Chapter 13

PULMONARY COLLECTINS AND DEFENSINS

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INTRODUCTION

Maintaining a sterile respiratory tract presents a unique host defense challenge determined, in part, by the large surface area of lung tissue that comes in direct contact with inhaled pathogens, particles, and gases. The constant exposure of the respiratory tract to microbial pathogens and associated inflammatory molecules is accommodated by a complex innate and acquired immune system that enhances clearance and killing of pathogens, while simultaneously attempting to minimize systemic acquired immune responses and local inflammation. Therefore, it is not surprising that a complex and multifaceted innate immune system has evolved to protect the lung against a large variety of pathogens. Pulmonary cells synthesize a repertoire of host defense molecules that bind, opsonize, or kill various pathogenic organisms. In addition to the contributions of lung parenchymal cells to host defense, phagocytes, leukocytes, mast cells, eosinophils, and lymphocytes also synthesize mediators of innate host defense. Lung parenchymal cells produce numerous small molecules and proteins with antimicrobial activities, including reactive oxygen and nitrogen species, lysozyme, lactoferrin, defensins, phospholipases, complement components, proteinase inhibitor, and secretory IgA. Respiratory epithelial cells also express two members of the collectin family of mammalian lectins, surfactant protein-A (SP-A) and surfactant protein-D (SP-D) that contribute to distinct aspects of innate host defense in the lung (1). This chapter will discuss the critical roles played by both SP-A and SP-D in the orchestration of innate defense, alveolar macrophage function, and modulation of inflammatory responses in the respiratory tract. Lung cells also produce antimicrobial peptides that play key roles in the response to respiratory epithelial compromise and microbial invasion. This chapter will also discuss the antimicrobial mechanisms of these peptides and specifically the role of defensins in acute lung injury (ALI).

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SP-A and SP-D: Structure and Synthesis

SP-A and SP-D are members of the collectin family of the mammalian C-type lectins that also includes mannose-binding protein (MBP) and conglutinin (1). MBP is produced by the liver and secreted into the serum. Children carrying mutations in the MBP gene are more susceptible to recurrent infections (2), supporting the concept that the collectins are an important part of innate immunity against microbial pathogens. The collectins, including SP-A and SP-D, are involved in innate host defense against a variety of bacterial and viral pathogens. Collectins form multimeric structures resembling C1q (the first component of the complement cascade), consisting of multimeric collagenous amino-terminal domains and globular carboxy-terminal, carbohydrate binding domains. The C-type lectins bind carbohydrate surfaces of many microorganisms mediating phagocytosis and killing by phagocytic cells (1).

SP-A is an abundant pulmonary surfactant-associated protein encoded by two genes located on chromosome 10 (3). The primary translation product generated from the SP-A mRNAs encodes a leader sequence that is cleaved during processing, a distinct N-terminal collagen-like, hydrophobic neck, and C-terminal C-type lectin domains (4). The mature peptide forms trimers that assemble to form an octadecamer that is the predominant airway form (5). SP-A binds various lipids and glycolipids including dipalmitoylphosphatidylcholine (DPPC) and binds to surface receptors on alveolar type II (ATII) cells and macrophages (1). In the human, SP-A synthesis is initiated in the developing respiratory epithelium in the second trimester of gestation, and is expressed by ATII cells, Clara cells, and subsets of cells in tracheo-bronchial glands in the adult lung (6).

SP-D was first identified as a component of pulmonary surfactant. It is now well known that SP-D is also expressed by a variety of non-pulmonary tissues in humans and other mammals (7). SP-D is encoded by a single gene located in close proximity to SP-A and other members of the collectin family of mammalian lectins, located on chromosome 10. SP-D mRNAs encode a complex polypeptide, that contains a signal peptide, a distinct amino-terminal collagen-like, α-helical coiled coil neck, and C-terminal C-type lectin domains (8). In the human lung, SP-D is expressed primarily in ATII cells and in serous cells in tracheal-bronchial glands. SP-D binds to phosphatidylinositol of pulmonary surfactant lipids (1).

SP-A and SP-D: Phagocytosis and Killing of Microorganisms

In vitro studies support a role for SP-A in enhancing microbial phagocytosis by macrophages. SP-A acts as opsonin and can directly stimulate macrophage activity (1). SP-A binds a variety of microorganisms *in vitro* (Table 1). SP-A also enhances calcium-dependent neutrophil uptake

of *E. coli*, *S. pneumoniae* and *S. aureus* (9). In addition to binding microorganisms, SP-A is chemotactic for alveolar macrophages and peritoneal macrophages (10). The structure and activity of SP-A *in vitro* strongly supports a role for SP-A in clearance of microbial pathogens from the lung as part of the innate immunity. As will be discussed below, an *in vivo* role for SP-A-mediated innate immunity has now been established by the use of SP-A deficient mice.

Table 1. Pathogens bound by SP-A (References 1, 11-17)

Gram-positive bacteria	Gram-negative bacteria
Group A *Streptococcus*	E. *coli* J5
Group B *Streptococcus*	*H. influenzae* (type a)
S. aureus	*K. pneumoniae*
S. pneumoniae	*P. aeruginosa*
Viruses	Fungi
Adenovirus	*A. fumigatus*
Cytomegalovirus	*C. neoformans*
Herpes simplex virus, Type 1	
Influenza virus A (H3N2)	Parasites
Respiratory syncytial virus	*P. carinii*

In vitro studies also support a role for SP-D in microbial phagocytosis by alveolar macrophages. Similar to SP-A, SP-D can bind Gram-negative and Gram-positive bacteria, viruses, fungi and parasites in a calcium-dependent manner (Table 2), and also enhances calcium-dependent uptake of *E. coli*, *S. pneumoniae* and *S. aureus* by neutrophils (9). In addition to binding microorganisms, SP-D is chemotactic for monocytes and neutrophils at lower concentrations than required for SP-A-dependent chemotactic activity (18). These *in vitro* findings strongly support the role of SP-D in binding and opsonization of microbial pathogens in the lung.

Table 2. Pathogens bound by SP-D (References 1, 17, 19, 20)

Gram-positive bacteria	Gram-negative bacteria
Group *B Streptococcus*	*E. coli*
	H. influenzae
	K. pneumoniae
	P. aeruginosa
	S. minnesota
Viruses	Fungi
Influenza virus A	*A. fumigatus*
Respiratory syncytial virus	*C. neoformans*
	Parasites
	P. carinii

SP-A and SP-D: Production of Free Radicals

Reactive oxygen and nitrogen species are generated by phagocytic cells and have an important role in killing of ingested and extracellular pathogens, and also have a role in both intracellular and intercellular signaling pathways. *In vitro* studies provide conflicting results regarding the role of SP-A in regulating oxygen radical production. In two different studies, SP-A stimulated the release of oxygen radicals from alveolar macrophages (21, 22). In contrast, other studies have found that SP-A decreases PMA-stimulated superoxide production in canine neutrophils and alveolar macrophages (23), and in rat alveolar macrophages (24). The conformation of SP-A may be important in the regulation of oxygen radical production by phagocytic cells and may explain, in part, the differences found in these *in vitro* studies. Stimulation of oxygen radicals by SP-A was not seen with rat peritoneal macrophages, rat or human neutrophils, or human peripheral blood monocytes (21) suggesting that SP-A regulation of oxygen radical production is specific for alveolar macrophages.

Nitric oxide (NO) is a reactive nitrogen species, which also has a role in phagocytic cell-mediated host defense (see Chapters 6 and 7). A variety of inflammatory stimuli increase alveolar macrophage NO production through the induction of inducible NO synthase. SP-A has variable effects on NO production. Similar to the observations regarding reactive oxygen species, SP-A does not stimulate NO production by peritoneal macrophages or ATII cells (25). Purified SP-A is often contaminated with lipopolysaccharide (LPS), which may explain differing results between *in vitro* studies, since LPS can stimulate immune cell function. SP-A treated to remove LPS does not stimulate NO production by macrophages (26), however, SP-A inhibits production of NO in macrophages stimulated with LPS (27). Alveolar macrophages stimulated with interferon-γ (IFN-γ), IFN-γ plus LPS (27), or IFN-γ followed by *Mycoplasma pulmonis* infection (28) generate more NO in the presence of SP-A. In contrast, SP-A inhibits NO production by IFN-γ activated macrophages incubated with *Mycobacterium tuberculosis* (29). These studies suggest that SP-A exerts differential effects on the production of reactive nitrogen species by alveolar macrophages depending on the stimulus and the activation state of the immune cells.

SP-D, similar to SP-A, enhances the production of oxygen radicals as assessed by monitoring lucigenin-dependent chemiluminescence by rat alveolar macrophages (30). This response appears to be specific for alveolar macrophages, as SP-D does not stimulate the production of oxygen radicals by neutrophils (31). SP-D, treated to remove endotoxin, does not stimulate production of NO by alveolar macrophages (26). In addition, SP-D does not affect the production of NO by macrophages stimulated with either LPS or IFN-γ (27). Further studies with SP-D are necessary to determine its role in regulating production of reactive oxygen and nitrogen species.

SP-A and SP-D: Lymphocyte Activation

T cells are active participants and regulators of acute pulmonary inflammation due to the release of cytokines. SP-A enhances proliferation of concanavalin A (Con A) stimulated spleen lymphocytes (32, 33). In contrast, SP-A suppresses phytohemagglutinin (PHA)-driven T cell proliferation and anti-CD-3 stimulated T cell proliferation (33). The three mitogens used in these studies have slightly different binding sites, but similar biochemical pathways for T-cell activation and proliferation (34). PHA and Con A bind to T-cells via the T-cell receptor, whereas anti-CD-3 binds to the T cell via the CD3-complex. These studies suggest that SP-A disrupts co-stimulatory signals unique or dominant in PHA- and anti-CD3-mediated T cell activation, and support a role for SP-A in regulating T cell responses depending on the mitogen encountered in the lung.

SP-D, similar to SP-A, modulates activation of T cells. SP-D has an inhibitory effect on Con-A, PHA, and anti-CD3 T cell proliferation (35). In addition, SP-D has a suppressive effect on allergen-stimulated lymphocyte proliferation of lymphocytes obtained from asthmatic and healthy children (36). These results suggest that SP-D plays an important role in regulating T cell responses in the lung.

SP-A Dependent Cytokine Production

Cytokines are a diverse group of biologically active proteins, many of which are thought to contribute to the pathogenesis of ALI by increasing the production of substances that promote local and systemic inflammatory processes. SP-A stimulates production of the cytokines TNF-α, IL-1α, IL-1β, and IL-6 by human peripheral blood mononuclear cells. SP-A also stimulates the production of TNF-α by rat peripheral blood mononuclear cells, alveolar macrophages, and splenocytes (1). Other studies have found that SP-A inhibits TNF-α release by alveolar (37) and interstitial (38) macrophages stimulated with LPS, inhibits the production of IL-2 by stimulated human lymphocytes (33), and inhibits IL-8 production by stimulated human eosinophils (39). Alveolar macrophages infected with *Candida albicans* generate less proinflammatory cytokines (TNF-α, IL-1β, macrophage inflammatory protein-1α, and monocyte chemoattractant protein-1) in the presence of SP-A (40). The reported variable effects of SP-A on cytokine release *in vitro* may be due to differences in study conditions and purification methods of SP-A. Despite these limitations, however, the studies support a role for SP-A in modulating cytokine expression in the lung during ALI. Relatively less is known regarding SP-D-dependent regulation of cytokine production.

SP-A Gene Deficient Mice

To investigate the role of SP-A in the regulation of immune function *in vivo*, our laboratory generated mice in which the SP-A gene was ablated by homologous recombination (SP-A deficient mice). Lung function is unchanged in SP-A deficient mice, thereby providing a powerful model for assessing the *in vivo* role of SP-A in host defense. SP-A deficient mice have increased susceptibility to infection by various pathogens including Group B *Streptococcus* (11), *Pseudomonas aeruginosa* (41), *Haemophilus influenza* (19), *Klebsiella pneumoniae* (42), *Mycoplasma pulmonis* (28), respiratory syncytial virus (43), adenovirus (14), influenza A virus (*LeVine et al unpublished data*), and *Pneumocystis carinii* (*Linke et al unpublished data*). Alveolar macrophages from the SP-A deficient mice display impaired phagocytosis and have an impaired ability to generate reactive oxygen species (11). In addition, inflammation is increased in the lungs of SP-A deficient mice following bacterial and viral infection (11, 43). These data, together with many *in vitro* studies, suggest that SP-A may opsonize bacteria and viruses and enhance their phagocytosis and clearance from the airspaces, thereby providing an early line of defense against pulmonary infection (Figure 1).

SP-D Gene Deficient Mice

To investigate the role of SP-D in the regulation of immune function *in vivo*, our laboratory also generated mice in which the SP-D gene was ablated by homologous recombination (SP-D deficient mice). Surfactant lipid pools and alveolar macrophages are increased in SP-D deficient mice. In the absence of SP-D the mice develop progressive emphysema. In addition, SP-D deficient mice have increased lung production of reactive oxygen species and metalloproteinases, both of which are postulated to play important roles in the development of emphysema (44). The phenotype observed in SP-D deficient mice supports an important role for SP-D in surfactant and lung architectural homeostasis.

With regard to SP-D and *in vivo* immune function, SP-D deficient mice efficiently cleared both Group B *Streptococcus* and *Haemophilus influenzae* from the lung (19). Despite the ability to clear bacteria from the lung, deficiency of SP-D is associated with increased inflammatory cell recruitment to the lung after infection. Alveolar macrophages from the SP-D deficient mice display impaired phagocytosis compared to macrophages from wild type mice. Oxygen radical production by the alveolar macrophages was markedly increased in SP-D deficient mice. In contrast to the efficient bacterial clearance, SP-D deficient mice are susceptible to both influenzae A (*LeVine et al, unpublished data*) and respiratory syncytial viral pneumonia, including increased expression of proinflammatory cytokines

and increased neutrophilic infiltrates (45). These finding suggest that SP-D plays a role in modulating cytokine production and inflammatory responses during viral and bacterial infections in the lung (Figure 1).

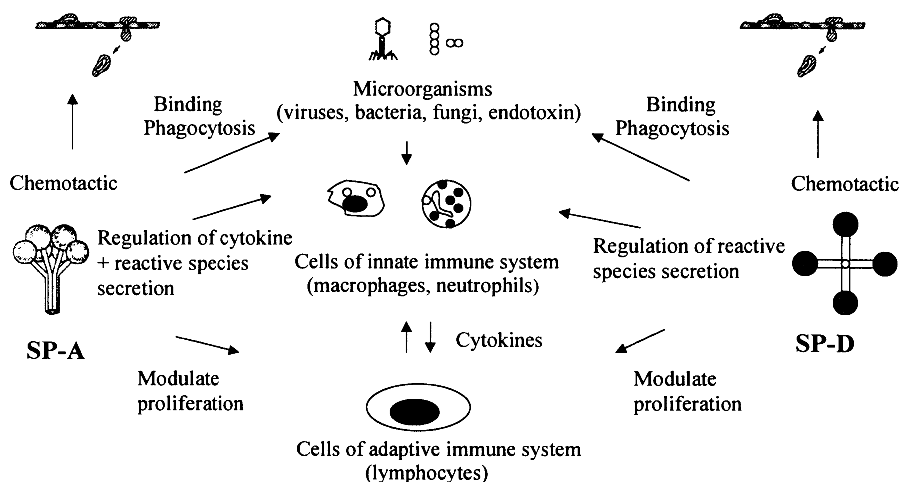

Figure 1. Model for SP-A and SP-D involvement in host defense in the lung. The surfactant-associated proteins SP-A and SP-D are members of a family of host defense lectins, designated collectins. There is increasing evidence that these pulmonary proteins are important components of the innate immune response to microbial challenge. SP-A and SP-D can bind to a broad spectrum of pathogens, including bacteria, viruses, fungi, parasites, as well as lipopolysaccharides (LPS, endotoxin) and allergens. In addition, SP-A and SP-D enhance the clearance of various pathogens by macrophages and neutrophils by aggregation of the pathogens or by direct interaction of the collectins with receptors on the phagocytic cells. The surfactant proteins have also been shown to have important roles in modulating the immune response including cytokine production, reactive species production, and lymphocyte proliferation.

SP-A and SP-D: Role in Lung Disease

SP-A levels are altered in a variety of pulmonary diseases. SP-A levels are lower than normal in both Gram-positive and Gram-negative bacterial pneumonia (1). SP-A levels relative to total protein are decreased in tracheal aspirates of children with bacterial pneumonia, viral pneumonitis, and ALI (46). In the lungs of patients with ALI, both lipid and SP-A levels are decreased and SP-A levels correlate with disease severity. Patients with severe lung injury have low levels of SP-A in the lung, however, with moderate lung injury SP-A levels return to more normal levels and actually increase to greater than control values 3 to 6 days after trauma (47). Similarly, SP-A concentrations are reduced in the lung of patients at risk for ALI who subsequently develop clinically apparent ALI and remain low for

as long as 14 days in patients with sustained ALI (48). Bronchoalveolar lavage (BAL) fluid from patients with ALI contains SP-A that is cleaved, possibly due to proteolytic cleavage by neutrophil elastase (49). In contrast, serum concentrations of SP-A increase in patients with ALI and acute cardiogenic pulmonary edema (1, 48). Increased serum levels of SP-A are thought to be due to SP-A leakage through the damaged pulmonary capillary endothelial/epithelial barrier into the serum.

Other conditions associated with reduced SP-A in the lung include infant respiratory distress syndrome, bronchopulmonary dysplasia, idiopathic pulmonary fibrosis, asthma, and cystic fibrosis (1, 50). In contrast, some pulmonary conditions are associated with increased SP-A levels in the lung including hyperoxia-related lung injury, AIDS-related pneumonia with *P. carinii*, silicosis, alveolar proteinosis, and hypersensitivity pneumonitis (1). Intratracheal instillation of LPS increases surfactant proteins in the lung associated with a proliferation of ATII cells (51) suggesting that increased protein synthesis and possibly changes in protein turnover may contribute to the increased SP-A levels secondary to LPS stimulation.

The regulation of SP-D production in the setting of lung disease has not been investigated as thoroughly as the regulation of SP-A. SP-D levels in BAL fluid are normal in patients at risk for ALI and those that develop ALI, however, serum SP-D levels increase in patients with ALI (48). These finding are in contrast to those with SP-A suggesting that SP-D may be regulated differently in the lungs of patients with ALI. In animal models, silica, LPS, and *P. carinii* infection increase SP-D levels in the lung. SP-D levels are increase in BAL fluids of patients with alveolar proteinosis, but not with idiopathic pulmonary fibrosis, interstitial pneumonia secondary to collagen-vascular diseases, or pulmonary sarcoidosis (1).

SP-A and SP-D: Summary Comments

The lung is constantly exposed to toxins, antigens, and microbes that can be efficiently cleared without the generation of destructive inflammatory responses. The pulmonary collectins SP-A and SP-D, traditionally associated with pulmonary surfactant function (i.e. lowering alveolar surface tension), are now also recognized as central modulators of innate host defense and inflammation in the lung. SP-A and SP-D are mammalian lectins capable of recognizing pathogens, leading to binding and enhancing phagocytosis of microbes. Furthermore, SP-A and SP-D modulate production of reactive oxygen species involved in microbial killing, and orchestrate cellular inflammatory responses to diverse types of respiratory pathogens, including bacteria and viruses.

Defensins: Structure and Synthesis

Antimicrobial substances used by host cells range from simple inorganic compounds (e.g. hydrogen peroxide, hypochlorous acid, NO) to relatively complex antimicrobial peptides. Some antimicrobial peptides are enzymes (proteases, and hydrolytic enzymes such as phospholipase, glycosidases, and lysozyme) that can digest the protective layers of microbes, while other antimicrobial peptides directly disrupt biological membranes. Antimicrobial peptides have been identified in organisms as diverse as humans, frogs, insects, plants, and protozoa. The antimicrobial peptides are microbicidal at micromolar concentrations against a wide range of target organisms. One family of antimicrobial peptides, the defensins, were named in 1985. Apart from antimicrobial activity, defensins also play a role in inflammation, wound repair, and specific immune responses (acquired immunity).

Mammalian defensins are cationic antimicrobial peptides characterized by the presence of six cysteine residues that form three intramolecular disulfide bonds. Defensins can be subdivided into two classes, the α-defensins and the β-defensins, based on alternative spacing of their six cysteine residues and differences in the alignment of the disulfide bridges. Despite their differences in sequence and disulfide bond pattern, the α- and β-defensins share a similar three-dimensional structure in solution (52). They also exhibit comparable antimicrobial activity, have genes closely clustered and are expressed in similar anatomical locations. Some of the defensin encoding genes are constitutively expressed, while others are inducible by infections and/or inflammatory agents.

The α-defensins are 29 to 35 amino acids in length and contain three disulfide bridges. α-Defensins are found in great abundance in the azurophilic granules of circulating neutrophils (5-18% of total protein) and in granule-containing Paneth cells of the small intestine (53-56). The human α-defensin family consists of four defensins isolated from neutrophils, HD-1 through HD-4, and two defensins expressed in the secretory granules of Paneth cells of the small intestine and epithelial cells of the female genital tract, HD-5 (55, 57) and HD-6 (58). Low level expression of HD-5 has been found in human nasal and bronchial epithelial cells (59).

The β-defensins are 36 to 42 amino acids in length with six cysteines in a spacing pattern and a disulfide alignment (60). In most tissues the constitutive level of β-defensin expression seems to be low, however, expression is increased during inflammation *in vivo* and *in vitro* in response to LPS (61). Two human β-defensins have been identified, HBD-1 (62) and HBD-2 (62). The gene encoding HBD-1 has been shown to be expressed in epithelial tissues including kidney, lung (both upper respiratory tract and parenchyma), pancreas, testis, gingival tissue, and vagina. HBD-2 is expressed in skin and tracheal mucosa (64). Expression of HBD-2 is

increased in cultured keratinocytes exposed to pro-inflammatory cytokines, bacteria, and fungi, suggesting that HBD-2 synthesis is induced during inflammation (65).

A cluster of genes on chromosome 8p22-23.1 encodes HBD-1 and HBD-2 immediately adjacent to the α-defensin locus (63,66). β-defensin molecules consist of a structurally rigid triple-stranded, anti-parallel β-sheet stabilized by intramolecular disulfide bonds, as demonstrated by NMR spectroscopy and x-ray crystallography (52). The mature β-defensin sequence consists of the carboxy terminus of a pre-pro-peptide (93 to 95 amino acids) containing an amino-terminal signal sequence followed by a 40 to 45 amino acid anionic pro-piece, which may be important for neutralization, processing, and folding of the cationic c-terminal peptide (67,68).

Antimicrobial Activity of Defensins

Defensins were originally identified based on their antimicrobial activity against Gram-negative and Gram-positive bacteria, fungi, and enveloped viruses (60). Microorganisms susceptible to human defensin-mediated killing are presented in Table 3. Human defensins 1 to 3 seem to be the major bacterial peptides in human neutrophils constituting 5-7% of total protein content in neutrophils and 30-50% of total protein content of the azurophilic granules. Human defensin 4 has a different antibacterial spectrum compared to HD 1-3 and is present in neutrophils at low concentrations (69). α-Defensins are microbicidal at 1-100 μg/mL (60) and are thought to have antimicrobial activity primarily in the phagolysosome where they reach high concentrations. Increasing salt concentrations competitively inhibit defensin activity (52). Epithelial cells have initial contact with microbes providing the first line of host defense prior to neutrophil recruitment. The β-defensin are expressed by epithelial cells and also have antimicrobicidal activity against Gram-positive and Gram-negative bacteria and fungi at concentrations of 10-100 μg/mL and HBD-2 is 10 times more potent than HBD-1 (60).

Defensins are microbicidal rather than static agents. Bacterial killing occurs in minutes and in most cases requires bacterial cell growth. Defensins interact with bacterial membranes by disrupting the order of the phospholipid bilayer causing loss of membrane integrity. The energy gradient across the membrane is destroyed, membrane damage occurs, and lysis of the bacteria follows. Studies supporting the interaction of defensins at the plasma membranes have demonstrated that defensins induce leakage of potassium and other cellular constituents (75). Defensins also form voltage-dependent, weakly anion-selective channels in planar lipid bilayer membranes (76).

Table 3. Antimicrobial Spectrum of Human Defensins (References 52, 60, 70-74)

Gram-positive bacteria	Gram-negative bacteria
Staphylococcus aureus	*Acinetobacter calcoaceticus*
	Capnocytophaga spp. strains
	Escherichia coli
	Pseudomonas aeruginosa
Viruses	Fungi
Cytomegalovirus	*Chlamydia trachomatis*
Herpes simplex virus, Type 1 and 2	*Cryptococcus neoformans*
Influenza virus A/WSN	*Giardia lamblia*
Vesicular stomatitis virus	

Role of Defensins in Inflammation

In addition to the antimicrobial activity, defensins may also play a role in inflammation and regulation of specific immune responses. The modulating effects of α-defensins on inflammation include chemotaxis, histamine release by mast cells, stimulation of wound repair, and apoptosis (77-79). Defensins are chemotactic for monocytes (78) and T lymphocytes (79), and stimulate cytokine production by T lymphocytes (80). Recent studies demonstrated expression of defensins (HD 1-3) in human T and natural killer cells (NK) (81). β-defensins attract immature dendritic cells and memory T cells, which initiate a primary and recall immune response, respectively (82). These findings provide evidence for a role of defensins as a link between the innate and adaptive immune responses.

α-Defensins are increased in disease states characterized by neutrophilic infiltrates. Defensin levels are increased in airway secretions with inflammatory lung diseases such as ALI, chronic bronchitis, α-1 antitrypsin deficiency, and cystic fibrosis (83). In addition, defensin levels are increase in the plasma of patients with sepsis or meningitis (84). In patients with diffuse panbronchiolitis, BAL fluid levels of defensins are increased in parallel with the neutrophil chemoattractant, IL-8 (85). *In vitro,* defensins induce IL-8 secretion in airway epithelial cells (86). Collectively, these data suggest that the defensins may stimulate chemokine expression and mediate the recruitment of neutrophils into the airways. In addition, defensins stimulate the release of leukotriene B_4 by neutrophils and IL-8 by macrophages (83), both neutrophil chemoattractants. *In vivo,* subcutaneous injection of defensins in mice produces a neutrophil and macrophage infiltrate (79) further supporting a role for defensins in regulating the inflammatory response that is typically seen during ALI.

Defensins in ALI

ALI is characterized by epithelial/endothelial injury and increased permeability, neutrophil accumulation in the airspaces, and increased pro-inflammatory cytokines within the lung compartment. Neutrophil-mediated lung injury is due, in part, to the serine proteases and neutrophil defensins which both affect the integrity of the epithelial layer, decrease the frequency of ciliary beating, increase secretion of mucus, and induce the synthesis of epithelial-derived mediators that may influence the amplification and resolution of inflammation. Neutrophil products that mediate tissue injury at sites of inflammation include the neutrophil serine proteinases, elastase, cathepsin G, proteinase 3, and the nonenzymatic defensins (87). To protect against the extracellular activity of serine proteinases, the lung is equipped with serine proteinase inhibitors. Defensins bind to members of the serine proteinase inhibitor (Serpin) family such as α-1 proteinase inhibitor (α1PI), alpha 1- antichymotrypsin, alpha 2-antiplasmin, and antithrombin III. When α-1PI, an inhibitor of neutrophil elastase, is bound to the defensins it is unable to bind and inactivate neutrophil elastase (88), which can injure lung tissue. *In vitro*, defensins cause lysis of airway epithelial cells. Co-incubation of elastase with defensin *in vitro* reduces defensin-induced cell lysis and prevents defensin-induced IL-8 synthesis (89).

In addition to the release of proteases by neutrophils, the production of reactive oxygen species can cause damage to the lung. Oxidative damage follows increased oxidative stress from reactive oxygen species from activated neutrophils and macrophages, and from the use of high oxygen concentrations during treatment. Glutathione, a potent antioxidant in the lung, protects against oxidant injury. Alterations in alveolar and lung glutathione metabolism are recognized as a central feature in ALI (87). *In vitro*, defensins decrease glutathione levels in airway epithelial cells (90), which may result in an imbalance in antioxidant versus pro-inflammatory responses to oxidative stress resulting in lung damage. In addition, the combination of defensins with hydrogen peroxide, both secreted by activated neutrophils, act synergistically to cause cell lysis (91) suggesting that defensins may have a pro-inflammatory role during ALI.

Complement activation, either within the lung or within the circulation, is associated with lung injury (see Chapter 5). C5 fragments (C5a and C5a-des-Arg) are especially potent PMN chemotaxins and activators. If complement activation is extensive, endothelial and type II epithelial damage can occur, accompanied by edema, hemorrhage, and fibrin deposition (87). Defensins have anti-inflammatory activities by inhibiting activation of the classical complement pathway (92) by binding to C1q (the first component of complement).

Extravascular fibrin deposition in ALI occurs, in part, due to depression of the fibrinolytic activity in the distal airspaces of the lung (87). Plasminogen concentrations in BAL fluid of patients with ALI are increased

compared with values in control patients, however, most of the plasminogen activator in BAL fluid is coupled to inhibitors (93). Defensins have been reported to inhibit fibrinolysis possibly by shielding of fibrin-bound plasminogen from activation by tissue type plasminogen activation (94).

LPS (endotoxin) has been implicated as an important agent in the induction of ALI. Endotoxin levels in the plasma of patients with ALI, or patients at risk that subsequently develop ALI, are increased compared with values measured in patients who do not develop ALI (95). *E. coli* endotoxin enhances complement-mediated neutrophil activation and superoxide production, and tissue injury (87). *In vitro*, LPS and TNF-α induce expression of defensins in tracheal epithelial cells (61). The inducible response of the defensins may represent a mechanism to recognize Gram-negative bacteria at epithelial surfaces and mount a host defense response important in preventing colonization and/or subsequent infection and inflammation.

Data regarding BAL levels of β-defensins is limited. In healthy volunteers, HBD-1 and HBD-2 are present in BAL fluid. Both HBD-1 and HBD-2 are present in BAL fluid of patients with cystic fibrosis or idiopathic pulmonary fibrosis (83). Plasma from patients with bacterial pneumonia contains greater concentrations of HBD-2 compared to plasma from healthy controls (96). *In vitro*, HBD-2 is inducible in airway epithelial cells either upon contact with *Pseudomonas aeruginosa*, and with stimulation by LPS, TNF-α, or IL-1β (97).

The bactericidal activity of the β-defensins is affected by local salt concentrations. In this regard, the airway surface fluid from patients with cystic fibrosis lacks antimicrobial activity believed to be due to the increased salt concentrations, which may decrease the activity of hBD-1 and hBD-2 (98). Recently the effect of salt concentration effect on the β-defensins has been questioned and other factors such as mucin or DNA have been proposed to inhibit bactericidal activity and could be of equal importance in inactivation of defensins in lung disease associated with cystic fibrosis. Future studies are necessary to further characterize the role of the β-defensins in ALI.

Summary

ALI may be the result of direct damage to the lung by the inciting disease or agent, or alternatively, injury may be the result of indirect damage resulting from the formation of oxidants, release of proteases, or release of mediators as part of the host response to the inciting agent. Since the lung is the usual site of entry of respiratory pathogens the pulmonary collectins and defensins play an important role in initial host defense and regulation of inflammation in the lung. Insufficient expression of collectins and defensins

may predispose to disease and may be markers for prediction of disease. In addition, further investigation of the role of collectins and defensins in ALI may provide novel therapeutic agents for prevention and treatment of pulmonary infections.

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