Pulmonary Defense and the Human Cathelicidin hCAP-18/LL-37

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Abstract

Antimicrobial peptides form an important component of the innate immune system. The cathelicidin family, a key member of the antimicrobial peptide defenses, has been highly conserved throughout evolution. Though widespread in mammals, there is currently only one identified human example, hCAP-18/LL-37. The cathelicidins have been found to have multiple functions, in addition to their known antimicrobial and lipopolysaccharide-neutralizing effects. As a result, they profoundly affect both innate and adaptive immunity. Currently, antimicrobial peptides are being evaluated as therapeutic drugs in disease states as diverse as oral mucositis, cystic fibrosis, and septic shock. One such peptide, the cathelicidin hCAP-18/LL-37, is reviewed in detail in the context of its role in lung physiology and defense.

Key Words

The Innate Immune System

Innate immunity refers to the inherited nonspecific defense mechanisms that acutely recognize pathogens and incite an inflammatory response. Innate immunity is not pathogen-specific, but rather identifies general classes of pathogens using conserved pathogen-recognition motifs that activate host defenses *(1)*. Plants and many animals do not possess an adaptive immune system and instead rely soley on their innate immune systems *(2)*. Antimicrobial peptides form a central component of this defense system. They have strong microbicidal activity and are found throughout the animal and plant kingdom. They provide an ever-ready defense against bacterial infections; they do not need to be "mobilized," unlike the cellular components of adaptive immunity. Without them, bacterial invasion would be inevitable.

Classification

Two major families of antimicrobial peptides have been described in mammals: the

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defensins and the cathelicidin peptides *(3)*. Defensins are cationic molecules with both hydrophobic and charged regions. They possess six cysteine residues, which result in the formation of three intramolecular disulphide bridges *(4)*. Three groups of defensins are described: the α, β and the θ defensins. The second family, the cathelicidins, are α-helical structures without cysteine residues. hCAP-18/LL-37 is the only current known human example *(5)*.

Discovery of the Cathelicidin Family of Antimicrobial Peptides

The cathelicidin (*cathe*psin *L in*hibitor) family of peptides, so named because of a highly conserved pro-region called the cathelin-like sequence, was first isolated from bovine neutrophils while researchers sought to identify potential antibacterial components of leukocytes *(6)*. Since then, cathelicidins have been found in many other animal species, including humans *(5)*. Recently, similar peptides have been isolated from hagfish, suggesting that the precursors of modern cathelicidins existed over 250 million years ago *(7)*.

Gene Structure and Processing

Human cathelicidin has a high degree of homology with other mammalian species. The gene has been mapped to chromosome 3 and has four separate exons *(8)*. The first exon encodes a signal piece and a part of the pro-peptide sequence, while exon 2 and 3 code for another portion of the pro-piece. The fourth exon encodes for the C-terminal amino acids that make up the effector peptide, and an untranslated region (Fig. 1). The human cathelicidin is synthesized as pre-pro-protein *(9)*. After removal of the signal peptide component by the endoplasmic reticulum (ER), the pro-peptide is stored as an inactive form in granules in human myeloid-derived cells. Following exocytosis, the pro-protein is activated by proteinase-3 at a specific site between an alanyl and leucyl residue (Fig. 2), releasing the antimicrobial peptide from the cathelin domain *(10)*. However, in different tissues, different proteases may activate LL-37. For example, LL37, present in seminal fluid, is activated by prostate-derived protease gastricsin when incubated at a pH corresponding to the vaginal pH, suggesting a potential role for preventing infection following sexual intercourse *(11)*. Although LL-37 is stored in neutrophil granules, high concentrations of this peptide are present in the plasma. Values of 1.2 µg/mL have been reported *(12)*. Neutrophils, which store cathelicidin intracellularly in an inactive form, generally do not release their granule proteins until they are outside the circulation and thus are unlikely to be the source of the high level seen. The high level of LL-37 maintained in the plasma appears to be owing to complexing of the C-terminal end to lipoproteins (very low-density lipoprotein [VLDL], low-density lipoprotein [LDL] and high-density lipoprotein [HDL]), which prevents renal clearance of the protein. The exact role of the binding to lipoproteins is unknown, but it has been postulated to protect cells from the cytotoxicity of high levels of cathelicidin, or to maintain high levels in the serum as an antibacterial defense mechanism, or both.

Tissue Expression and Regulation of Gene Expression

Cathelicidins have been found in every mammal studied thus far, and appear to be an essential component of their immune system. Recently, a rare inherited disorder, Morbus Kostmann syndrome, where afflicted patients die within 1 yr of birth from bacterial infections, has been found to be at least, in part, due to a deficiency of LL-37 *(13)*.

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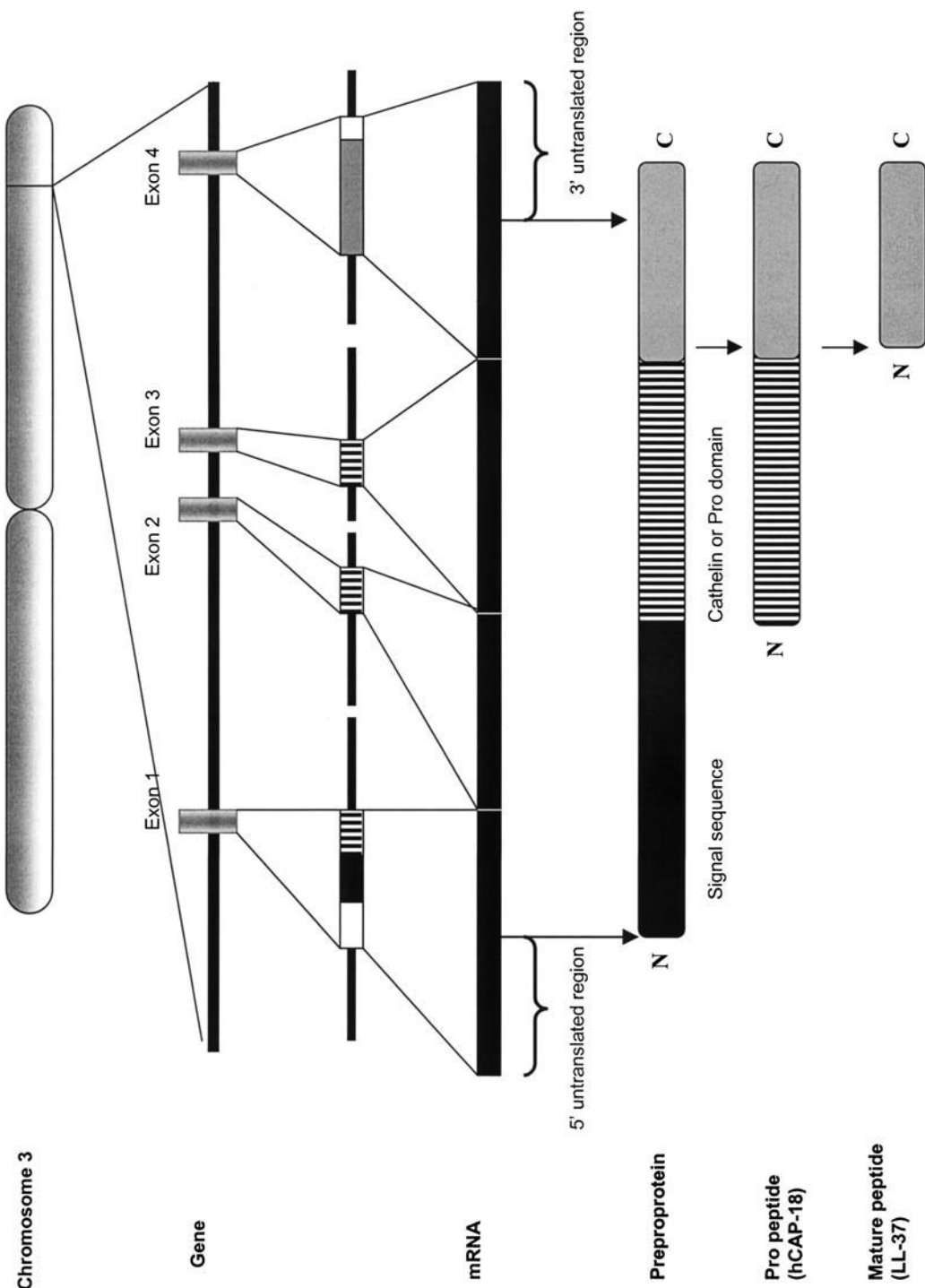
Fig. 1. The LL-37 gene, located on chromosome 3, has four separate exons. In the diagram, the gene has the following components: white box, untranslated region; black box, signal sequence; striped box, pro-sequence; gray box, mature peptide; white box, 3' untranslated region. Exon 4 encodes for the mature peptide and a small untranslated region.

Pre- /signal peptide

METQRASLSLGRWSLWLLLLGLVVPSASA

Pro peptide

QVLSYKEAVLRAIDGINQRSSDANLYRLLDLDPRPTMDGDPDTPKPVSFYVKETVCPRTTQQSPEDCDFKKDGLVKRCMGTVTLNQARGSFDISCDKDNKRFA

Mature C terminal peptide

LLGDFFRKSK EKIGKEFKRI VQRIKDFLRN LVPRTES

Fig. 2. Amino-acid sequence of the three components of the human cathelicidin. The cathelicidin is synthesized as a pre-pro-protein. The signal sequence is removed during processing and the inactive propeptide is stored in granules in neutrophils. Cleavage of the pro-sequence results in the release of LL-37, the mature C terminal peptide.

Though initially isolated from neutrophils, subsequent research has shown LL-37 to be widely distributed in many different cells and tissues *(14)*. By *in situ* hybridization, and polymerase chain reaction (PCR), it is expressed in NK cells, γδT cells, and mast cells. It is also expressed in squamous epithelium of the mouth, tongue, esophagus, intestine, cervix, vagina, sweat and salivary glands, bronchial epithelial cells, and bronchial glands. It is a major component of the antibacterial component of the vernix caseosa protecting the fetus and newborn *(15)*.

Initially, LL-37 gene expression in neutrophils was thought to be restricted to the myeloid stage of neutrophil maturation with subsequent downregulation with terminal-cell differentiation *(16,17)*. However, subsequent studies demonstrated that bovine neutrophils upregulate the Bac5 gene (analogous to LL-37) upon stimulation with lipopolysaccharides (LPS), or heat-killed bacteria with increased protein expression *(18)*.

Regulation of gene expression can play a significant role in predisposing tissues to infection. For example, in psoriatic skin there is evidence for increased LL-37 message and protein production. Consequently, there is a relatively low incidence of skin infections. In contrast, in atopic dermatitis, there is a significant decrease in LL-37 mRNA expression and protein production. This may account for the higher incidence of skin infections in atopic individuals *(19)*. Indeed, cutaneous injury, in general, leads to an increase in the synthesis and release of LL-37 from both keratinocytes and from granulocytes that are recruited to the injury site *(20)*.

Additional factors that modulate cathelicidin gene expression have recently been studied in newborn infants. There, the skin shows significant increased expression of both LL-37 mRNA and protein in contrast to adult skin *(21)*. What drives this increased expression is unknown. It may represent an evolutionary compensation for the relative lack of fully functional neutrophils in the newborn period. In a porcine model, the pig cathelicidin PR-39 is induced by LPS in bone marrow cells, as evidenced by increased mRNA expression at 3 h. Peak protein expression was evident after 12 h of incubation with a 10-fold increased in protein expression *(22)*.

Functional Activity of Cathelicidin

Although all the functional activities of cathelicidins are probably not known, at present the following classes of activities have been described (Table 1): (1) immunomodulatory effects, (2) chemoattraction, (3) angiogenesis, and (4) antimicrobial effects.

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Table 1. Functions of H/CAP-18/ LL-37

Antimicrobial activity
  Bacteria
    Microbicidal activity for Gram-positive organisms *(39,43,57,59,80)*
    Microbicidal activity for Gram-negative organisms *(39,43,57,59,80)*
    Microbicidal activity for spirochaetes *(45,81)*.
  Virus
    Virucidal for Vaccinia *(40)*
  Fungus
    Fungicidal but only at high concentrations *(41)*
Chemoattraction
  Neutrophils *(35,36)*
  Monocytes *(35,36)*
  T cells *(34–36)*
  Mast cells *(37)*
Immunomodulatory effects
  Effects on LPS
    Binds to LPS *(82)*
    Inhibits LPS-induced cytokine release *(23)*
    Protects mice from LPS-induced death *(23,24)*
  Effects on macrophages
    Inhibits LPS-induced cytokine release from macrophages *(23,83)*
    Stimulates gene expression of cytokines, chemokines, and chemokine receptors *(25)*
  Effects on Dendritic cells
    Stimulated DC endocytosis, phagocytic activity, receptor expression, and cytokine secretion that
      promote a Th1 response *(26)*
  Effects on mast cells
    Stimulates release of histamine *(27)*
Growth and repair effects
  Angiogenesis and arteriogenesis
    Via binding to FPRL1, a G protein-coupled receptor *(38)*
  Effects on epithelial cells
    Promotes re-epithelialization of wounded skin *(28)*

Immunomodulation

The cathelicidins have numerous effects on the immune system. One of their earliest recognized functions was their ability to bind directly to LPS, thus inhibiting its biological activity, including cytokine release from macrophages *(23)*. Indeed, in vivo studies have shown that synthetic cathelicidins can attenuate the lethality of antibiotic-induced endotoxin release in mice. Infected mice treated with injections of cathelicidin had lower endotoxin and tumor necrosis factor (TNF) production, despite the fact that bacterial blood counts were unchanged from control mice. This suggests that the beneficial effects in septic mice were owing to its LPS-neutralizing activity rather than to its antibacterial activity *(24)*.

With regard to macrophages, it has been known for some time that cathelidins can suppress LPS-induced cytokine release *(23)*. Further investigations have demonstrated that macrophages upregulate a number of genes,

especially chemokines and their receptors, in response to LL-37 exposure *(25)*. Gene array has shown over 30 genes to be upregulated in macrophages following 4-h exposure to LL-37 at a concentration of 50 µg/mL. Specific genes upregulated include receptors such as platelet-derived growth factor (PDGF) receptor, transforming growth factor β (TGF-β) receptor 1, granulocyte colony-stimulating factor (G-CSF) receptor, and interferon γ (IFN-γ) receptor, interleukin-10 (IL-10), macrophage colony stimulating factor (MCSF), interferon, chemokines such as MCP-3, and chemokine receptors including CXCR-4. Of note, although LL-37 causes chemokine release, resulting in recruitment of inflammatory cells, this occurred without the release of TNF-α. This may allow for the removal of invading microbes while limiting the amount of inflammation they induce.

Recently it has been reported that LL-37 can modulate the type of inflammatory response through its effects on dendritic cells (DCs). In vitro, LL-37-stimulated DCs showed significantly enhanced endocytosis, phagocytic activity, receptor expression, and increased secretion of cytokines that promote a Th1 response *(26)*. Further functions recently identified are the ability of LL-37 to induce histamine release from mast cells *(27),* and a capacity to promote epithelial repair *(28).*

We recently evaluated what role cathelicidins may play in interleukin-1β (IL-1β) physiology in monocytes. IL-1β is a pro-inflammatory cytokine whose processing and release is tightly regulated *(29)*. It is a key early component of the inflammatory response. Human epithelium shows little response to LPS exposure with regard to the production of the defensin family of antimicrobial peptides *(30)*. However, monocyte-derived cells stimulated with LPS promoted a significant production of β defensins in human epithelial cultures. This increased defensin mRNA production is mediated by IL-1β,

released from monocytic type cells *(31)*. The porcine cathelicidins have also been shown to induce IL-1β release *(32)*. Working with human monocytes, we have demonstrated that LL-37 enhances IL-1β processing and accelerates the release of mature IL-1β. This activity was inhibited with $P2X_7$ inhibitors, suggesting that the effect of LL-37 on IL-1β is mediated through activation of the $P2X_7$ receptor *(33)*. This again highlights the ability of antimicrobial peptides not only to function as part of the innate immune system, but also to form important links to the adaptive immune response.

Chemoattraction

The chemoattractant effect of LL-37 was first described by Chertov et al. *(34)*. They observed, in vitro, that LL-37 had a dose-dependent chemotactic effect on peripheral-blood monocytes. Similar findings were noted for freshly isolated neutrophils and T lymphocytes *(35)*. Experiments suggested this interaction occurs through a G-coupled protein receptor, formyl peptide receptor-like 1 (FPRL1). Also, the chemotactic effect was not inhibited with human serum, administered at a dose that was known to block the antimicrobial effects of LL-37 *(36)*. Further studies have demonstrated that LL-37 also has chemotactic effects on mast cells. These cells, normally resident in tissue, increase at sites of inflammation, an effect believed mediated through G protein-phospholipase C signaling pathway *(37)*. This ability of cathelicidin to attract inflammatory cells integrates the innate and adaptive components of the immune system and helps coordinate and amplify the immune response.

Angiogenesis

Because neoangiogenesis is an important component for repair and wound healing, the finding that LL-37 has angiogenic activity, both

in vivo and in vitro, may not be as surprising as first thought, given that it is released at sites of infection and inflammation. Using the chorioallantoic membrane (CAM) model of angiogenesis, Koczulla et al. *(38).* showed new vessel growth after exposure to a 5 μg pellet of LL-37. A control peptide had no such effect. Also, the placement of a LL-37 pellet in a rabbit muscle resulted in both angiogenesis (new blood vessel formation) and arteriogenesis (growth of existing vessels). In vitro experiments demonstrated that the cathelicidin caused a dose-dependent proliferation of human umbilical vein epithelial cells (HUVECs). Furthermore, LL-37 caused enhanced sprouting of endothelial cells from cultured hamster aortic root rings. The mechanism whereby LL-37 exerts its angiogenic effect is believed to be through binding to FPRL1, a G protein-coupled receptor. Other cellular responses to LL-37 are mediated by this receptor *(36).* Supporting this finding was the observation that pertussis-toxin (a G protein-coupled receptor inhibitor) eradicated the angiogenic effects of LL-37 on HUVECs. Additionally, a synthetic FPRL1 agonist hexapeptide (Su peptide) was able to mimic the proliferative effects of LL-37 when applied to HUVECs. Thus, the finding that an antimicrobial peptide has angiogenic activity, in addition to chemotactic activity, highlights the role of LL-37 not only in host defense, but also wound repair.

Antimicrobial Activity

Though the cathelicidin, LL-37, has many different functions, its primary activity is to kill Gram negative and Gram-positive bacteria *(39).* Fungicidal and viricidal effects have also been documented *(40,41).*
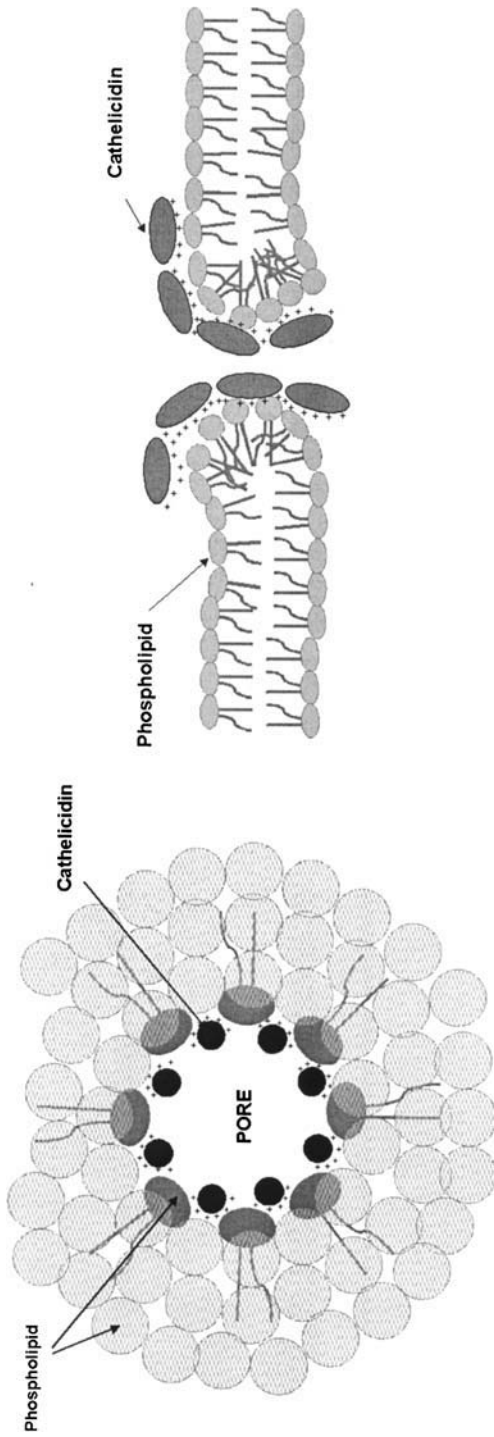
Mechanism of Action

LL-37, an amphipathic α-helix peptide, induces disruption of lipid bilayers. Having a

net-positive charge, it becomes orientated parallel to the polar/nonpolar interface of lipid bilayers. Though initially thought to cause a detergent-like effect on the lipid bilayer, recent studies suggest one of a number of proposed mechanisms to be responsible: the barrel stave model, the carpet model, and the toroidal pore model. The toroidal pore model suggest that, after carpeting the biomembrane, these molecules induce positive curvature strain, which may lead to toroidal peptide-lipid pores (Fig. 3) *(42).* Following disruption of the membrane, the membrane potential is lost, causing alteration of intracellular ion concentration and biomolecules.

The antimicrobial activity of LL-37 has been demonstrated both in vivo and in vitro. The minimum inhibitory concentration (MIC) of these peptides is in the range of 0.1–100 μg/mL. Concentrations three times this range are sufficient to induce damage in mammalian cells. The microbicidal activity is altered depending on the pH and saline concentration. For Gram-positive organisms, Turner et al. demonstrated a MIC of 1–100 μg/mL *(39).* Travis et al. reported a MIC of 0.1–5 μ*M* for *Escherichia coli,* staphlococcus, methicillin resistant *Staphylococcus aureus,* and pseudomonas *(43).* In cystic fibrosis (CF) patients, the MIC for commonly isolated bacteria varied from 0.8 μg/mL to 32 μg/mL *(44).* Spirochetes appear to be less susceptible, requiring higher concentrations *(45).*

Interestingly, the pro-sequence, or cathelin-like, domain of the immature LL-37 peptide has also been found to have antimicrobial activity, following enzymatic cleavage from the C-terminus domain (LL-37). Furthermore, it can also inactivate the cysteine proteinase cathepsin L, suggesting a potential role for suppressing proteinase-induced tissue injury during an inflammatory response *(46).*

Fig. 3. Putative mechanism of membrane disruption. Several mechanisms have been proposed. The toroidal pore model is shown. LL-37 is believed to induce positive curvature strain, which results in pore formation across lipid bilayers.

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The Development of Microbicidal Resistance

Although a rare event, a number of organisms have developed mechanisms for inactivating, or circumventing, the microbicidal effect of the cathelicidins. For example, Neisseria gonorrhea has an energy dependent efflux pump, the mtr pump (*m*ultiple *t*ransferable *r*esistance) that enhances resistance to a range of membrane-damaging compounds. This mtr pump allows *Neisseria gonorrhoeae* to withstand the damaging effect of cationic antimicrobial proteins like LL-37 *(47).* Hemophilus influenza, on the other hand, uses a form of molecular mimicry to circumvent the assault by LL-37. It does so by expressing phosphorylcholine on its cell surface. Because this phospholipid is also found on host-cell membrane in the form of phosphatidylcholine, it camouflages itself as a eukaryotic cell and escapes much of the lethality induced by antimicrobial peptides, which work by targeting differences between bacterial and host membranes *(48).* Other bacteria such as Proteus mirabilis, Pseudomonas aeruginosa, Streptococcus pyogenes, and Enterococcus fecalis use an assortment of proteinases to degrade and inactivate LL-37 *(49).* Some bacteria, such as Shigella, can downregulate LL-37 expression in epithelial cells and monocytes, thus enhancing its invasiveness *(50).* In certain diseases, the microenvironment may affect the functioning of antimicrobial peptides. For example, in CF, F-actin and DNA, present in the airway lumen, can bind and inactivate the microbicidal effects of LL-37. Reversal of this assocation can result in restored antimicrobial activity *(51).* In the case of the defensins, the high-salt microenvironment dramatically diminishes their antimicrobial effects *(52).*

The Respiratory System Defense and the Role of Cathelicidins

Because of its large surface area and its intimate relationship to the outside atmosphere, the respiratory tract has evolved many different mechanisms to prevent the development of respiratory infections. Layers of defenses protect against infecting organisms and, in most instances, this barrier is so effective that the area below the larynx is normally sterile *(53).* These protections include; continuous saliva production, mucosal epithelial cell barrier and desquamation of infected cells, the cough reflex, the mucociliary escalator *(54),* lung epithelial lining fluid, alveolar macrophages, and the ability to recruit activated neutrophils if required *(55).* Despite these extensive protections, organisms frequently breach these barriers. Indeed, pneumonia remains a leading cause of death in the United States *(56).*

There are several routes whereby pathogens can gain access to the lungs, including colonization and spread from the pharynx and mouth, hematogenous dissemination through the bloodstream, or inhalation of airborne organisms. In most instances, colonization and subsequent aspiration, or inhalation of infected airway secretions, is the usual mode of spread.

As noted previously, lung-lining fluid has been found to have microbicidal activity *(57),* a feature first noted by Alexander Fleming in his studies of respiratory secretions. Since then, respiratory secretions have been shown to contain many different antimicrobial molecules. They include lysozyme, lactoferrin, secretory leukoprotease inhibitor, defensins, in addition to the cathelicidins. These molecules may have the capacity to act synergistically. In this regard, human beta defensins have been shown to be present in surface epithelium and submucosal glands of human airways *(58).* Likewise, the cathelicidin family has been shown, by *in situ* hybridization, to be present diffusely throughout the proximal airway epithelium and in serous and mucous cells of the submucosal glands *(59).*

Alveolar macrophages form the majority of inflammatory cells present in a normal adult lung, and are a major barrier to any potential pathogens. These cells use genetically predetermined "pattern recognition receptors" to identify invading organisms. Once binding occurs, the cells attempt to phagocytose the pathogen. A further effect is the release of cytokines, which attract neutrophils, and cause activation of the adaptive immune response. Toll-like receptors (TLRs) have recently been identified as being a major class of receptors that respond to conserved microbial molecules called pathogen-associated molecular patterns (PAMPs). For example, TLR-4 recognizes and binds Gram-negative bacterial LPS (60). TLRs act as a bridge between the innate and adaptive immunity (61). DCs, sentinel cells present in the skin and mucosa, possess TLR receptors. Upon stimulation, they function as antigen-presenting cells (APCs) to T cells, which allows for the clonal expansion of T and B cells. These expanded clones then can interact specifically with particular microorganisms. As previously noted, LL-37 has multiple effects on macrophages, increasing cytokine production, receptor expression, and chemokine release. Through its effects on DCs, it can promote a TH1 response. This again demonstrates how the innate and adaptive immune systems are interlinked.

The adaptive immune response complements the innate immune response. Although it takes time to develop, it brings the power of specificity to bear on the invading organism. It results in the production of specific T cells, or immunoglobulins directed against the inciting pathogen. Development of adaptive immunity has been divided into three components: (1) recognition and processing of a particular antigen; (2) stimulation, proliferation, and differentiation of specific lymphocyte clones to mature effector cells; and (3) an effector phase, where specific clones of activated lymphocytes enhance the inflammatory response to eliminate pathogens.

In the lung, most particulate antigens are never presented to the immune system, because they are rapidly cleared by the mucociliary escalator, or by alveolar macrophages, and are transported out of the lung by the mucociliary escalator and by coughing. If the antigenic organism escapes this surveillance, then it can be processed by APCs. Although alveolar macrophages can serve as APCs, they do so only poorly. Most of the processing is done by DCs, which reside in the lung interstitium (62). Once antigen is identified by APCs, it is presented, in a processed form, in conjunction with the class II major histocompatibility complex (MHC) surface protein, to T cells, specifically precursor T helper cells. This results in the effector, or activation phase of the adaptive immune response.

Once activated, these TH0 cells produce IL-2 and show increased expression of surface CD40 ligand, which allows a more efficient binding of APCs. A number of co-stimulatory events are necessary between the APC and the T cells to ensure effective clonal expansion. At this time, these cells can differentiate into either Th1 or Th2 cells. These act to orchestrate the innate and adaptive immune response. The exact factors that determine which type of response will develop are gradually being elucidated (63,55). A TH1 response results in release of IFN-γ and TNF. In the lung, this results in a neutrophil-predominant response. In contrast, a TH2 response produces clones of lymphocytes that secrete IL-4, IL-5, and IL-13.

Clinical Examples of Cathelicidin Function and Respiratory System Defenses

The important role played by antimicrobial peptides, under normal and diseased condi-

tions, is evident throughout the respiratory tract. In the oral cavity, salivary glands secrete antimicrobial peptides, including LL-37. In chronic infection of the salivary glands, this expression can be significantly upregulated *(64)*. Cathelicidin deficiency may be one reason why patients with xerostomia are predisposed to dental and oral cavity infections *(65)*. This may also make them more susceptible to colonization with pathogenic bacteria with the risk of subsequent bronchitis or pneumonia.

Gingival tissue can also produce antimicrobial peptides, including beta-defensin-1 (hBD-1) and hBD-2. α defensins and LL-37 are produced by polymorphonuclear leukocytes migrating through the gingival tissue *(66)*. Thus, the oral cavity with its stratified epithelium, peptide defenses, and adaptive immune response, can in most instances withstand the onslaught of potential pathogens in the healthy state. However, when the defenses are perturbed, as is the case in patients who are subject to endotracheal intubation, or chemotherapy-induced mucositis, infections frequently develop. For example the incidence of ventilator-associated pneumonia is approx 3% for each day intubated *(67)*.

An important role for antimicrobial peptides has also been suggested in chronic inflammation of the sinuses. Immunohistochemistry and reverse transcription polymerase chain reaction (RT-PCR) demonstrate that LL-37 peptide is present in the surface epithelium, and in serous and mucous cells of the submucosal glands. Infiltrating inflammatory cells were significantly more positive for LL-37 in cases than in controls *(68)*.

The vital role that antimicrobial peptides play in the lower respiratory tract is highlighted by specific disease entities such as CF. CF is a common autosomal recessive hereditary disorder in people of Northern European descent. In most instances, it is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene on chromosome 7. The CFTR gene encodes a protein that functions as a cAMP-regulated chloride channel in the apical membrane of epithelial cells. Mutation of this protein results in chronic respiratory infections and chronic inflammation with resultant progressive damage to the lungs. The reason for this predisposition for infections seems to result from the high salt content of CF airway secretions, which inactivate many lung antimicrobial peptides *(69)*. The β defensins, a major antimicrobial component in the lung, are inactivated in this environment, which compromises the hosts ability to eradicate infectious organisms *(70)*. 217} Although progress has been made in the treatment of CF, novel therapies are needed in order to prevent the inevitable progressive lung destruction resulting from infections and inflammation. Correction of the CFTR defect in animals results in a return of normal bactericidal killing, but progress in translating this finding to humans has been problematic *(71)*. In an effort to overcome this problem, cathelicidin peptides, being less affected by salt concentrations, have been evaluated for their capacity to kill resistant bacteria frequently found in the CF airways. Cathelicidins from different species have been found to have activity against a large array of bactericidal isolates from CF patients. Some of these peptides may be capable of being synthesized at a pharmaceutical scale for production and administered via aerosolization *(44)*.

In a novel approach to the problem of peptide inactivation in CF lungs, using a bronchial xenograph model, Bals et al. demonstrated that LL-37 is present in equal concentrations in the lung-lining fluid of both normal and CF xenografts. CFTR gene transfer, using an adenoviral vector, to cor-

rect the epithelial-cell defect, restored the microbicidal killing without altering LL-37 concentrations. Overexpression of the LL-37 peptide, by means of an adenoviral vector in CF xenografts, increased LL-37 concentrations in the lung-lining fluid and also corrected the microbicidal deficit *(72)*. This approach of increasing LL-37 levels through gene transfer or through aerosolization may eventually be used clinically.

In normal hosts, levels of LL-37 have been evaluated in diseased states. For example, in newborn infants, elevated levels of LL-37 were present in the tracheal aspirates of those who developed pulmonary infections. Interestingly, elevated levels were also seen in response to systemic infections. This effect may be mediated through inflammatory cytokines such as IL-1, IL-6, or TNF-α *(73)*. Other chronic inflammatory lung diseases also demonstrate elevated levels of antimicrobial peptides. Sarcoidosis patients, who have an inflammatory response characterized by a T-helper type 1 response, have elevated levels of LL-37 in bronchoalveolar lavage (BAL) fluid, in comparison to normal controls. Furthermore, immunohistochemistry also identified LL-37 signal in alveolar macrophages and bronchial epithelial cells *(74)*.

Recent studies in our laboratory have suggested that LL-37 is involved in the release of IL-1β from inflammatory cells *(33)*. Given the important role of IL-1β in ARDS, we sought to determine levels of LL-37 in the early inflammatory phase of ARDS *(75)*. In our group of ARDS patients, with a mean APACHE II score of 28, we found significantly elevated levels of LL-37 in the BAL fluid of ARDS cases, in comparison to normal controls (26 ± 8 ng/mL of BAL fluid (mean $\pm$ SEM) vs 3.5 ± 8 ng/mL) using a LL-37 enzyme-linked immunosorbent assay (ELISA) *(76)*. Levels correlated with A-a gradient (a reflection of the severity of lung injury) but not BAL neutrophil count, suggesting it is not simply the release of pre-formed LL-37 from extravascular neutrophils that accounts for the higher levels. Local production from epithelial cells or tissue macrophages may be responsible.

Future Directions

It is prescient to think that replacing a deficiency of cathelicidin may have therapeutic implications. Though synthetic cathelicidin mimics have not yet been developed, a related antimicrobial peptide has been successfully synthesized. A protegrin, initially isolated from porcine leukocytes, which appears to be analogous to the human defensins, was used as a template to manufacture an analog, called iseganan *(77)*. It is currently in Phase III clinical trials for the treatment of oral mucositis secondary to systemic chemotherapy. Other prospective uses of iseganan include control of respiratory pathogens in patients with CF and reduction of oral bacteria to prevent ventilator-associated pneumonia. However, in order to advance the production and clinical testing of peptide-based therapeutics, technical hurdles of synthesizing large quantities of complexly folded peptides must first be overcome *(78)*. Recently, the use of cathelin-like peptides coupled to human IgG has been shown to be effective in protecting mice from polymicrobial sepsis. The benefit was owing not simply from the LPS neutralizing capacity, but also the ability of the construct to kill bacteria and sterilize the blood *(79)*.

In conclusion, the identification of the important role that cathelicidins play in both the innate immune and adaptive immune systems, in addition to its effects on blood vessel formation and wound healing, suggest that these molecules could be used to generate pharmaceutical compounds with the potential to impact many diverse clinical conditions.

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