Endogenous Production of Antimicrobial Peptides in Innate Immunity and Human Disease

Richard L. Gallo, MD, PhD, and Victor Nizet, MD

Address
Departments of Medicine and Pediatrics, Division of Dermatology, University of California San Diego, and VA San Diego Healthcare System, San Diego, CA, USA.
E-mail: rgallo@vapop.ucsd.edu

Current Allergy and Asthma Reports 2003, **3:**402–409
Current Science Inc. ISSN 1529-7322

Antimicrobial peptides are diverse and evolutionarily ancient molecules produced by all living organisms. Peptides belonging to the cathelicidin and defensin gene families exhibit an immune strategy as they defend against infection by inhibiting microbial survival, and modify hosts through triggering tissue-specific defense and repair events. A variety of processes have evolved in microbes to evade the action of antimicrobial peptides, including the ability to degrade or inactivate antimicrobial peptides, or suppress host production of the peptide in response to infection. Animal models and clinical investigations have shown that an absence of cathelicidin or defensin antimicrobials can lead to disease. In this article, we review important recent advances in understanding the biology of antimicrobial peptides and their role in normal immunity and human disease.

Introduction

Endogenous antimicrobial peptides (AMPs) are small amino acid–based molecules that have the capacity to kill microbes and are produced by the host organism. The existence of such molecules has been known for decades, but, recently, interest in the AMPs has increased from the perspective of both the basic scientist attempting to understand immunity, and the clinician attempting to better diagnose and treat disease. Animal models, and unique human populations, have revealed that AMPs constitute an important first-line defense in the mammalian immune system. In this review, we discuss essential aspects of the biology of AMPs; experimental models that have revealed their significance; and recent observations that suggest that defects in the expression or function of specific AMPs might explain fundamental aspects of the pathophysiology of human diseases as diverse as atopic dermatitis, cystic fibrosis, *Shigella*-mediated dysentery, and dental decay.

The definition of an AMP has been loosely applied to any peptide with the capacity to inhibit the growth of microbes. AMPs might exhibit potent killing or inhibition of a broad range of microorganisms, including gram-positive and -negative bacteria as well as fungi and certain viruses. More than 800 such AMPs have been described, and an updated list can be found at the website: http://www.bbcm.units.it/~tossi/pag1.htm. Some of these AMPs have now been demonstrated to protect diverse organisms, including plants, insects, and mammals, against infection. AMP sequence analysis has shown that the peptide immune system is evolutionarily ancient, and the conservation of AMP gene families throughout the animal kingdom further supports their biologic significance.

Antimicrobial peptides function within the conceptual framework of the innate immune system. The term "innate immunity" describes defense events that do not require prior exposure to the pathogen for recognition of danger from infection. Lower organisms, including plants and insects, rely almost exclusively on innate defense strategies for protection. In mammals, studies of the relative contribution of innate immunity to overall immune surveillance have focused in large part on cell-mediated events, such as phagocytosis by neutrophils and macrophages. Following internalization, compartmentalized production of reactive oxygen intermediates and proteases leads to microbial killing. However, an essential element to the concept of innate immunity is immediate responsiveness to danger. Recruitment of neutrophils and macrophages takes time, usually hours, whereas pathogenic bacteria might replicate rapidly in vivo. AMPs have emerged as key elements for inhibition of microbial proliferation prior to recruitment of cellular immune defense elements.

Cathelicidins and Defensins: Two Broad Groups of Antimicrobial Peptides

The diverse AMP gene families have little in common aside from the techniques used for their discovery and the observation that they can kill microbes. Most of the peptides are highly cationic and have a structure that organizes charged residues separately from hydrophobic residues. This structural characteristic is thought to be critical to the ability of the peptide to interact with the microbial membrane and

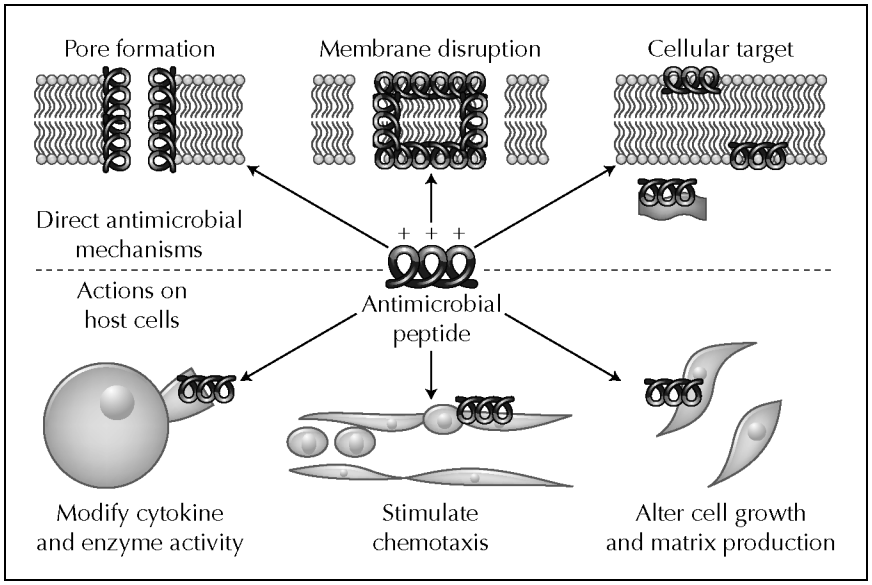

Figure 1. Diverse biologic activities of mammalian antimicrobial peptides contributing to innate immune defense.

initiate antimicrobial effects. Although many AMPs might prove to be important in immune defense, to date members of the cathelicidin and defensin gene families have been studied most extensively in mammals. Selected aspects of the biochemistry of these AMPs are described to show how their function can affect human disease.

Cathelicidins

Cathelicidins were so named because they all contain a conserved "cathelin" precursor domain [1]. They are organized as an N-terminal signal peptide, a highly conserved prosequence, and a structurally variable cationic peptide at the C-terminus. The prosequence resembles cathelin, a protein originally isolated from porcine neutrophils as an inhibitor of cathepsin L (hence, the name cathelin). The conservation of the cathelin domain is striking between species, indicating that various members of the family evolved from the duplication of a common ancestor gene.

In humans and in mice, the cathelicidin family is limited to a single gene product, whereas in domesticated farm animals, such as pigs, cattle, and sheep, there are multiple cathelicidin genes. They are all similar in the precursor domain but have very different C-terminal peptides. The C-terminal peptides derived from cathelicidins were among the earliest mammalian AMPs to show potent, rapid, and broad-spectrum antimicrobial activity. The stored cathelicidin is present as a larger pre-proprotein then enzymatically processed for activation of the two parts [2]. The processed C-terminal peptides have been assigned a variety of other names based on their unique sequence.

The human cathelicidin, LL-37 / hCAP18, was cloned from RNA isolated from human bone marrow [3]. The mature AMP is referred to as LL-37 because it begins with two leucine residues and is 37 amino acids long. hCAP18 was independently named to be recognized as a cationic AMP whose mass before proteolytic processing is approximately 18 kDa [4]. LL-37 / hCAP18 has a hydrophobic N-terminal domain in a α-helical conformation, most pronounced in the presence of negatively charged lipids. Processing of LL-37 from the C-terminus of the cathelicidin precursor occurs through the actions of enzymes such as neutrophil elastase and proteinase 3, and is essential for activation of this AMP [5,6].

Both halves of the processed human cathelicidin are active. The cathelin domain can function as both an antimicrobial protein and a protease inhibitor [7,8]. The peptide domain (LL-37) has a different spectrum of antimicrobial activity from the cathelin domain, works well in synergy with other AMPs, and has direct effects to activate host cells. The ability of cathelicidins to both kill bacteria and modify the host immune response reflects a fundamental property of many AMPs. As illustrated in Figure 1, an AMP can kill microbes by various mechanisms, depending on the structure of the peptide and the nature of the microbial target. In addition, the peptide can influence the host immune response through a variety of eukaryotic cell interactions. For example, LL-37 acts as a chemoattractant by binding to the formyl-peptide-receptor-like-1 (FPRL-1) [9••]. LL-37 can recruit mast cells [10], then be produced by the mast cell to kill bacteria [11]. LL-37 participates in innate immunity both by direct antimicrobial activity and by recruitment of cellular defenses; such multifunctionality is a repeating theme of the AMP field.

Nonhuman cathelicidins deserve special attention because of their usefulness in modeling human cathelicidin function and their potential for use in novel drug design. In mice, the sole cathelicidin peptide is named CRAMP, for cathelin-related antimicrobial peptide [12]. Like LL-37, CRAMP is abundantly expressed in bone marrow and neutrophils and forms a similar structure in the lipid phase. As discussed later, studies of CRAMP in the murine model have provided direct evidence of the importance of cathelicidins in immune defense. Pigs have many cathelicidins, including protegrins, PR-39, prophenins, and porcine myeloid AMPs.

Protegrins (PG-1 to PG-5) are highly cationic and contain two intrachain disulfide bonds and an amidated C-terminus. PG-1 is active against gram-positive and -negative bacteria in vitro and in vivo. PR-39 is a proline- and arginine-rich peptide, with broad-spectrum antibacterial properties, that was originally purified from porcine intestine [13]. PR-39 was one of the first antimicrobial peptides that demonstrated multiple functions in mammals [14]. PR-39 induces proteoglycan synthesis, which is critical for wound repair; exhibits chemoattractant activity for leukocytes; and exerts immunomodulatory (anti-inflammatory) actions in vivo.

Defensins

Defensins are a widely dispersed family of AMPs. These cationic peptides contain six to eight cysteine residues that form characteristic disulfide bridges. They are found in mammals, and distantly related defensins appear in insects and plants. The alignment of disulfide bridges and the molecular structure classifies mammalian defensins into three distinct sub-families: α-defensins, β-defensins, and θ-defensins; the latter group is absent in humans.

α-Defensins have three disulfide bridges in a 1-6, 2-4, 3-5 alignment. Human neutrophils express four distinct α-defensins (α-defensin-1 to -4) also referred to as human neutrophil peptides (HNP-1 to -4). They are stored in azurophilic granules of neutrophils as fully processed (~ 3 kDa), mature peptides. Two additional human defensins (HD-5 and -6) are expressed in Paneth cells of the small intestinal crypts [15] and in epithelial cells of the female urogenital tract. Homologous peptides were also found in Paneth cells of the mouse small intestine granulocytes and referred to as "cryptdins" (crypt defensins) [16]. Paneth cells store α-defensins as propeptides. Like cathelicidins, α-defensins exert action on both microbes and the host. For example, HNP 1–3 have been shown to increase the expression of tumor necrosis factor (TNF)-α and interleukin (IL)-1 in human monocytes that have been activated by bacteria (*Staphylococcus aureus*), or reduce expression of the vascular adhesion molecule-1 (VCAM-1) in human umbilical vein endothelial cells activated by TNF-α [17].

The β-Defensins have six cysteine motifs connected by three disulfide bridges but spaced differently from those in α-defensins. The disulfide bonds are C1-C5, C2-C4, and C3-C6. β-Defensins have been identified from many cell types, including epithelial cells and neutrophils. Four types are now known in humans and named human beta defensin (HBD)-1 to -4. It is likely that more will be found in humans based on a recent genomic analysis that suggests many β-defensins genes have yet to be discovered [18]. β-Defensins have a broad spectrum of antimicrobial activity under optimal culture conditions and additional immune-related cellular functions. For example, HBD-2 binds to chemokine receptor CCR6, is chemotactic for immature dendritic cells and memory T-cells [19], and promotes histamine release and prostaglandin D2 production in mast cells [20]. These studies suggest that a role for β-defensin in allergic reactions might indicate that AMPs could serve as vaccine adjuvants to enhance antibody production.

Experimental Evidence that Antimicrobial Peptides Influence Infectious Disease Outcome

An expanding body of experimental research points to an important role for AMPs in mammalian defense against microbial pathogens. These studies have been done in a variety of infectious disease model systems, and can be grouped into three complementary lines of investigation: 1) correlations between the in vitro sensitivity or resistance of a microbe to AMPs and its pathogenic potential; 2) demonstrations of the therapeutic potential of AMP administration against microbial colonization or disease; and 3) evidence that blocking AMP production increases immune susceptibility of the host to infection. Data collected from each of these approaches are summarized in this section.

Several pathogenic bacteria exhibit intrinsic resistance to mammalian AMPs, and an array of phenotypic strategies to survive AMP killing has been characterized (Fig. 2). One common pattern of antimicrobial resistance involves bacterial modification of normally anionic cell-wall constituents with cationic molecules; the net effect of these substitutions is to decrease the attraction of positively charged AMPs to the bacterial surface. Cell-wall charge alterations associated with AMP resistance in gram-negative bacteria include covalent modification of the lipopolysaccharide (LPS) lipid A by acylation or aminoarabinose [21,22] or the expression of LPS-associated phosphatidylcholine [23]. AMP resistance in the gram-positive pathogen *S. aureus* is mediated by incorporation of D-alanine within lipotechoic acid or L-lysine within phosphatidylglycerol [24,25•]. *Salmonella typhimurium* and *Neisseria gonorrhoeae* utilize efflux pumps to expel AMPs, avoiding accumulation of bactericidal levels [26,27]. *Salmonella enterica*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Proteus mirabilis* produce proteinases that are capable of inactivating AMPs, either through direct degradation or by triggering release of neutralizing sulfated polysaccharides from host proteoglycans [28–31]. Going one step further and targeting the source, *Shigella* spp. evade AMP killing by downregulating epithelial cell production of defensin and cathelicidin [32••].

Isogenic bacterial mutants have been generated that exhibit increased AMP sensitivity in vitro compared with the wild-type parent strain. These mutants have been studied in animal models to show that AMP resistance itself contributes to virulence potential. Dlt- and MprF- mutants of *S. aureus* lack D-alanine and L-lysine cell wall modifications, respectively, and are more sensitive to AMP activity in vitro. Both types of mutants are more readily phagocytosed by neutrophils and less able to establish systemic infection in mice. *S. enterica* mutants in PmrA lack the aminoarabinose modification of LPS associated with AMP resistance and are less virulent in a murine enteric infection model [28]. The PhoP-PhoQ regulatory system controls multiple virulence

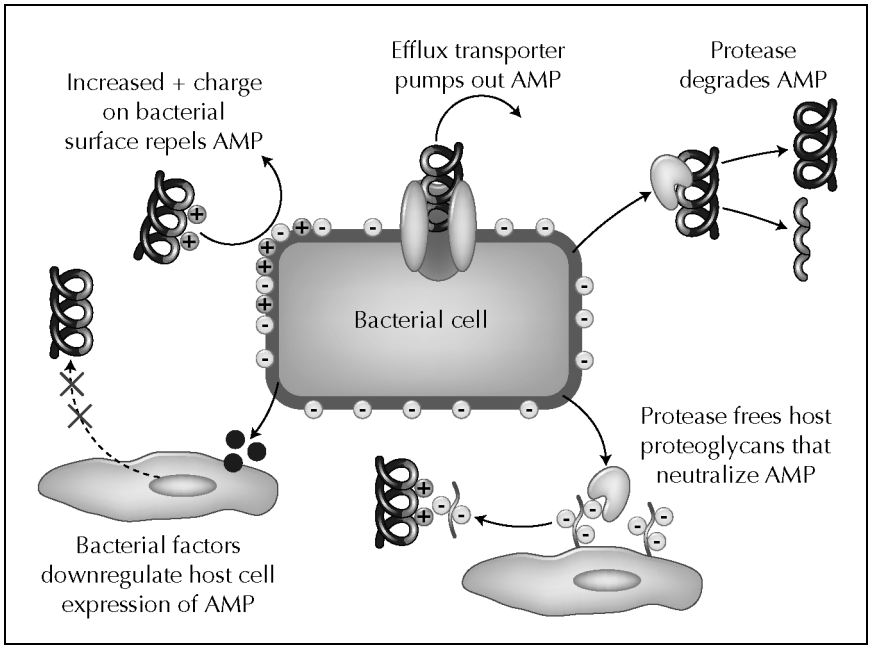

Figure 2. Mechanisms by which pathogenic microorganisms might resist antimicrobial peptide killing. AMP—antimicrobial peptide.

phenotypes in *Salmonella* spp, including a K^+-coupled AMP efflux pump and the AMP protease, PgtE [26]. *S. typhimurium* mutants in the PhoP-PhoQ regulatory system are more sensitive to AMP killing and significantly less virulent in murine enteric infections. Finally, a study of *Streptococcus pyogenes* pathogenicity approached the issue from the opposite perspective. This bacterium is normally sensitive to AMP killing, but resistant mutants can be selected by serial exposure to AMP in vitro. Upon subcutaneous inoculation of mice, the AMP-resistant *S. pyogenes* mutant was found to induce more severe necrotizing skin infections than the wild-type parent strain [33••].

Additional experimental evidence indicates that augmentation of AMP levels on host mucosal surfaces can retard bacterial colonization and enhance resistance to infection. In these studies, increased AMP levels are achieved through gene therapy or by exogenous administration of purified or recombinant peptide. For example, the airway surface fluid of cystic fibrosis xenografts failed to kill *P. aeruginosa* or *S. aureus*; however, exposure of the xenografts to an adenovirus expressing human cathelicidin restored bacterial killing to normal levels [34]. Mice treated intratracheally with this adenoviral–AMP vector had a lower bacterial load and diminished inflammatory response after *P. aeruginosa* challenge. Gene therapy with a retroviral vector carrying human β-defensin-2 enhanced the ability of several mouse and human cell lines to kill *Escherichia coli*. In lambs with pneumonia, intratracheal administration of cathelicidin AMP reduced the concentration of bacteria in pulmonary tissues and fluid [35]. Topical administrations of a gel containing a cationic AMP has also been shown to decrease *Trichomonas vaginalis* colonization of vaginal epithelium in mice [36], block *P. aeruginosa* superinfection of experimental burns in rats [37], and inhibit development of wound infections in pigs [38].

The overexpression or supplementation studies described earlier do not prove that the endogenous level of AMP production by a mammalian host is sufficient to establish effective innate immunity against bacterial pathogens. The application of knockout-mouse technology to the field of AMP research is beginning to provide such evidence. A mouse deleted for the *Cnlp* gene encoding the only mouse cathelicidin CRAMP exhibits normal growth and development and produces normal numbers of circulating leukocytes [33]. However, blood and isolated mast cells from $Cnlp^{-/-}$ mice are notably deficient in killing the pathogen *S. pyogenes*, and knockout mice challenged with this bacterium develop much more severe and persistent necrotizing skin infections than their wild-type littermates [11,33••]. Therefore, cathelicidin, which is induced adaptively in the skin following cutaneous injury or *S. pyogenes* infection [39], appears to serve an important innate defense function. Defb1, a murine homologue to human β-defensins, is expressed on respiratory epithelium. A Defb1-/- mouse showed delayed clearance of *Haemophilus influenzae* from the lung following nebulization, but normal clearance of *S. aureus* administered in a similar fashion [40,41].

Additional data linking endogenous AMP peptide production to innate immune defense come from studies in which specific host pathways for AMP activation or inactivation are manipulated. For example, a specific inhibitor of neutrophil elastase was used to block the proteolytic activation of protegrin AMPs in porcine skin. Administration of this inhibitor significantly decreased the ability of pigs to clear bacteria from skin wounds, and subsequent treatment with the calculated amount of intact protegrin normalized in vivo antimicrobial activity [42•]. Shedding of heparan sulfate cell-surface proteoglycans can interfere with AMP function. The pathogen *P. aeruginosa*, through its virulence factor

Table 1. Alerations of antimicrobial peptide activity in human disease conditions

Increased production (due to infection)	
Sinusitis	β-defensin
Pyelonephritis	α-defensin
Empyema	β-defensin
Neonatal pneumonia	Cathelicidin, β-defensin
Staphylococcus aureus colonization	β-defensin
Mycobacterium avium pneumonia	α-defensin, β-defensin
Bacterial vaginosis	β-defensin
Condyloma accuminata	Cathelicidin
Verruca vulgaris (common warts)	Cathelicidin
Pelvic inflammatory disease	β-defensin
Helicobacter pylori gastritis	β-defensin
HIV infection (nonprogressors)	α-defensin
Increased production (due to injury)	
Acute respiratory distress syndrome (ARDS)	α-defensins
Acute skin wounds	Cathelicidin
Increased production (idiopathic)	
Psoriasis	Cathelicidin, β-defensin
Lichen planus	β-defensin
Sarcoidosis	Cathelicidin
Idiopathic pulmonary fibrosis	α-defensins
Diffuse panbronchiolitis	α-defensins
Decreased production	
Atopic dermatitis	Cathelicidin, β-defensin
Burn wounds	β-defensin
Kostmann syndrome	Cathelicidin
Shigella dysentery	Cathelicidin
Chronic skin ulcers	Cathelicidin
Impaired function	
Cystic fibrosis	Cathelicidin, β-defensin

LasA, enhances in vitro shedding of the proteoglycan syndecan-1 [29]. Newborn mice deficient in syndecan-1 resist *P. aeruginosa* lung infection but become susceptible when treated with syndecan-1 derived heparan sulfate chains [43].

Antimicrobial Peptide Production and Function in Human Clinical Disease

In the previous section, we review mounting experimental evidence that points to a prominent role for AMPs in mammalian innate immunity. Appreciation of this research has sparked considerable interest for exploring the importance of AMPs in influencing the outcome of human disease. AMP production is increased adaptively in response to a number of specific infections and in response to acute damage to the epithelial barrier (Table 1). Conversely, increased production of these molecules is also associated with certain chronic inflammatory disorders, reflecting the dual functions of several AMPs in immune activation. Investigators are also asking whether decreased AMP production or interference with AMP function might be evident in primary or acquired immunodeficiencies, or explain the increased infectious risks associated with certain injuries (*eg,* burns) or chronic diseases (*eg,* cystic fibrosis). Our emerging knowledge base linking alterations in AMP activity to human disease conditions is reviewed in this section.

A variety of infectious disease conditions in humans trigger local production of AMPs by epithelial cells and infiltrating leukocytes. In the upper respiratory tract, α-defensins are increased in chronic infective rhinitis and *S. aureus* nasal colonization [44], whereas β-defensins are higher in the maxillary sinus fluid of patients with sinusitis than in normal controls [45]. In the lower respiratory tract, high concentrations of cathelicidin and β-defensins are found in the bronchoalveolar lavage (BAL) fluid of newborn infants with pneumonia [46]. Such increase in AMPs was correlated with high levels of IL-8 and influx of circulating neutrophils. Levels of α- and β-defensin are also increased in the respiratory tract of patients with pulmonary *Mycobacterium avium-intracellulare* infection, in which plasma β-defensin measurement might itself serve as a useful marker of disease activity [47].

Respiratory-tract concentrations of defensin AMPs are also increased with the diffuse alveolar damage of acute respiratory distress syndrome (ARDS), which might have infectious or noninfectious etiologies, as well as idiopathic inflammatory lung diseases, such as diffuse panbronchiolitis and idiopathic pulmonary fibrosis (IPF) [48]. It is interesting to speculate that an undesired or dysregulated overproduction of AMP leads to unwanted proinflammatory effects and contributes to the basic pathogenesis of these inflammatory lung disorders. Experiments in mice

support such a hypothesis: intratracheal instillation of purified defensins leads to acute lung dysfunction, neutrophil influx, and release of inflammatory mediators, such as TNF-α and macrophage inflammatory protein-2 into BAL fluid [49]. Neutrophil defensins might also trigger lung remodeling by inducing epithelial-cell proliferation through an epidermal growth factor (EGF) receptor–independent, MAP kinase signaling pathway [48].

The chronic inflammation of cystic fibrosis (CF) is also associated with increased levels of AMP in respiratory tract secretions. However, the CF airway surface fluid is diminished in its ability to kill bacteria. This defect is reflected in chronic, high-level bacterial colonization and recurrent pneumonia with organisms such as *P. aeruginosa*. The bacteria-killing ability of CF airway fluid is restored when its salt concentration is lowered to normal levels, suggesting that the abnormally high salt concentrations produced by the defective CF transmembrane conductance regulator might be responsible [50]. The bacterial killing ability of epithelial-derived AMPs such as the human β-defensins and cathelicidin are inactivated by high salt concentrations [51,52], suggesting a defect in this component of innate immune defense might be responsible for the chronic pulmonary infections seen in CF patients.

In the gastrointestinal tract, significant increases in the constitutive expression of β-defensin have been seen in the gastric epithelium of patients with gastritis or gastric cancers induced by *Helicobacter pylori* [53,54]. The induced AMP inhibits the growth of *H. pylori* in vitro, suggesting it plays an antibacterial role. Strong immunostaining for α-defensins or β-defensins is seen in the colonic epithelium of patients with active ulcerative colitis or Crohn's disease [55], but the functional significance of this expression has yet to be established. Enteropathogenic bacteria also modulate expression of AMP in the colonic epithelium. Human fetal intestinal xenografts infected intraluminally with *S. typhi* show increased human β-defensin-2 expression [56]. In contrast, biopsies of patients with bacillary dysentery produced by *Shigella* spp. reveal downregulation of β-defensin-1 and cathelicidin production, suggesting a potential virulence property of the bacteria to subvert innate immune defenses of the gastrointestinal mucosa [32••].

Upregulation of AMP production was also found with infections of the urogenital tract. Human β-defensin-1 is expressed constitutively in normal renal tissue, whereas induction of β-defensin-2 gene and protein expression is demonstrated only in tubulus epithelia with chronic pyelonephritis [57]. Women with pelvic inflammatory diseases secondary to *N. gonorrhoeae*, *T. vaginalis*, or *Chlamydia trachomatis* had higher median levels of neutrophil defensins in the vagina than did uninfected women, and the mean levels of these AMPs were strongly associated with the presence of endometritis [58].

Many skin conditions have been examined for changes in the pattern of AMP expression at the epidermal barrier. Cathelicidin expression is increased in the inflammatory skin lesions of erythema toxicum neonatorum, with immunolocalization of the peptide within CD15-expressing neutrophils, EG2-expressing eosinophils, and CD1a-expressing dendritic cells [59]. Cathelicidin expression is also induced within the epidermis during development of verruca vulgaris or condyloma accuminata, suggesting that AMPs represent a component of the immunologic response to papillomavirus infection [60]. Differential expression of AMPs seems to play a determinative role in the susceptibility of chronic inflammatory skin disorders to infectious complications. In psoriasis, cathelicidin and β-defensin levels are elevated, and secondary infection is rare, whereas in atopic dermatitis, cathelicidin expression of the same AMPs is deficient, and bacterial or viral superinfection is common [61••]. Especially relevant in this regard is the proven activity of human cathelicidin against *S. pyogenes* [39] and the synergistic activity of human cathelidicin and β-defensin against *S. aureus* [61••], the leading agents of superficial and invasive skin infection in humans.

Expression of β-defensin is absent in full-thickness burn wounds and blister fluid from partial thickness burns [62], evidence of an innate immune defect that might contribute in the greatly increased risk of burn wound infection and sepsis. Cathelicidin is produced in high levels in postwound skin and is strongly expressed in healing skin epithelium; antibody against this AMP inhibits re-epithelialization in a dose-dependent manner [39,63]. In chronic ulcers, cathelicidin levels are low and absent in the ulcer-edge epithelium [63]. Therefore, it seems that cathelicidin AMPs are important in successful wound closure, and defects in their production can be correlated with development of chronic ulcers.

An association between oral disease and AMPs was shown in patients with Kostmann syndrome, a severe congenital neutropenia. These patients are treated with granulocyte-colony stimulating factor to increase neutrophil counts within the normal range. Nevertheless, patients with Kostmann syndrome experience frequent infections and develop periodontal disease. Functional analysis of neutrophils isolated from these patients demonstrated normal oxidative burst function, but revealed a lack of measurable cathelicidin and diminished levels of α-defensins [64•]. Several of the original index cases in the Kostmann family were recently shown to harbor mutations in the neutrophil elastase gene, suggesting a mechanism for lack of AMP processing and activation [65]. Discovery that impaired AMP peptide function contributes to an inherited immunodeficiency will no doubt spark exploration of the role of endogenous AMP in other human immunologic disorders, including the acquired immunodeficiency syndrome (AIDS). Interestingly, human α-defensin can exhibit potent antiviral activity, as demonstrated by its ability to reduce experimental adenoviral infectivity by 95% [66]. CD8 T lymphocytes from long-term, stably-infected patients with HIV-1 release α-defensins that suppress HIV-1 replication [67], and it is possible that an impaired response of these

AMPs is associated with more rapid disease progression in other infected individuals.

Conclusions

Antimicrobial peptides represent an evolutionarily ancient defense strategy. However, only recently has the production of cathelicidin and defensin peptides been recognized as an important component in human innate immunity. Several investigations have now documented diverse biologic functions of AMPs in microbial killing and augmentation of cellular immune functions. Emerging clinical studies have also begun to associate changes in AMP production or function with human infectious diseases, inflammatory syndromes, or immune deficiencies. In the coming years, we will likely learn more about how impaired AMP activity on mucosal tissues predisposes to infectious complications, how resistance to AMP killing represents a defining feature of certain invasive microbial pathogens, and how dysregulated AMP expression might contribute to chronic inflammatory disorders. A thorough understanding of these associations might pave the way for therapeutic strategies involving administration of exogenous AMP or modulation of endogenous AMP production.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. 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.
2. Zanetti M, Litteri L, Griffiths G, *et al.*: **Stimulus-induced maturation of probactenecins, precursors of neutrophil antimicrobial polypeptides.** *J Immunol* 1991, **146:**4295–4300.
3. Agerberth B, Gunne H, Odeberg J, *et al.*: **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:**195–199.
4. Larrick JW, Lee J, Ma S, *et al.*: **Structural, functional analysis and localization of the human *CAP18* gene.** *FEBS Lett* 1996, **398:**74–80.
5. Gudmundsson GH, Agerberth B, Odeberg J, *et al.*: **The human gene *Fall39* and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes.** *Eur J Biochem* 1996, **238:**325–332.
6. Sorensen OE, Follin P, Johnsen AH, *et al.*: **Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3.** *Blood* 2001, **97:**3951–3959.
7. Zarember KA, Katz SS, Tack BF, *et al.*: **Host defense functions of proteolytically processed and parent (unprocessed) cathelicidins of rabbit granulocytes.** *Infect Immun* 2002, **70:**569–576.
8. Zaiou M, Nizet V, Gallo RL: **Antimicrobial and protease inhibitory functions of the human cathelicidin (hCAP18/LL-37) prosequence.** *J Invest Dermatol* 2003, **120:**810–816.
9. •• De Y, Chen Q, Schmidt AP, *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:**1069–1074.
Antimicrobial peptides act on the host as a chemotactic factor and kill bacteria. This study identified a receptor on host cells for human cathelicidin that might enable its chemotactic activity.
10. Niyonsaba F, Iwabuchi K, Someya A, *et al.*: **A cathelicidin family of human antibacterial peptide LL-37 induces mast cell chemotaxis.** *Immunology* 2002, **106:**20–26.
11. 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:**2274–2278.
12. Gallo RL, Kim KJ, Bernfield M, *et al.*: **Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse.** *J Biol Chem* 1997, **272:**13088–13093.
13. Agerberth B, Lee J, Bergman T, *et al.*: **Amino acid sequence of PR-39: isolation from pig intestine of a new member of the family of proline-arginine-rich antibacterial peptides.** *Eur J Biochem* 1991, **202:**849–854.
14. Gallo RL, Ono M, Povsic T, *et al.*: **Syndecans, cell surface heparin sulfate proteoglycans, are induced by a proline-rich antimicrobial peptide from wounds.** *Proc Natl Acad Sci U S A* 1994, **91:**11035–11039.
15. Jones DE, Bevins CL: **Paneth cells of the human small intestine express an antimicrobial peptide gene.** *J Biol Chem* 1992, **267:**23216–23225.
16. Ouellette AJ, Hsieh MW, Cano-Gauci DF, *et al.*: **Mouse Paneth cell defensins: primary structures and antibacterial activities of cryptdin isoforms expressed in a single intestinal crypt.** *Infect Immun* 1994, **62:**5040–5047.
17. Chaly YV, Paleolog EM, Kolesnikova TS, *et al.*: **Neutrophil alpha-defensin human neutrophil peptide modulates cytokine production in human monocytes and adhesion molecule expression in endothelial cells.** *Eur Cytokine Netw* 2000, **11:**257–266.
18. 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 U S A* 2002, **99:**2129–2133.
19. Yang D, Chertov O, Bykovskaia SN, *et al.*: **Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6.** *Science* 1999, **286:**525–528.
20. Befus A, Mowat C, Gilchrist M, *et al.*: **Neutrophil defensins induce histamine secretion from mast cells: mechanisms of action.** *J Immunol* 1999, **163:**947–953.
21. Guo L, Lim KB, Poduje CM, *et al.*: **Lipid A acylation and bacterial resistance against vertebrate antimicrobial peptides.** *Cell* 1998, **95:**189–198.
22. Gunn JS, Ryan SS, Van Velkinburgh JC, *et al.*: **Genetic and functional analysis of a PmrA-PmrB–regulated locus necessary for lipopolysaccharide modification, antimicrobial peptide resistance, and oral virulence of *Salmonella enterica* serovar typhimurium.** *Infect Immun* 2000, **68:**6139–6146.
23. Lysenko ES, Gould J, Bals R, *et al.*: **Bacterial phosphorylcholine decreases susceptibility to the antimicrobial peptide LL-37/hCAP18 expressed in the upper respiratory tract.** *Infect Immun* 2000, **68:**1664–1671.
24. Peschel A, Otto M, Jack RW, *et al.*: **Inactivation of the Dlt operon in *Staphylococcus aureus* confers sensitivity to defensins, protegrins, and other antimicrobial peptides.** *J Biol Chem* 1999, **274:**8405–8410.
25. • Peschel A, Jack R.W, Otto M, *et al.*: ***Staphylococcus aureus* resistance to human defensins and evasion of neutrophil killing via the novel virulence factor MprF is based on modification of membrane lipids with l-lysine.** *J Exp Med* 2001, **193:**1067–1076.
Bacteria have several strategies for altering their sensitivity to killing by antimicrobial peptides. This study shows that the bacterial surface charge alters the virulence of *Staphylococcus aureus* by altering binding defensins.
26. Parra-Lopez C, Lin R, Aspedon A, *et al.*: **A salmonella protein that is required for resistance to antimicrobial peptides and transport of potassium.** *Embo J* 1994, **13:**3964–3972.
27. Shafer WM, Qu X, Waring AJ, *et al.*: **Modulation of *Neisseria gonorrhoeae* susceptibility to vertebrate antibacterial peptides due to a member of the resistance/nodulation/division efflux pump family.** *Proc Natl Acad Sci U S A* 1998, **95:**1829–1833.
28. Guina T, Yi EC, Wang H, *et al.*: **A PhoP-regulated outer membrane protease of *Salmonella enterica* serovar typhimurium promotes resistance to alpha-helical antimicrobial peptides.** *J Bacteriol* 2000, **182:**4077–4086.

29. Park PW, Pier GB, Preston MJ, *et al.*: **Syndecan-1 shedding is enhanced by LasA, a secreted virulence factor of *Pseudomonas aeruginosa*.** *J Biol Chem* 2000, **275:**3057–3064.
30. Schmidtchen A, Frick IM, Bjorck L: **Dermatan sulphate is released by proteinases of common pathogenic bacteria and inactivates antibacterial alpha-defensin.** *Mol Microbiol* 2001, **39:**708–713.
31. Schmidtchen A, Frick IM, Andersson E, *et al.*: **Proteinases of common pathogenic bacteria degrade and inactivate the antibacterial peptide LL-37.** *Mol Microbiol* 2002, **46:**157–168.
32.•• Islam D, Bandholtz L, Nilsson J, *et al.*: **Downregulation of bactericidal peptides in enteric infections: a novel immune escape mechanism with bacterial DNA as a potential regulator.** *Nat Med* 2001, **7:**180–185.

Indentification of another virulence strategy for bacteria. In this case, shigella express a factor that suppresses the production of antimicrobial peptides in the gut.

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

Demonstration that mammals depend on the expression of antimicrobial peptides for immune defense. Knock-out mice lacking cathelicidin become more susceptible to infection, and bacteria engineered to be more resistant become more virulent.

34. Bals R, Weiner D, Meegalla R, *et al.*: **Transfer of a cathelicidin peptide antibiotic gene restores bacterial killing in a cystic fibrosis xenograft model.** *J Clin Invest* 1999, **103:**1113–1117.
35. Brogden KA, Kalfa VC, Ackermann MR, *et al.*: **The ovine cathelicidin SMAP29 kills ovine respiratory pathogens in vitro and in an ovine model of pulmonary infection.** *Antimicrob Agents Chemother* 2001, **45:**331–334.
36. Lushbaugh WB, Blossom AC, Shah PH, *et al.*: **Use of intravaginal microbicides to prevent acquisition of *Trichomonas vaginalis* infection in Lactobacillus-pretreated, estrogenized young mice.** *Am J Trop Med Hyg* 2000, **63:**284–289.
37. Chalekson CP, Neumeister MW, Jaynes J: **Improvement in burn wound infection and survival with antimicrobial peptide D2A21 (Demegel).** *Plast Reconstr Surg* 2002, **109:**1338–1343.
38. Ceccarelli AV, Cole AM, Park AK, *et al.*: **Therapeutic effect of a pig-derived peptide antibiotic on porcine wound infections.** *Comp Med* 2001, **51:**75–79.
39. Dorschner RA, Pestonjamasp VK, Tamakuwala S, *et al.*: **Cutaneous injury induces the release of cathelicidin antimicrobial peptides active against group A *Streptococcus*.** *J Invest Dermatol* 2001, **117:**91–97.
40. Moser C, Weiner DJ, Lysenko E, *et al.*: **Beta-defensin 1 contributes to pulmonary innate immunity in mice.** *Infect Immun* 2002, **70:**3068–3072.
41. Morrison G, Kilanowski F, Davidson D, *et al.*: **Characterization of the mouse beta defensin 1, Defb1, mutant mouse model.** *Infect Immun* 2002, **70:**3053–3060.
42.• Cole AM, Shi J, Ceccarelli A, *et al.*: **Inhibition of neutrophil elastase prevents cathelicidin activation and impairs clearance of bacteria from wounds.** *Blood* 2001, **97:**297–304.

Further demonstration that the activity of antimicrobial peptides is important to mammalian defense. Inhibition of the processing and activation of antimicrobial peptides enables the prolonged survival of bacteria in pig wounds.

43. Park PW, Pier GB, Hinkes MT, *et al.*: **Exploitation of syndecan-1 shedding by *Pseudomonas aeruginosa* enhances virulence.** *Nature* 2001, **411:**98–102.
44. Cole AM, Tahk S, Oren A, *et al.*: **Determinants of *Staphylococcus aureus* nasal carriage.** *Clin Diagn Lab Immunol* 2001, **8:**1064–1069.
45. Carothers DG, Graham SM, Jia HP, *et al.*: **Production of beta-defensin antimicrobial peptides by maxillary sinus mucosa.** *Am J Rhinol* 2001, **15:**175–179.
46. Schaller-Bals S, Schulze A, Bals R: **Increased levels of antimicrobial peptides in tracheal aspirates of newborn infants during infection.** *Am J Respir Crit Care Med* 2002, **165:**992–995.
47. Ashitani J, Mukae H, Hiratsuka T, *et al.*: **Plasma and BAL fluid concentrations of antimicrobial peptides in patients with *Mycobacterium avium*-intracellulare infection.** *Chest* 2001, **119:**1131–1137.
48. Aarbiou J, Ertmann M, van Wetering S, *et al.*: **Human neutrophil defensins induce lung epithelial cell proliferation in vitro.** *J Leukoc Biol* 2002, **72:**167–174.
49. Zhang H, Porro G, Orzech N, *et al.*: **Neutrophil defensins mediate acute inflammatory response and lung dysfunction in dose-related fashion.** *Am J Physiol Lung Cell Mol Physiol* 2001, **280:**L947–L954.
50. Smith JJ, Travis SM, Greenberg EP, *et al.*: **Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid.** *Cell* 1996, **85:**229–236.
51. Goldman MJ, Anderson GM, Stolzenberg ED, *et al.*: **Human beta-defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis.** *Cell* 1997, **88:**553–560.
52. Bals R, Wang X, Wu Z, *et al.*: **Human beta-defensin 2 is a salt-sensitive peptide antibiotic expressed in human lung.** *J Clin Invest* 1998, **102:**874–880.
53. Bajaj-Elliott M, Fedeli P, Smith GV, *et al.*: **Modulation of host antimicrobial peptide (beta-defensins 1 and 2) expression during gastritis.** *Gut* 2002, **51:**356–361.
54. Uehara N, Yagihashi A, Kondoh K, *et al.*: **Human beta-defensin-2 induction in *Helicobacter pylori*-infected gastric mucosal tissues: antimicrobial effect of overexpression.** *J Med Microbiol* 2003, **52:**41–45.
55. Cunliffe RN, Kamal M, Rose FR, *et al.*: **Expression of antimicrobial neutrophil defensins in epithelial cells of active inflammatory bowel disease mucosa.** *J Clin Pathol* 2002, **55:**298–304.
56. O'Neil DA, Porter EM, Elewaut D, *et al.*: **Expression and regulation of the human beta-defensins hBD-1 and hBD-2 in intestinal epithelium.** *J Immunol* 1999, **163:**6718–6724.
57. Lehmann J, Retz M, Harder J, *et al.*: **Expression of human beta-defensins 1 and 2 in kidneys with chronic bacterial infection.** *BMC Infect Dis* 2002, **2:**20.
58. Wiesenfeld HC, Heine RP, Krohn MA, *et al.*: **Association between elevated neutrophil defensin levels and endometritis.** *J Infect Dis* 2002, **186:**792–797.
59. 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* 2002, **147:**1127–1134.
60. Conner K, Nern K, Rudisill J, *et al.*: **The antimicrobial peptide LL-37 is expressed by keratinocytes in condyloma acuminatum and verruca vulgaris.** *J Am Acad Dermatol* 2002, **47:**347–350.
61.•• Ong PY, Ohtake T, Brandt C, *et al.*: **Endogenous antimicrobial peptides and skin infections in atopic dermatitis.** *N Engl J Med* 2002, **347:**1151–1160.

The susceptibility of patients with atopic dermatitis to bacterial infections might be explained by a lack of expression of antimicrobial peptides.

62. Ortega MR, Ganz T, Milner SM: **Human beta defensin is absent in burn blister fluid.** *Burns* 2000, **26:**724–726.
63. Heilborn JD, Nilsson MF, Kratz G, *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:**379–389.
64.• Putsep K, Carlsson G, Boman HG, *et al.*: **Deficiency of antibacterial peptides in patients with morbus Kostmann: an observation study.** *Lancet* 2002, **360:**1144–1149.

Patients with the inherited neutrophil abnormality of Kostmann syndrome show frequent oral infections. This susceptibility might be explained by the lack of cathelicidin antimicrobial peptides in their saliva.

65. Aprikyan AA, Carlsson G, Stein S, *et al.*: **Neutrophil elastase mutations in severe congenital neutropenia patients of the original Kostmann family.** *Blood* first edition paper, prepublished online January 16, 2003; DOI 10:1182/blood-2002-04-1255.
66. Bastian A, Schafer H: **Human alpha-defensin 1 (HNP-1) inhibits adenoviral infection in vitro.** *Regul Pept* 2001, **101:**157–161.
67. Cole AM, Hong T, Boo LM, *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:**1813–1818.