial cells. Eur Cytokine Netw 11:257–266, 2000

Cole AM, Ganz T, Liese AM, Burdick MD, Liu L, Strieter RM: Cutting edge: IFN-inducible ELR- CXC chemokines display defensin-like antimicrobial activity. J Immunol 167:623–627, 2001a

Cole AM, Shi J, Ceccarelli A, Kim YH, Park A, Ganz T: Inhibition of neutrophil elastase prevents cathelicidin activation and impairs clearance of bacteria from wounds. Blood 97:297–304, 2001b

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 47:347–350, 2002

Cumberbatch M, Dearman RJ, Uribe-Luna S, Headon DR, Ward PP, Conneely OM, Kimber I: Regulation of epidermal Langerhans cell migration by lactoferrin. Immunology 100:21–28, 2000

Cutuli M, Cristiani S, Lipton JM, Catania A: Antimicrobial effects of alpha-MSH peptides. J Leukoc Biol 67:233–239, 2000

Di Nardo A, Vitiello A, Gallo RL: Cutting edge: Mast cell antimicrobial activity is mediated by expression of cathelicidin antimicrobial peptide. J Immunol 170:2274–2278, 2003

Domachowske JB, Dyer KD, Adams AG, Leto TL, Rosenberg HF: Eosinophil cationic protein/RNase 3 is another RNase A-family ribonuclease with direct antiviral activity. Nucleic Acids Res 26:3358–3363, 1998a

Domachowske JB, Dyer KD, Bonville CA, Rosenberg HF: Recombinant human eosinophil-derived neurotoxin/RNase 2 functions as an effective antiviral agent against respiratory syncytial virus. J Infect Dis 177:1458–1464, 1998b

Dorschner RA, Lin KH, Murakami M, Gallo RL: 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, 2003

Dorschner RA, Lopez-Garcia B, Gallo RL: Skin microenvironment dictates susceptibility of bacteria to antimicrobial peptides. J Invest Dermatol 122: A133, 2004

Dorschner RA, Pestonjamasp VK, Tamakuwala S, *et al*: Cutaneous injury induces the release of cathelicidin anti-microbial peptides active against group A Streptococcus. J Invest Dermatol 117:91–97, 2001

Fang XM, Shu Q, Chen QX, Book M, Sahl HG, Hoeft A, Stuber F: Differential expression of alpha- and beta-defensins in human peripheral blood. Eur J Clin Invest 33:82–87, 2003

Frohm M, Agerberth B, Ahangari G, Stahle-Backdahl M, Liden S, Wigzell H, Gudmundsson GH: 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, 1997

Gallo RL, Ono M, Povsic T, Page C, Eriksson E, Klagsbrun M, Bernfield M: Syndecans, cell surface heparan sulfate proteoglycans, are induced by a proline-rich antimicrobial peptide from wounds. Proc Natl Acad Sci USA 91:11035–11039, 1994

Glaser R, Harder J, Bartels J, Christophers E, Schroder J-M: Psoriasin (S100a7) is a major and potent *E. coli*-selective antimicrobial protein of healthy human skin. J Invest Dermatol 117:768, 2001

Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK: The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. Mol Cell 10:1033–1043, 2002

Goldman MJ, Anderson GM, Stolzenberg ED, Kari UP, Zasloff M, Wilson JM: Human beta-defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis. Cell 88:553–560, 1997

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 276:5707–5713, 2001

Harder J, Schroder JM: RNase 7, a novel innate immune defense antimicrobial protein of healthy human skin. J Biol Chem 277:46779–46784, 2002

Harwig SS, Ganz T, Lehrer RI: Neutrophil defensins: Purification, characterization, and antimicrobial testing. Methods Enzymol 236:160–172, 1994

Heilborn JD, Nilsson MF, Kratz G, Weber G, Sorensen O, Borregaard N, Stahle-Backdahl M: The cathelicidin anti-microbial 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, 2003

Kashima M: H1 histones contribute to candidacidal activities of human epidermal extract. J Dermatol 18:695–706, 1991

Kieffer AE, Goumon Y, Ruh O, *et al*: The N- and C-terminal fragments of ubiquitin are important for the antimicrobial activities. Faseb J 17:776–778, 2003

Koczulla R, von Degenfeld G, Kupatt C, *et al*: An angiogenic role for the human peptide antibiotic LL-37/hCAP-18. J Clin Invest 111:1665–1672, 2003

Kowalska K, Carr DB, Lipkowski AW: Direct antimicrobial properties of substance P. Life Sci 71:747–750, 2002

Lambert RW, Campton K, Ding W, Ozawa H, Granstein RD: Langerhans cell expression of neuropeptide Y and peptide YY. Neuropeptides 36:246–251, 2002

Liu L, Roberts AA, Ganz T: By IL-1 signaling, monocyte-derived cells dramatically enhance the epidermal antimicrobial response to lipopolysaccharide. J Immunol 170:575–580, 2003

Marchini G, Lindow S, Brismar H, *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 147:1127–1134, 2002

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 12:418–425, 2003

Murakami M, Lopez-Garcia B, Braff M, Dorschner RA, Gallo RL: Postsecretory processing generates multiple cathelicidins for enhanced topical antimicrobial defense. J Immunol 172:3070–3077, 2004

Nizet V, Ohtake T, Lauth X, *et al*: Innate antimicrobial peptide protects the skin from invasive bacterial infection. Nature 414:454–457, 2001

Nomura I, Gao B, Boguniewicz M, Darst MA, Travers JB, Leung DY: Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. J Allergy Clin Immunol 112: 1195–1202, 2003

Ong PY, Ohtake T, Brandt C, *et al*: Endogenous antimicrobial peptides and skin infections in atopic dermatitis. N Engl J Med 347:1151–1160, 2002

Oono T, Huh WK, Shirafuji Y, Akiyama H, Iwatsuki K: Localization of human beta-defensin-2 and human neutrophil peptides in superficial folliculitis. Br J Dermatol 148:188–191, 2003

Ortega MR, Ganz T, Milner SM: Human beta defensin is absent in burn blister fluid. Burns 26:724–726, 2000

Parish CA, Jiang H, Tokiwa Y, *et al*: Broad-spectrum antimicrobial activity of hemoglobin. Bioorg Med Chem 9:377–382, 2001

Philpott MP: Defensins and acne. Mol Immunol 40:457–462, 2003

Rosen H, Michel BR: Redundant contribution of myeloperoxidase-dependent systems to neutrophil-mediated killing of *Escherichia coli*. Infect Immun 65:4173–4178, 1997

Schittek B, Hipfel R, Sauer B, *et al*: Dermcidin: A novel human antibiotic peptide secreted by sweat glands. Nat Immunol 2:1133–1137, 2001

Schutte BC, Mitros JP, Bartlett JA, *et al*: Discovery of five conserved beta-defensin gene clusters using a computational search strategy. Proc Natl Acad Sci USA 99:2129–2133, 2002

Simpson AJ, Maxwell AI, Govan JR, Haslett C, Sallenave JM: Elafin (elastase-specific inhibitor) has anti-microbial activity against gram-positive and gram-negative respiratory pathogens. FEBS Lett 452:309–313, 1999

Sohnle PG, Hunter MJ, Hahn B, Chazin WJ: Zinc-reversible antimicrobial activity of recombinant calprotectin (migration inhibitory factor-related proteins 8 and 14). J Infect Dis 182:1272–1275, 2000

Sorensen OE, Cowland JB, Theilgaard-Monch K, Liu L, Ganz T, Borregaard N: Wound healing and expression of antimicrobial peptides/polypeptides in human keratinocytes, a consequence of common growth factors. J Immunol 170:5583–5589, 2003

Sorensen OE, Follin P, Johnsen AH, Calafat J, Tjabringa GS, Hiemstra PS, Borregaard N: Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. Blood 97:3951–3959, 2001

Stenger S, Hanson DA, Teitelbaum R, *et al*: An antimicrobial activity of cytolytic T cells mediated by granulysin. Science 282:121–125, 1998

Takahashi M, Horiuchi Y, Tezuka T: Presence of bactericidal/permeability-increasing protein in human and rat skin. Exp Dermatol 13:55–60, 2004

Takahashi M, Tezuka T, Katunuma N: Inhibition of growth and cysteine proteinase activity of Staphylococcus aureus V8 by phosphorylated cystatin alpha in skin cornified envelope. FEBS Lett 355:275–278, 1994

Tang YQ, Yeaman MR, Selsted ME: Antimicrobial peptides from human platelets. Infect Immun 70:6524–6533, 2002

Tasiemski A, Hammad H, Vandenbulcke F, Breton C, Bilfinger TJ, Pestel J, Salzet M: Presence of chromogranin-derived antimicrobial peptides in plasma during coronary artery bypass surgery and evidence of an immune origin of these peptides. Blood 100:553–559, 2002

Wingens M, van Bergen BH, Hiemstra PS, *et al*: Induction of SLPI (ALP/HUSI-I) in epidermal keratinocytes. J Invest Dermatol 111:996–1002, 1998

Yang D, Chertov O, Bykovskaia SN, *et al*: Beta-defensins: Linking innate and adaptive immunity through dendritic and T cell CCR6. Science 286: 525–528, 1999

Yang N, Strom MB, Mekonnen SM, Svendsen JS, Rekdal O: The effects of shortening lactoferrin derived peptides against tumour cells, bacteria and normal human cells. J Pept Sci 10:37–46, 2004

Zaiou M, Nizet V, Gallo RL: Antimicrobial and protease inhibitory functions of the human cathelicidin (hCAP18/LL-37) prosequence. J Invest Dermatol 120:810–816, 2003