

Defensins: Key players or bystanders in infection, injury, and repair in the lung?

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Antimicrobial peptides have been identified as key elements in the innate host defense against infection. Recent studies have indicated that the activity of antimicrobial peptides may be decreased in cystic fibrosis, suggesting a major role for these peptides in host defense against infection. One of the most intensively studied classes of antimicrobial peptides are defensins. Defensins comprise a family of cationic peptides that in human subjects can be divided into the α - and β -defensin subfamilies. The α -defensins are produced by neutrophils and intestinal Paneth's cells, whereas β -defensins are mainly produced by epithelial cells. Although studies on β -defensins have so far focused on their antimicrobial activity, studies on α -defensins have suggested a role of these peptides in inflammation, wound repair, and specific immune responses. α -Defensins, which accumulate in airway secretions of patients with various chronic inflammatory lung disorders, were shown to be cytotoxic toward airway epithelial cells and to induce chemokine secretion in several cell types. Furthermore, the capacity of α -defensins to promote bacterial adherence to epithelial cells in vitro further supports a role for these peptides in the pathogenesis of chronic obstructive pulmonary disease and cystic fibrosis. Increased numbers of neutrophils are also present in the airways of patients with asthma, suggesting that neutrophils are involved in the pathogenesis of this disease. Because defensins are able to induce histamine release by mast cells and increase the airway hyperresponsiveness to histamine, it is tempting to speculate that defensins may also contribute to the inflammatory processes in asthma. Besides these proinflammatory effects, α -defensins may also display anti-inflammatory activities, including regulation of complement activation and proteinase inhibitor secretion. Finally, defensins may be involved in wound repair because defensins increase epithelial cell proliferation. Thus recent defensin research has revealed potential links between the innate and acquired immune system. (*J Allergy Clin Immunol* 1999;113:1-8.)

Key words: Defensins, antimicrobial peptides, neutrophils, epithelial cells, infection, innate immunity, host defense, immune response, inflammatory lung disease

Abbreviations used

α 1-PI:	α 1-Proteinase inhibitor
BAL:	Bronchoalveolar lavage
COPD:	Chronic obstructive pulmonary disease
hBD:	Human β -defensin
HD:	Human defensin
HNP:	Human neutrophil peptides
SLPI:	Secretory leukocyte proteinase inhibitor

Host defense against infection involves a multitude of factors and cells that together form the elements of innate and acquired immunity. Components of innate immunity that have been studied intensively are antimicrobial peptides. These peptides, which have now been identified as key elements in the innate defense against infection, are often cationic, display a large variety of structural diversity, and are produced by phagocytes, epithelial cells, and other cell types. Recent studies have indicated that the activity of antimicrobial peptides in the lungs of patients with cystic fibrosis may be decreased, suggesting that these peptides are involved in the pathogenesis of pulmonary infections. This hypothesis has led to new research initiatives in the study of antimicrobial peptides in the lung. A substantial portion of these studies has been focused on defensins, a family of antimicrobial peptides that have been identified in plants, animals, and human subjects. Besides their involvement in the host defense against infection, defensins are also thought to play a role in inflammation, wound repair, and specific immune responses (acquired immunity). This review describes recent developments in defensin biology that may be important in understanding their role in infection and inflammation in the lung.

The peptide structure of the cationic defensins is characterized by the presence of 6 cysteine residues that form 3 intramolecular disulfide bridges. On the basis of the pairing of these cysteine residues in the disulfide bridges, the human defensin family is divided into the α - and β -defensin subfamilies. The first human α -defensins were described in 1985, and this family now comprises 6 members.^{1,2} Four of them, called human neutrophil peptides (HNPs) 1 to 4, are located in the azurophilic granules of the neutrophil. Two, named human defensin (HD)-5 and HD-6, are present in the secretory granules of the intestinal Paneth's cells and in epithelial cells of the female genital tract.³ HD-5 and HD-6 are not further

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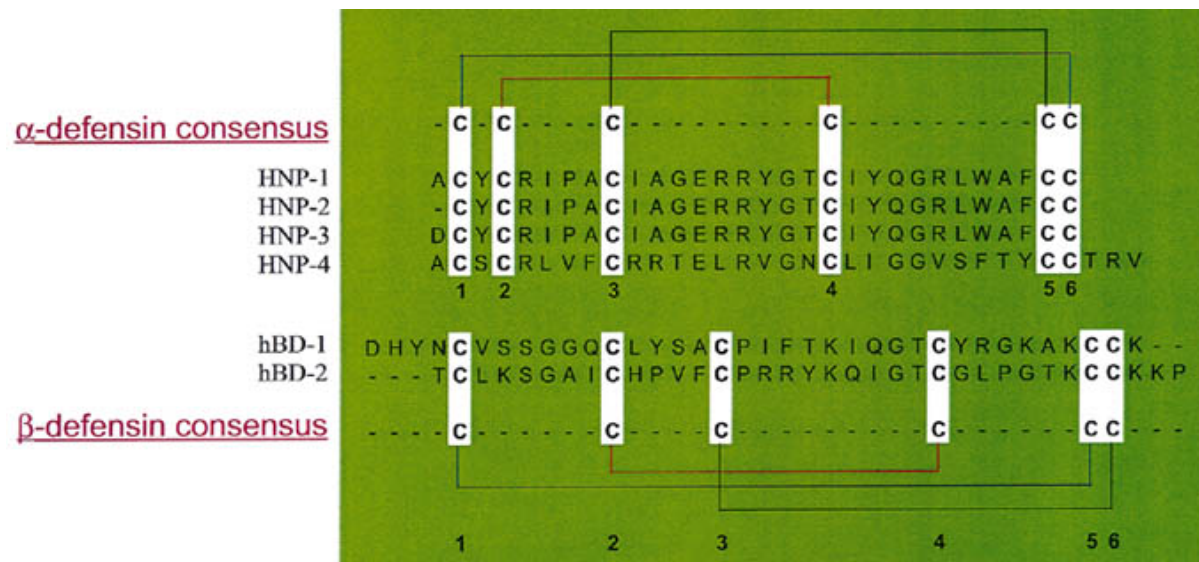


FIG 1. Primary amino acid and consensus sequences for the α - and β -defensins. Boxes indicate the highly conserved cysteines that are numbered 1 to 6. The disulfide linkages of these bridges for α -defensins are established as 1-6, 2-4, and 3-5, whereas the disulfide linkages of the bridges for β -defensins are 1-5, 2-4, and 3-6. The amino acid sequences of the α -defensins HD-5 and HD-6 are not shown.

discussed in this review because there are no data on their expression in lung tissue. In the early 1990s, a second class of defensin was identified in bovine neutrophils and in epithelial cells of bovine tongue (lingual antimicrobial peptides) and trachea (tracheal antimicrobial peptide).⁴ These peptides, which are structurally different from the α -defensins, were termed β -defensins. Additional research revealed that the expression of these epithelial-derived β -defensins increased during inflammation *in vivo* and *in vitro* in response to bacterial LPS.⁵ The finding that defensins are expressed in the epithelial cells that are at the forefront of defense against invading microorganisms, and the notion that the epithelial expression of these peptides is regulated in response to exposure to bacterial products, has led to an intense search for β -defensins in human epithelial cells. The first human β -defensin (hBD-1) was isolated in 1995 from 480 L of hemofiltrate,⁶ and this defensin was later shown to be present in epithelial cells in various organs, including the lung.^{7,8} Two years later, the second human β -defensin (hBD-2) was isolated from psoriatic scales.⁹ Proinflammatory cytokines, bacteria, and fungi were found to increase the expression of this defensin in cultured keratinocytes.

GENE AND PEPTIDE STRUCTURE

Human neutrophil defensins are encoded by a cluster of genes on chromosome 8p23, which include the genes for all known α -defensins except HNP-2.¹⁰⁻¹² Because the HNP-2 peptide lacks the N-terminal amino acid present in the HNP-1 and HNP-3 peptides, it is thought that

HNP-2 is a proteolytic product of either one or both of the HNP-1 or HNP-3 peptides.

Human neutrophil defensins (HNPs 1-4) are small, cationic, and arginine-rich peptides that lack enzymatic activity.¹² The peptides contain 6 conserved cysteine residues that participate in 3 characteristic intramolecular disulfide bridges that are considered to be essential for their antimicrobial activity. Whereas the first 3 peptides (HNPs 1-3) are very similar and differ only in a single N-terminal amino acid, HNP-4 is markedly different in its overall sequence and amino acid composition, sharing only 32% homology with HNPs 1 to 3.¹³ During neutrophil development, the peptides are produced as a result of processing of a propeptide, which itself lacks antimicrobial activity.¹⁴

As observed for the α -defensins, the genes for hBD-1 and hBD-2 are also located on chromosome 8, loci p23.^{15,16} The localization of the gene for hBD-1 is about 100 to 150 kb from that of the gene for HNP-1,¹⁵ suggesting that they share a common ancestral gene. The gene for hBD-1 is highly homologous to other mammalian β -defensins. β -Defensins differ from α -defensins at the level of gene, complementary (c)DNA, and prepropeptide sequences.⁴ The mature hBD-1 peptide consists of 36 amino acids and contains the 6 characteristic cysteine residues.¹⁵ Multiple forms of hBD-1, ranging in length from 36 to 47 amino acids, which differ in their antimicrobial activity, have been purified from human urine.¹⁷ Cysteine pairing distinguishes β - from α -defensins; whereas in α -defensins they are linked 1-6, 2-4, and 3-5, in the β -defensins they are connected 1-5, 2-4, and 3-6 (Fig 1). Because of this highly conserved

6-cysteine motif, members of both subfamilies of defensins have a very similar molecular conformation. The hBD-2 gene spans about 2 kb and is located about 500 to 600 kb from hBD-1.¹⁶ The observation that the distance between the hBD-2 gene and the other defensin genes is larger than that between the other defensin genes led the authors to speculate that in the future additional defensin genes could be identified in this region.

REGULATION OF β -DEFENSIN EXPRESSION

As outlined above, the different defensins are expressed in selected cell types, but thus far it appears that β -defensins are the only defensins expressed in epithelial cells in various tissues. Whereas the expression of human α -defensins and that of the β -defensin hBD-1 is constitutive, hBD-2 expression is induced on epithelial stimulation. In foreskin-derived keratinocytes, the hBD-2 expression rapidly (within 1 hour) increased on stimulation by TNF- α and persisted for more than 48 hours.⁹ In addition, bacteria (gram-negative and gram-positive), as well as fungi (*Candida albicans*), strongly enhanced hBD-2 expression, suggesting that epithelial cells reinforce their antimicrobial defense on contact with microorganisms. This is supported by in vivo data, showing increased hBD-2 protein levels in plasma of patients with bacterial pneumonia¹⁸ and bronchoalveolar lavage (BAL) fluid from patients with cystic fibrosis or inflammatory lung disease.¹⁹ Increased expression of hBD-2 in response to proinflammatory stimuli could in part be explained by the presence of several nuclear factor κ B consensus binding sites in the promoter of the hBD-2 gene, which are absent in hBD-1.¹⁶ The hBD-1 gene contains nuclear factor IL-6 and IFN- γ consensus sites,^{20,21} suggesting that inflammatory mediators may also influence hBD-1 expression. However, studies aimed to analyze the regulation of β -defensin expression in epithelial cells have not revealed modulation of hBD-1 expression.^{8,19,22}

The expression of β -defensins is observed in several organs, but the intensity of expression varies markedly between tissues. The highest hBD-1 messenger (m)RNA expression was detected in the kidney,²² the pancreas,²² and the female reproductive tract.¹⁷ Less, but constitutive, hBD-1 expression was present throughout the conducting airways of the human lung and was detected from proximal bronchi to distal bronchioles in alveolar epithelial cells and in the submucosal glands.¹⁹ Furthermore, in vitro data show hBD-1 expression in cultures of primary human epithelial cells derived from trachea, bronchi, the small airways, and epithelial cells of the mammary gland.^{8,19,22} hBD-2 is mainly expressed in the skin, trachea, and lungs, whereas less expression is observed in the kidney, uterus, and salivary gland tissue.⁹

ANTIMICROBIAL ACTIVITIES

Defensins were originally identified on the basis of their antimicrobial activity, which is directed against

gram-negative and gram-positive bacteria, fungi, and enveloped viruses.^{1,2} Because defensins (HNPs 1-3) constitute 5% to 7% of the total protein content of the human neutrophil and 30% to 50% of the total protein content of the azurophilic granules, it is thought that they are the most abundant antimicrobial proteins present in the neutrophil. In contrast, HNP-4 accounts for approximately 1% of the total defensin content and is less antimicrobial. The microbicidal α -defensin concentration ranges between 1 and 100 μ g/mL and is optimal in low-ionic strength media in the presence of nutrients and in the absence of serum.¹ Therefore α -defensins are thought to exert their antimicrobial activity primarily in the phagolysosome, where they reach high concentrations (in the milligram per milliliter range), rather than in the extracellular milieu.

The mechanism by which α -defensins may kill bacteria is mainly studied on gram-negative bacteria and is thought to be a 2-step process that requires a metabolically active target cell.^{1,2} In the first phase cationic defensins bind to the outer membrane of the bacteria, which results in disruption of the normal barrier property of the membrane. Next, voltage-dependent ion-permeable membrane channels are formed, which lead to membrane permeabilization.^{23,24} During this phase of membrane permeabilization, defensins become internalized and ultimately lead to cell death. Although the mechanism of defensin-induced cell death needs to be explored, it has been suggested that defensin-induced DNA damage, interference with protein synthesis, or both are involved.¹ Defensins do not have antiviral activity against viruses without envelopes, which suggests that the membrane interaction is essential for antimicrobial defensin function.

In addition to α -defensins, β -defensins were also originally discovered because of their antimicrobial activity and display a broad antimicrobial activity against several gram-positive and gram-negative bacteria and certain fungi.^{9,25} Similar to the α -defensins, the antimicrobial activity of hBD-1 and hBD-2 ranges between a concentration of 0.1 to 50 μ g/mL, and it appears that hBD-2 is 10 times more potent than hBD-1. Furthermore, the antimicrobial activity of the β -defensins is decreased in the presence of high sodium chloride concentrations.¹⁹ Whether the antimicrobial activity is also prevented in the presence of serum proteins is still unknown. Also unknown is how β -defensins kill their targets. On the basis of their similarity to α -defensins, it is most likely that β -defensins also kill their targets by creating membrane channels in the target membrane.

THE POTENTIAL ROLE OF DEFENSINS IN THE PATHOGENESIS OF INFLAMMATORY LUNG DISEASE

Initial studies on α -defensins focused on their antimicrobial activity, but subsequent studies revealed that α -defensins may also play a role in inflammation, wound repair, and regulation of the specific immune response

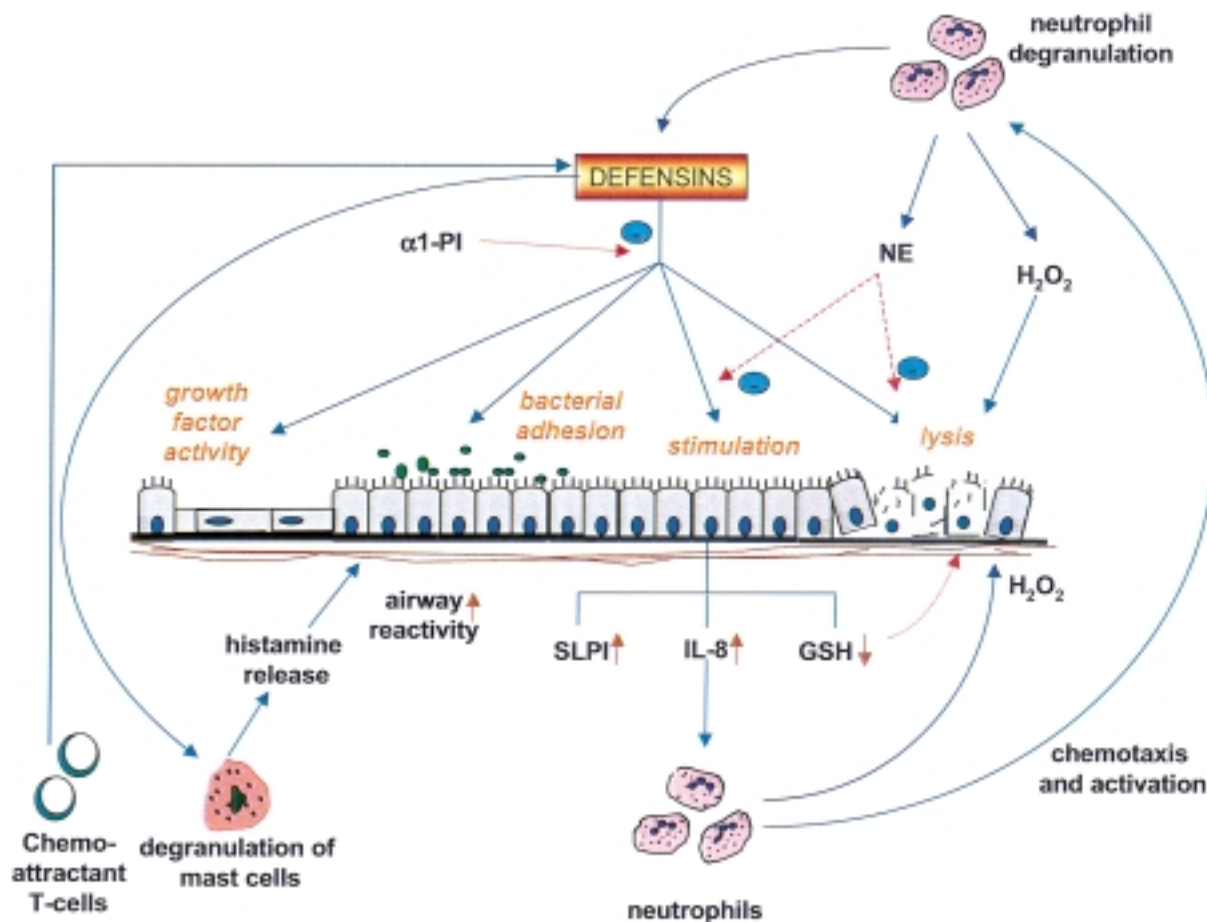


FIG 2. Possible role of neutrophil α -defensins in inflammation and wound repair processes. NE, Neutrophil elastase; GSH, glutathione.

(Table I and Fig 2). Studies on human β -defensins that started in the mid-1990s have so far only focused on their antimicrobial activity. However, on the basis of the structural similarity with α -defensins, it is likely that β -defensins have activities that are distinct from their antimicrobial activity.

α -Defensins

Increasing evidence indicates that the levels of α -defensins are increased in patients with various neutrophil-dominated inflammatory disorders. Elevated defensin levels are observed in the plasma of patients with sepsis or meningitis²⁶ and in patients with idiopathic pulmonary fibrosis.²⁷ In addition, defensins accumulate in the airway secretions of patients with inflammatory lung diseases, such as cystic fibrosis,²⁸ chronic bronchitis,²⁹ α 1-antitrypsin deficiency,³⁰ and adult respiratory distress syndrome.³¹ Furthermore, in the BAL fluid of patients with diffuse panbronchiolitis,²⁷ the defensin levels are increased and found to strongly correlate with the increased IL-8 levels also present in the airway secretions of these patients. This correlation may be explained by the neutrophil attractant activity of IL-8 but also by an effect of α -defensins on IL-8 synthesis.

Recently, we have shown that defensins induce IL-8 synthesis in airway epithelial cells,³² suggesting that defensins may contribute to the perpetuation of an inflammatory response by stimulation of local chemokine release. Furthermore, defensins have been shown to stimulate the production of the neutrophil chemoattractants leukotriene B₄ and IL-8 by alveolar macrophages.³³ Other in vitro studies have shown that neutrophil defensins display chemotactic activity for monocytes³⁴ and T cells,³⁵ but not for neutrophils. In vivo studies also point to a role of defensins in leukocyte recruitment. Subcutaneous injection of defensins in mice results in both a neutrophil and mononuclear infiltrate,³⁵ and defensins may increase the clearance of a bacterial infection in mice in vivo, possibly by recruiting leukocytes to the site of infection.³⁶ Defensins may not only be involved in the regulation of the innate and specific immune responses by regulating cellular influx but have also been shown to induce cytokine release by T cells, such as IFN- γ , IL-6, and IL-10.³⁷ In line with this, defensins were shown to increase systemic IgG, but not IgA, responses on intranasal delivery together with the antigen in mice.³⁷

In a variety of neutrophil-dominated inflammatory

TABLE I. Expression and antimicrobial and other activities of the human α - and β -defensins

	Expression	Antimicrobial spectrum	Other activities
α-Defensins			
HNP-1, HNP-2, HNP-3	Neutrophils	Gr ⁻ and Gr ⁺ bacteria, fungi, enveloped viruses	Cytotoxicity; induction of chemokine/cytokine synthesis in airway epithelial cells and T cells; increased SLPI release by airway epithelial cells; decreased intracellular GSH levels in airway epithelial cells; enhancement of bacterial adherence to airway epithelial cells; induction of LTB ₄ and IL-8 release by alveolar macrophages; induction of histamine release by mast cells; chemotaxis of monocytes and T cells; modulation of cell proliferation; modulation of antibody responses; inactivation of members of the serpin family; inhibition of complement activation; inhibition of fibrinolysis
HNP-4	Neutrophils	Gr ⁻ and Gr ⁺ bacteria, fungi, enveloped viruses	Unknown
HD-5, HD-6	Paneth's cells, epithelial cells of female intestinal tract	Gr ⁻ and Gr ⁺ bacteria, fungi	Unknown
β-Defensins			
hBD-1	Epithelial cells of various origin (constitutive expression)	Gr ⁻ and Gr ⁺ bacteria	Unknown
hBD-2	Epithelial cells of various origin, keratinocytes (inducible expression)	Gr ⁻ and Gr ⁺ bacteria, fungi	Unknown

Gr⁻, Gram negative; Gr⁺, gram positive; GSH, glutathione; LTB₄, leukotriene B₄.

lung disorders, but also in asthma, an increase in epithelial permeability and epithelial injury is a frequent finding.^{38,39} Although this has been attributed to the effect of proteinases, defensins may also contribute to airway epithelial cell damage. This is supported by in vitro studies showing that defensins, in concentrations that are likely to be relevant to the in vivo situation, cause lysis of airway epithelial cells.⁴⁰ Defensins may also promote epithelial cell damage by binding to members of the serine proteinase inhibitor (serpin) family, such as the α 1-proteinase inhibitor (α 1-PI). This binding results in an inability of α 1-PI to bind and inactivate the injurious neutrophil elastase.²⁹ Recent observations from our laboratory suggest that the defensin-mediated inactivation of α 1-PI is possibly counterbalanced in part by an increase in the levels of another elastase inhibitor, the secretory leukocyte protease inhibitor (SLPI). This is based on the finding that defensins increase SLPI protein release by airway epithelial cells, an increase that was not abolished in the presence of α 1-PI.⁴¹ Because both elevated SLPI and α 1-PI levels can be detected in the BAL fluid of patients with chronic obstructive bronchitis and emphysema,^{42,43} our finding may be relevant to the in vivo situation.

In an attempt to delineate the mechanisms that underlie defensin-induced IL-8 production in airway epithelial cells, we recently observed that defensins decrease glutathione levels in airway epithelial cells.⁴⁴ Glutathione is

a potent antioxidant present in the lung and conveys protection against endogenous and exogenous oxidants. The defensin-induced decrease in glutathione may lead to an increased susceptibility of epithelial cells for oxidant-mediated injurious effects, as is indicated by the observation that defensins and H₂O₂ interact synergistically regarding cell lysis.⁴⁵ Various studies have shown that in the lungs of patients with neutrophil-dominated diseases, such as chronic obstructive pulmonary disease (COPD), there are signs of increased oxidative stress and a decreased antioxidant capacity,^{46,47} which leads to a disturbed oxidant-antioxidant balance. Defensins may contribute to this imbalance by reducing glutathione levels in airway epithelial cells.

It has to be noted that most studies on neutrophil defensins are in vitro studies, and therefore the extracellular activity of defensins in vivo remains largely to be established. Because defensins are cationic peptides, it is clear that on neutrophil degranulation, part of the defensins will become rapidly complexed with anionic substances, such as nucleic acids and mucopolysaccharides. They will also bind to various serum proteins, including members of the serine proteinase inhibitor (serpin) family, such as α 1-PI,²⁹ an event that is known to restrict the cytolytic activity of defensins against epithelial cells²⁹ and microorganisms (van Wetering et al, unpublished data) and their ability to induce chemokine synthesis.³² In contrast, in the presence of serum,

defensins retain their chemotactic activity for T cells.³⁵ Furthermore, their ability to induce SLPI secretion by airway epithelial cells is not affected by purified α 1-PI.⁴¹ However, because high defensin levels are found in, for example, purulent sputum of patients with cystic fibrosis,²⁸ it is feasible that these concentrations overwhelm the defensin-binding components and that the actions of defensins observed in vitro may also occur in vivo. This is of particular interest in patients with α 1-PI deficiency, in whom BAL fluid defensin levels are markedly increased compared with normal subjects.³⁰

In addition to these proinflammatory activities, defensins may also contribute to bacterial colonization, as observed in COPD. One of the characteristics of these patients with COPD is that they have recurrent lower respiratory tract infections, in which *Haemophilus influenzae* is frequently found. Defensins were found to increase the adherence of *H influenzae* to cultured airway epithelial cells.⁴⁸ Whereas defensins may thus promote bacterial colonization, another possibility is that defensin-stimulated adherence of *H influenzae* results in exposure to high concentrations of epithelial cell-derived antimicrobial peptides, such as β -defensins or SLPI,⁴⁹ and thus promotes removal of the bacteria. Because defensins are present in airway secretions and at the epithelial surface of lung tissue,²⁷ these findings may be of clinical importance.

Besides the possible contribution of defensins to the pathogenesis of neutrophil-mediated inflammatory lung diseases, defensins might also play a role in asthma. Recent data indicate that increased numbers of neutrophils are present in the airway secretions and airway walls of patients with an asthma exacerbation.⁵⁰ Furthermore, it has been shown that elevated cationic proteins are associated with airway responsiveness. Because defensins are the most abundant cationic protein present in the neutrophil, it is suggested that defensins may contribute to the airway hyperresponsiveness.⁵¹ This is supported by 2 observations. First, defensins have been shown to increase the histamine release by human mast cells.⁵² Second, the tracheal responsiveness to histamine is enhanced in isolated guinea pig trachea,⁵³ indicating that defensins induce an increase in airway hyperresponsiveness.

In addition to its antimicrobial and potential proinflammatory activities, defensins also display anti-inflammatory activities. They inhibit activation of the classical pathway of complement activation by binding to C1q⁵⁴ and have been reported to inhibit fibrinolysis.⁵⁵ These findings may be relevant in view of the high concentration of defensins found in the plasma of patients with sepsis.²⁶ Furthermore, at concentrations that are well below those causing most of their proinflammatory activities, defensins induce epithelial cell proliferation at low, nontoxic concentrations in vitro⁵⁶ and may promote epithelial wound repair in vivo. In line with these observations, we recently demonstrated that at high concentrations, defensins increase the resistance of epithelial cells to neutrophil elastase- or cathepsin G-induced detachment.⁴⁰

β -Defensins

Although the presence of β -defensins is detected in several tissues, data regarding protein levels are limited. Various studies have indicated that both hBD-1 and hBD-2 play an important, although somewhat different, role in the mucosal defense in the lung. This is indicated by the observation that the expression of both hBD-1 and hBD-2 varies between organs but also within one organ. Furthermore, although both hBD-1 and hBD-2 are present in the BAL fluid of patients with cystic fibrosis or idiopathic pulmonary fibrosis, only hBD-1 is also present in healthy volunteers.¹⁹ These data suggest that hBD-1 is important in host defense in the absence of inflammation, whereas hBD-2 is important during inflammation. One of the characteristics of cystic fibrosis is the presence of chronic bacterial colonization in the airways that initiates a chronic inflammatory response. It appeared that because of the increased salt concentration, the airway surface fluid from airway epithelial cells derived from patients with cystic fibrosis lacks antimicrobial activity.^{7,57} In addition, it was shown that blocking of hBD-1 expression abolished the antibacterial activity in airway surface fluid of bronchial xenografts.⁷ These data indicate that in airway secretions from these patients, the activity of antimicrobial peptides, including that of hBD-1 and hBD-2, may be decreased as a result of its increased salt concentration.⁵⁷

Whereas the cystic fibrosis studies clearly demonstrate the potential importance of antimicrobial peptides, such as β -defensins, in host defense against infection, other activities of β -defensins have received little attention so far. On the basis of their homology with α -defensins and the wide variety of activities described for the members of this defensin subfamily, it is likely that future studies will reveal similar activities of β -defensins.

CONCLUDING REMARKS

Although defensins were identified on the basis of their antimicrobial activity, the spectrum of potential defensin activities is now rapidly increasing (Table I). Studying this remarkable family of peptides has not only contributed to new insights into the pathogenesis of chronic inflammatory disorders, such as cystic fibrosis, but also revealed new links between the innate and acquired immune system. Several important questions need to be addressed in the near future. These studies are likely to expand the presently known spectrum of activities of β -defensins. Research aimed to investigate the contribution of both α - and β -defensins to host defense against infection, inflammation, specific immune responses, and wound repair processes in vivo are warranted. In addition, although asthma research in the past has mainly focused on the role of eosinophils, increasing evidence indicates that neutrophils are also involved. Therefore it would be of interest to study the role of defensins regarding possible interactions with eosinophils and their involvement in the severity of the disease.

Finally, studying defensins may contribute to the development of new treatments of infections that are clearly required in view of the increasing problem of acquired resistance of microbes for conventional antibiotics.

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