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Defensins and cathelicidins in lung immunity

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Defensins were first identified in 1985 and are now recognized as part of a large family of antimicrobial peptides, divided into three categories: α-, β-, and θ-defensins. These defensin classes differ in structure, sites of expression and biological activities. Human α-defensins include peptides that are expressed primarily in neutrophils, whereas human β-defensins are widely expressed in epithelial cells, including those lining the respiratory tract. Defensins were first studied for their broad spectrum activity against bacteria, fungi and viruses; however, it is now clear that they also recruit inflammatory cells and promote innate and adaptive immune responses. Recent evidence shows that defensins have anti-inflammatory effects as well. Hence, defensins can participate in all phases of an immune response in the lung, including initial killing of pathogens and mounting – and resolution –- of an immune or inflammatory response. The cathelicidin, LL-37, is an antimicrobial peptide produced by neutrophils and respiratory epithelial cells that has similar roles in lung immunity as the defensins. A major challenge for the coming years will be to sort out the relative contributions of defensins and LL-37 to overall immune responses in the lung and to determine which of their many *in vitro* activities are most important for lung immunity.

Keywords: defensins, cathelicidins, LL-37, lung immunity

INTRODUCTION

Innate immunity is particularly important in the respiratory tract due to continual exposure to particulates and pathogens entering through inhalation or other contact with the nasal or oral surfaces. Ideally, innate defense mediators would inhibit infection while inducing minimal inflammation to avoid damage to the delicate alveolar surface. For this reason, restriction of infection or inflammation to the upper respiratory tract is also important. In instances where infection cannot be aborted at the earliest phase, innate mediators provide a vital link to rapid, but controlled, generation of an adaptive immune response. Innate mediators can also contribute to effective resolution of the adaptive response. We will attempt to summarize how antimicrobial peptides appear to participate in all of these phases of lung immunity.

Neutrophil defensins were first reported by Ganz *et al.*[1] in 1985. Production of a defensin ('tracheal antimicrobial peptide' or TAP) by respiratory epithelial cells (bovine tracheal epithelium) was first reported in 1991 by Diamond *et al.*[2] The TAP was subsequently shown to be a member of the β-defensin family (homologue of hBD2).[3] A cathelicidin was first identified as a lipopolysaccharide (LPS) binding protein in rabbit granulocytes in 1991.[4] Since these discoveries, there has been a tremendous expansion in research related to these antimicrobial peptides, demonstrating broad-spectrum antimicrobial activities and modulation of innate and adaptive immune responses.[3] This review will specifically focus on the role of defensins and the antimicrobial peptide LL-37 in human lung immunity. In general, defensins and LL-37 have two major functions in host defense: direct inhibition of pathogens and modulation of other innate and adaptive immune

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responses. Although there is considerable evidence that defensins and LL-37 function to promote immune responses (hence, they have been called 'alarmins'), recent findings also indicate that they can have anti-inflammatory effects that may be important and beneficial.

General overview of the human defensins and LL-37

Table 1 provides a general overview of the structure and expression of human defensins and LL-37. The human defensins include β-defensins (hBDs) that are expressed widely in skin and mucosal epithelia and six α-defensins that include four human neutrophil peptides (HNPs 1–4) and two epithelial α-defensins (HD5 and HD6). The hBDs and HD5 and HD6 are expressed predominantly by mucosal epithelial cells where they contribute to initial host defense against infection and also to maintenance of epithelial integrity. We will not deal extensively with HD5 and HD6 since their role in lung immunity appears to be limited, in contrast to the important roles of HNPs 1–4 and hBDs. The HNPs are predominantly expressed by neutrophils and to a lesser extent in other bone marrow derived cells that are resident in, or recruited to, inflamed or infected sites in the body. The HNPs are constitutively expressed and packaged in azurophil granules of neutrophils from which they can be released in large quantities during neutrophil activation. In general, hBDs are expressed by secretion from epithelial cells rather than by degranulation and are secreted in mature form on to the surface of, or lining fluid surrounding, these cells. The hBD is constitutively expressed in most instances, whereas epithelial expression of hBDs 2–4 is generally inducible in response to infectious or inflammatory stimuli.

The only human cathelicidin is hCAP18/LL-37, which has similar biology to defensins and also plays important roles in mucosal defense. Like the defensins, hCAP18/LL-37 is a member of a large family of cationic antimicrobial peptides expressed in many species and has a broad spectrum of antimicrobial activity and many immunomodulatory effects. The cathelicidins contain a signal peptide, a cathelin-like domain and antimicrobial domain (LL-37). hCAP18/LL-37 is produced by neutrophils, macrophages and various epithelial cells as well. The antimicrobial domain is released by cleavage by proteases and this domain is termed LL-37. We also consider another class of defensins, called θ-defensins or retrocyclins. These have a cyclic structure and are expressed in non-human primates.[5] Humans express θ-defensin mRNA but lack the corresponding peptides because the human θ-defensin (*DEFT*) gene contains a stop codon in the signal sequence that aborts translation. Retrocyclins are synthetic humanized θ-defensin peptides whose sequences are based on those found in human *DEFT* genes and are of interest for their therapeutic potential in bacterial or viral lung infections.

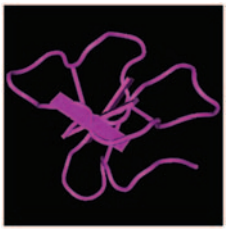
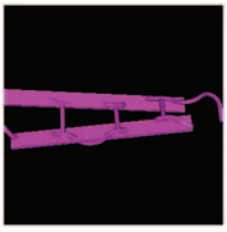
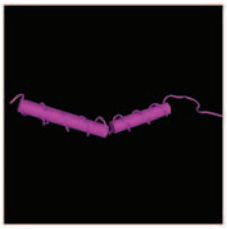
Antimicrobial peptides have the ability to kill a broad range of pathogens, including bacteria, viruses, fungi and protozoa. In some cases, the direct pathogen killing function of defensins results from membrane permeabilization. They also can inhibit the action of bacterial toxins.[3] In return, bacteria have devised mechanisms to combat activity of antimicrobial peptides, including alteration of bacterial surface components to reduce binding of the peptides or to depress expression of HBDs by epithelial cells.[6] An ability of defensins to induce bacterial or viral aggregation has been described and this could play a role in reducing infectious titers and promoting clearance from the airway.[7,8] The ability of defensins to induce aggregation of pathogens may depend on assembly of dimeric or larger defensin complexes.[8] We will not explore, in depth, the biochemistry or antimicrobial mechanisms of action of defensins here; however, these fascinating areas have been the subject of recent excellent reviews.[9,10]

Expression and biological effects of defensins and LL-37: in vitro *studies using respiratory epithelial cells, neutrophils or macrophages*

Table 2 provides an overview of various functional properties of human defensins and LL-37. Antimicrobial peptides contribute to the barrier function of intact respiratory epithelia. The respiratory epithelium itself is an important component of the initial innate response to lung infection, as recently confirmed by Shornick *et al.*[11] in a study showing that mice that lacked stat 1 in the epithelium, but had bone marrow containing stat 1, had strongly impaired response to respiratory viral infection, while the inverse was not true. In addition to generating cytokines, the respiratory epithelium produces hBDs and LL-37 that can directly inhibit infection while triggering recruitment of immune cells.[3] The hBD1 is constitutively expressed and not generally up-regulated, whereas the others are up-regulated during infections or inflammation. All of the peptides are expressed in a variety of other epithelia and have broad-spectrum antimicrobial activity. The hBD2 is most highly expressed in lung, whereas hBD4 is expressed most highly in testes and stomach, LL-37 in bone marrow, and hBD3 in skin and tonsil. An important question that has not been evaluated in depth is relative levels of secretion of the defensins at different levels of the respiratory tract during infection.

Regulation of hBD2 expression by respiratory epithelial cells can be induced by LPS through a mechanism involving CD14, Toll-like receptor (TLR)4 and nuclear factor (NF)-κB.[12,13] Respiratory synctitial virus (RSV)

Table 1. Overview of human antimicrobial peptides: structures, sites of expression and gene copy numbers

Antimicrobial peptide	Structure[a]	Structural features	Sites and mechanisms of expression	Gene copy numbers
Alpha-defensins HNP 1–4		Dimerize in solution	Neutrophil azurophil granules; constitutive expression, released upon degranulation	HNPs 1–3 have variable copy numbers and related variation in protein expression
Alpha-defensins HD5 and HD6		HD6 dimerizes	Epithelial cells (GI, GU); constitutive expression, secreted	
Beta-defensins hBD 1–4			Epithelial cells (respiratory, skin, GI, GU, eye); hBD1 constitutive expression, hBDs 2–4 inducible, secreted	hBDs 2–4 can vary from 2 to 12 copies
Theta-defensins retrocyclins 1 and 2		Cyclic structure, beta hairpin braced by 3 disulfide bonds, retrocyclin 2 trimerizes	Primate bone marrow[b]	
Cathelicidin LL-37		Curved amphipathic helix-bend-helix	Neutrophil specific granules and epithelial cells (respiratory, skin, *etc*.); inducible; vitamin D regulates expression	No copy number polymorphism (2 copies per diploid genome)

[a]The structures shown are those of HNP1, HD6, HBD1, retrocyclin-2, and LL-37. They were obtained on PubMed and the PDB ID#s are, respectively: 3GNY, 1ZMQ, 1KJ5, 2ATG, and 2K60.
[b]Bioactive retrocyclin 1 was produced *in vitro* by human epithelial cells treated with aminoglycoside.

also stimulates production of hBD2 in A549 cells through a NF-κB-dependent process that involves RSV-stimulated tumor necrosis factor (TNF)-α production.[14] The hBD3 is induced by bacteria and TNF-α in primary tracheal epithelial cells.[15] Recent studies have demonstrated that vitamin D is important in regulation of LL-37 expression. Respiratory epithelial cells convert vitamin D to its active metabolites, contain vitamin D receptors, and elaborate cathelicidins and CD14 in response to active metabolites of vitamin D.[16]

Table 2. Functions of antimicrobial peptides

Direct inhibition of infection	Bacterial, fungal and viral killing Aggregation of bacteria and viruses
Recruitment of neutrophils and monocytes	LL-37 chemotactic for neutrophils and monocytes hBDs chemotactic for monocyte/macrophages
Promotion of adaptive immune responses	LL-37, HNPs and hBDs chemotactic for T-cells hBDs and HNPs chemotactic for dendritic cells and up-regulate activation of dendritic cells
Reducing inflammatory injury	LL-37 binds to LPS and reduces its inflammatory effects Reduction of pro-inflammatory cytokine release by macrophages (LL-37 and HNPs) Reduction of neutrophil oxidant production (HNPs)
Promotion of phagocytosis	Promotion of macrophage phagocytosis of bacteria (HNPs) Promotion of neutrophil and monocyte phagocytosis of influenza virus or bacteria (HNPs and retrocyclins)
Binding to DNA and RNA	LL-37 binds self DNA and RNA and promote dendritic cell activation by these nucleic acids

This response pathway may be important in defense against *Mycobacterium tuberculosis*[17] or respiratory virus infection (since the pathway is potentiated by dsRNA).[18]

In addition to their direct antimicrobial and antiviral activities, hBDs and LL-37 bind to specific receptors on trafficking immune cells to stimulate recruitment of inflammatory cells to the mucosa as recently reviewed.[9,19] In brief, The hBDs are chemotactic for dendritic cells (DCs) and T-cells via interaction with CCR6.[20] The hBD2 can activate DCs via binding to TLR4[21] and hBD3 can similarly activate antigen presenting cells by interacting with TLR1 and TLR2.[22] hBDs can also stimulate Mast cell migration and activation.[23]

Circulating immune cells, especially neutrophils, are recruited to the lung in response to chemotactic stimuli and provide an abundant source of HNPs and further increments in LL-37. The HNPs also have chemotactic effects for immature DCs, T-cells and Mast cells.[24–26] In addition, HNPs stimulate IL-8 and IL-1β mRNA and IL-8 protein production by human bronchial epithelial cells.[27] High concentrations (>20 μg/ml) of HNPs also can be cytotoxic for respiratory epithelial cells.[27] HNP1 induces mucin production in NCI-H292 cells (with additive increases in presence of LPS).[28] The HNPs also induced oxidant production in lung explants and the combination of lung explant tissue and defensins had markedly greater antibacterial activity than the HNPs alone.[29] Although macrophages store only minor amounts of HNPs, they can acquire HNPs via ingestion of neutrophils and these HNPs co-localize with *M. tuberculosis* in early endosomes and contribute to inhibition of bacterial growth.[30] It is possible that this finding is representative of a more wide-spread phenomenon in which defensins or LL-37 can be taken up by various cell types and exert inhibition on growth of intracellular pathogens. For example, HD5 has been shown to be taken up by cervical epithelial cells.[31]

Although the bulk of literature on immunomodulation by HNPs deals with their ability to promote immune responses or inflammation, there is also emerging evidence that they can down-modulate some aspects of inflammation. The HNPs promote uptake of bacteria and viruses by neutrophils or macrophages without increasing oxidant responses.[8] Similar findings were obtained with retrocyclins.[7] A recent study showed that HNPs released from dying and necrotic neutrophils dampen inflammation *in vitro* and *in vivo*, probably by inhibiting pro-inflammatory cytokine production by macrophages while promoting macrophage phagocytosis.[32] Hence, there is *in vitro* evidence for a variety of mechanisms through which HNPs can contribute to host defense, including direct antimicrobial effects, promotion of immune responses as well as aiding in resolution of inflammation.

LL-37 has a similar spectrum of activities as the defensins. LL-37 stimulates chemotaxis of neutrophils, monocytes and T-cells.[33] There is evidence for several specific receptors for LL-37 on these cells, including formyl peptide receptor like 1 (FPRL1) on neutrophils, monocytes and lymphocytes, GAPDH in the cytoplasm of monocytes, and CXCR2 on neutrophils.[3,34] LL-37 can promote inflammatory responses of macrophages in concert with IL-1β;[35] however, LL-37 also binds LPS and can inhibit responses to it through this mechanism and through direct interactions with monocytic cells that reduce TLR-mediated activation.[36] Of interest, recent studies also show that LL-37 binds to DNA and RNA and can promote the ability of these nucleic acids to activate DCs through TLRs.[37,38]

Roles and regulation of defensins and LL-37 based on murine models

One important challenge for future research is to determine the relevance of these various *in vitro* findings to the *in vivo* contributions of defensins and LL-37 to host defense or inflammation. Mouse models have begun to be helpful in this regard at least with respect to β-defensins and cathelicidins. Mouse homologues for hBDs and LL-37 have been identified as follows: hBD1 = mBD1, hBD2 = mBD3, hBD3 = mBD14, and hCAP/LL-37 = CRAMP.[3] Knockouts of the mBD1 and CRAMP genes have been accomplished. The results from the mBD1$^{-/-}$ mouse models (developed in two laboratories) have shown no obvious phenotypic changes in the absence of infection. Absence of mBD1 did result in delayed clearance of *Haemophilus influenzae* from the lung although inflammatory responses and survival were unchanged.[39] No difference in response to *Streptococcus pneumoniae* or *Staphylococcus aureus* lung infection were noted in mBD$^{-/-}$ mice.[39,40] The subtlety of the defects in these mice may indicate that deletion of several mBDs would be needed to show greater effects. The host defense defects documented in CRAMP$^{-/-}$ mice, thus far, have been for bacterial infections of skin and urinary tract.[3] One limitation of mouse models is that mouse neutrophils do not express peptides homologous to HNPs. Mouse models have been useful to study up-regulation of mBDs and CRAMP during respiratory bacterial infections.[41,42]

Based on *in vitro* findings and murine studies, antimicrobial peptides appear to play important roles in respiratory viral infections. The HNPs, HDs, hBD2, and retrocyclins all have antiviral activity against influenza virus.[3] Defensins also have activity against a variety of other respiratory viruses, including RSV, adenovirus, and parainfluenza virus and contribute to the respiratory epithelial cell response to rhinovirus.[3] Viral infections also increase expression of epithelial defensins. For instance, influenza A virus infection up-regulates expression of mBD3 and mBD4 in conducting airway epithelial cells in mice,[43] and parainfluenza virus increases expression of sheep BD1 in neonatal lambs.[44] In each case, up-regulation of BDs coincided with increased expression of surfactant protein D (SP-D), another innate immune protein with broad spectrum antibacterial and antiviral activity. Of interest, LTB4 treatment increases both cathelicidin and mBD3 production in mouse lung and this treatment results in improved outcome of influenza viral infection.[18]

There is evidence to suggest both pro- and anti-inflammatory effects of defensins *in vivo*. Instillation of HNPs into mouse lung caused inflammation and deterioration of lung function.[45] These findings suggest that, in some conditions, high levels of HNPs may be deleterious. Instillation of retrocyclins into mouse lung induced a milder degree of inflammation but this effect correlated with protection against mortality from SARS coronavirus infection.[46] As noted above, there are various ways that HNPs or LL-37 could trigger inflammation through direct actions on respiratory epithelial cells. An additional mechanism through which HNPs could promote inflammation is by impairing activity of surfactant protein (SP)-D. Surfactant-protein-D plays important roles in defense against influenza virus through direct viral inhibition and through reducing virus-induced inflammatory responses.[47,48] Surfactant protein-D and SP-A also contribute to maintenance of an anti-inflammatory milieu in the normal healthy lung. Severe influenza or SARS coronavirus infection are associated with severe inflammation with associated extensive neutrophil and monocyte influx and this inflammatory response appears to be deleterious in some models (*e.g.* H5N1 infection of mice).[49] One mechanism through which neutrophil influx could impair host defense is through degradation of SP-D through action of neutrophil proteases and HNPs. The HNPs 1–3 bind strongly to SP-D, generally reduce its antiviral activity and can cause it to precipitate out of broncho-alveolar lavage fluid.[50] Of interest, however, a recent study demonstrated that neutrophil influx occurring after influenza A virus infection of mice actually had a protective effect through reducing lung inflammation.[51] As noted above, HNPs released from dying and necrotic neutrophils dampen inflammation in mouse lungs.[32]

A long-standing conundrum is the fact that enormous numbers of activated neutrophils enter the lung during acute lung infections (*e.g.* bacterial pneumonia) without causing extensive injury, at least in acute and self-limited infections. This observation alone suggests that the large burden of HNPs and LL-37 delivered by neutrophils is not harmful and may, in fact, contribute to resolution of inflammation. Further follow-up studies regarding potential anti-inflammatory effects of these antimicrobial peptides will, therefore, be of great interest. It is clear from this summary of murine studies that much work needs to be done to clarify the effects of defensins and LL-37 *in vivo*. Even more complexity arises when trying to understand the role of antimicrobial peptides in human lung diseases.

Antimicrobial peptides in human respiratory diseases

Studies of cystic fibrosis reveal the complexity of trying to determine the role of defensins in lung disease. Children with cystic fibrosis are born with normal lung function but undergo steady deterioration marked by recurrent respiratory and bacterial viral infections and chronic lung infections with organisms like *Pseudomonas aeruginosa* and

Burkholderia cenocepacia. Broncho-alveolar lavage fluid (BALF) from cystic fibrosis patients has been shown to have reduced antibacterial activity and this appears, in part, to be due to high salt concentrations in the fluid and inhibition of defensin activity (which is salt sensitive).[52,53] There is also evidence of impaired up-regulation of hBD2 and increased degradation of hBDs in cystic fibrosis by cathepsins; in addition, the LPS of *B. cenocepacia* renders it resistant to antimicrobial peptides.[3] Levels of HNPs and LL-37 are elevated in cystic fibrosis BALF associated with persistent inflammation and infection.[54,55] Deficiency of α_1-antitrypsin is another condition in which neutrophil proteases levels are elevated in concert with HNPs.[56]

Elevated levels of hBD2 have been found in diffuse panbronchiolitis and bronchiolitis obliterans complicating lung transplantation. Elevated levels HNPs have been found in idiopathic pulmonary fibrosis and acute respiratory distress syndrome in these cases correlating with the degree of lung inflammation or injury.[3] Further studies obviously are needed to determine if the defensins are causative of inflammation or a response to other insults. The contributions of defensins or LL-37 to asthma have not been extensively studied, although subjects with asthma do have increased susceptibility to respiratory viral infections. hBD2 and LL-37 expression can be suppressed by Th2 cytokines, leading to reduced antimicrobial activity; therefore, alterations in antimicrobial peptide levels could contribute to features of asthma.[3]

Bacterial pneumonia, pulmonary tuberculosis and sepsis are associated with elevations in hBDs, HNPs and LL-37 levels in the lung fluids and blood.[3] How these defensins are processed or removed has not been studied extensively, although one mechanism of inhibition of HNPs in the lung involved ADP-ribosylation on arginine residues.[57,58] The HNP activities are also inhibited by serum proteins, whereas antimicrobial activities of LL-37 are not. There is evidence that LL-37 contributes to host defense against tuberculosis.[59] African-Americans have been shown to have increased susceptibility to tuberculosis which may relate to low levels of vitamin D and consequent reduced ability of macrophages to kill *M. tuberculosis*.[17] Treatment of macrophages with TLR agonists increases vitamin D receptors and results in increased production of LL-37 and other vitamin D responsive genes. The HNPs also have activity against mycobacteria and may contribute to host defense mediated by macrophages.[30] As noted above, there is evidence for a role of defensins and LL-37 in defense against viral infection in mice but there is little data regarding this from human investigations. Current or former tobacco smoking has been associated with reduced levels of hBD2 in pharyngeal washes and sputum of patients with acute pneumonia.[60]

Clearly, our understanding of the role of antimicrobial peptides in human lung diseases is limited at present and this should be a burgeoning area of study in the coming years. The role of antimicrobial peptides has been more definitively established in some gastrointestinal and skin diseases (*e.g.* inflammatory bowel diseases or atopic dermatitis).[3] Valuable information should be obtained through study of people with genetic variations (*e.g.* in NOD signaling or gene copy number polymorphisms) or characterization of gene expression profiles in various lung diseases. There are large variations in gene copy numbers of HNPs 1–3 and hBDs 2–4 (but not LL-37) and increased copy number of the HNPs has been correlated with increased production of these defensins by neutrophils.[61] These variations in gene copy number could be associated with certain illnesses (*e.g.* increased hBD copy number is associated with psoriasis[62]) or host defense against infection.

Interaction of defensins with other innate immune mediators

Antimicrobial peptides constitute only one component in a complex mixture of innate immune mediators in oral, nasal and respiratory lining fluids. There have been relatively few studies of how defensins and LL-37 interact with these other components.[63–65] As noted, HNPs 1–3 bind to SP-D and, in some cases, inhibit its activity against influenza virus. The hBDs show minimal binding to SP-D and have co-operative antiviral interactions with it.[7] Although retrocyclins also bind strongly to SP-D, they also have co-operative antiviral interactions.[7] Given the large number of innate mediators present in respiratory lining fluids, it will clearly be challenging to identify the specific contributions of defensins separate from other components. Nonetheless, further studies of this kind are an important priority. Further studies evaluating up-regulation of expression of innate mediators of various kinds by infection or inflammation should also be useful.

Therapeutic considerations of antimicrobial peptide research

Defensins have potential for therapy given their broad-spectrum antimicrobial and antiviral activity and the fact that they can be synthesized with minor modifications to enhance activity further. Defensins have cytotoxic effects at high concentrations that have also raised concerns about systemic administration.[66] Direct application to epithelial surfaces is perhaps more promising, as illustrated by treatment of diabetic foot ulcers.[67] One concern for direct applications of defensins in the lung would be the possibility of inducing

excessive inflammation as occurred with instillation of HNPs into the lung.[45] We have found that retrocyclins have strong antiviral activity against IAV and, unlike HNPs, do not interfere with the antiviral activity of SP-D despite binding to it.[7] Hence, retrocyclins may be a better candidate than HNPs for instillation in the respiratory tract. A recent study demonstrated reduced weight loss and mortality in mice treated with a retrocyclin by intranasal instillation 15 min before infection with a mouse-adapted version of the SARS coronavirus.[46] Surprisingly, the protection was not the result of inhibition of viral replication. In this model, the retrocyclin did induce some level of inflammation in the lung on its own, although it reduced generation of some pro-inflammatory cytokines triggered by the virus. Hence, retrocyclins may exert beneficial effects through modulation of inflammatory responses in the lung. Another study found benefit of a retrocyclin for treatment of H5N1 IAV infection in mice.[68] Another fascinating recent finding is that retrocyclins can be produced in human cervicovaginal secretions in response to aminoglycoside treatment.[69]

Treatment of some diseases through direct application in the lung may be technically challenging. Adenoviral vectors for delivery of antimicrobial peptides have been tested in animal models with mixed results.[70–72] Another approach would be to increase endogenous defensin generation through use of known regulatory stimuli. In a mouse model, administration of LTB4 increased defensin and cathelicidin generation and improved outcome of IAV infection.[18] Vascular endothelial growth factor promotes BD and SP-D production in the lung resulting in improved outcome of RSV infection.[73] The importance of vitamin D in LL-37 generation and other aspects of innate defense suggest that measurement of vitamin D levels and supplementation could be important for improving outcome of some infections. Finally, application of butyrate or some essential amino acids to epithelia has been shown to increase antimicrobial peptide generation.[74,75] Hence, there are a variety of indirect means through which antimicrobial peptide expression could be locally increased.

Conclusions

Our understanding of the role of antimicrobial peptides to lung immunity is in its infancy in part because of the important redundancy of innate immune mechanisms. An emerging theme in defensin biology is the ability of epithelial derived defensins (hBDs and LL-37) to be up-regulated upon exposure to pathogens or cytokines with the potential to inhibit infection directly, prior to evolution of a more extensive inflammatory response. The most well-developed model of this role for defensins is in the gastrointestinal tract (*e.g.* Crohn's disease appears to be a clear example of chronic inflammation resulting from a breakdown in initial epithelial barrier functions normally mediated by defensins); however, the evidence supports a similar role for defensins and LL-37 in the respiratory tract and other mucosal systems. In addition to their direct antimicrobial actions, β-defensins and LL-37 are also embedded in other physiological processes involved in maintaining epithelial integrity like wound healing and angiogenesis. There is considerable evidence of a role for defensins and LL-37 in recruitment of immune cells through specific receptors and chemokine like activity facilitating the propagation of an adaptive immune response. In this respect, it is conceivable that antimicrobial peptides might contribute to inflammation in a deleterious manner in some settings although there is little evidence for this thus far. In fact, there are several lines of evidence that defensins could down-regulate inflammation, either through inhibiting LPS-mediated responses, promoting phagocytosis while inhibiting oxidant responses of neutrophils or monocytes, or inhibition of pro-inflammatory cytokine secretion by macrophages in the presence of bacteria or LPS or other non-specific inflammatory stimuli. A major challenge for future research will be to determine which *in vitro* functions ascribed to antimicrobial peptides are most relevant *in vivo*. Studies involving therapeutic use of antimicrobial peptides for lung infections have just begun to emerge and appear to show promise for treatment of infection. These studies should be helpful in determining important *in vivo* activities of defensins and LL-37.

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