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Surfactant proteins and the inflammatory and immune response in the lung

I. BARBERI
S. D'ARRIGO
E. GITTO

N.I.C.U., Az. Osp. Universitaria - G. Martino - Università degli Studi di Messina, Italy

A B S T R A C T

Surfactant proteins are important for regulating surfactant activity and innate host defence; in particular, polymorphisms in intron 4 of the SP-B gene and dominant mutations of SP-C have been associated with bronchopulmonary dysplasia. The innate immune system is older and consists of soluble proteins, which bind microbial products and phagocytic leukocytes resembling primitive amoebae, which float through the bloodstream and migrate into tissues at sites of inflammation, or reside in tissue waiting for foreign material. The innate immune system is always active and is immediately responsive, ready to recognize and inactivate microbial products entering lungs and other tissues. Pro-inflammatory cytokines (interleukins IL-1 β , IL-6 and soluble ICAM-1) are present in lung lavage fluid from day 1 in premature infants with respiratory distress and reach a peak in the second week. IL-1 β induces the release of inflammatory mediators, activating inflammatory cells and up-regulating adhesion molecules on endothelial cells. ICAM-1 is a glycoprotein, promoting cell-to-cell contact. Direct contact between activated cells leads to further production of proinflammatory cytokines and other mediators. α -chemokine IL-8 is released, including neutrophil chemotaxis; the activated neutrophils mediate endothelial cytotoxicity, inhibit surfactant synthesis and release elastase.

Pulmonary surfactant serves two primary functions in the lung: 1) it is a *surface-acting agent* that lowers surface tension at the aqueous-air interface of the alveolar surface and 2) the surfactant, specifically its components hydrophilic surfactant proteins SP-A and SP-D (known as *collectins*), is an important component of the innate immune response in the lung.

Natural surfactant is a complex mixture of phospholipids, neutral lipids and proteins. Each of the components and their balance are important an adsorption, film formation, and film behavior at the alveolar surface. Mathematically, surface tension can be expressed by the Laplace law, which states that the pressure (P) within an elastic sphere is directly proportional to the tension (T) of the wall and inversely proportional to the radius (r) of the curvature: $P = 2 \times T/r$.

Applying this principle to the alveolus, P is the pressure inside the alveoli, T represents the surface tension at the interface, and r is the alveolar radius. The presence of surfactant in the liquid lining the alveoli allows the surface tension to change as the surface area of the alveolus changes. Surfactants are molecules that have an ener-

getic preference for interface because of their amphipathic nature. Pulmonary surfactant lowers surface tension in a dynamic rather than static manner. As the alveolar *bubble* size decreases during exhalation, the surfactant film is compressed and surface tension approaches zero. As the *bubble* radius increase with inspiration, the surfactant film is expanded and surface tension proportionately increases, compensating for the effects of the increasing radius. In surfactant-deficient states, some alveoli collapse while some patent alveoli overexpand, generating ineffective gas exchange within a nonhomogeneously inflated lung. The consequences of surfactant deficiency are seen commonly in the diffuse atelectasis in preterm infants with infant respiratory distress syndrome.

Four surfactant proteins have been identified, SP-A, B, C and D. SP-A and SP-D. Surfactant proteins (SP) are divided into the hydrophobic surfactant proteins SP-B and SP-C and the hydrophilic surfactant proteins SP-A and SP-D. While SP-B and SP-C lower the alveolar surface-tension, SP-A and SP-D primarily mediate the host-defence function of pulmonary surfactant. Surfactant protein A and SP-D are col-

lectins (collagen-lectins), which consist of four structural domains, including a collagenous domain and a C-terminal carbohydrate recognition domain (CRD). The CRD mediates interactions with the pathogen, thereby mediating cellular phagocytosis. Collectins bind to cell-surface receptors on alveolar macrophages and type II cells. Surfactant protein D knock-out mice progressively develop emphysema, pulmonary inflammation, accumulation of surfactant phospholipids and lipid-laden macrophages.

SP-B and SP-C can be extracted with the phospholipids in commercial surfactants, whereas the hydrophilic proteins SP-A and SP-D are lost during the extraction process. The presence of SP-B and SP-C is probably the primary reason for the greater efficacy of *natural* relative to synthetic surfactants. Although surfactant seems to be produced by type II cells in the lung, its synthesis can be affected by glucocorticoids, estrogens and androgens. Secretion of surfactant from type II cells can be stimulated by β -adrenergic and cholinergic agonists and by factors such as hyperinflation within the lung parenchyma. Surfactant has a quick turnover rate and is catabolized by type II epithelial and alveolar macrophages. Maternal corticosteroid treatment significantly improves fetal lung development by maturation of type II cells and enhancement of the gas exchange surface area. Maternal steroid treatment is considered a standard of care for the delivery of preterm infants between 24 to 34 weeks gestational age.

Surfactant protein D is primarily expressed and secreted by type II alveolar cells but is also detected in Clara cells and in the tracheal and bronchial glands of the lower airways.^{1,2} Although SP-D was first identified in the respiratory tract, current studies demonstrate the expression of SP-D in almost all mucosal surfaces, including epithelial cells in exocrine ducts, the mucosa of the gastrointestinal and genitourinary tract and recently in human tear fluid, protecting the eyes against *Pseudomonas aeruginosa* invasion.⁴ Surfactant protein D binds with high affinity to alveolar macrophages and improves the phagocytosis of bacteria by increasing the activity of recognition receptors on alveolar macrophages.⁵ Furthermore, alveolar macrophages participate in SP-D turnover by internalization and degradation of SP-D. High numbers of apoptotic alveolar macrophages are found in the lungs of SP-D-deficient mice.⁶ The intrapulmonary administration of human recombinant SP-D to these mice reduces the alveolar macrophage apoptosis,⁷ promotes the clearance of apoptotic alveolar macrophages⁸ and decreases lung inflammation.⁹ Surfactant protein D modulates the migration of monocytes and neutrophils mediated via the CRD domain. The SP-D was recently found to recognize DNA and RNA of apoptotic cells¹⁰

by both the CRD and the collagen-like region. Thereby, SP-D enhances the uptake of DNA by human monocytes and macrophages¹¹ and enables the clearance of DNA released by necrotic cells or pathogens and minimizes the generation of anti-DNA antibodies.¹¹ The pulmonary inflammation found in SP-D deficiency might be owing to the fact that apoptotic cells and their DNA cannot be cleared and act pro-inflammatory in the alveolar space.⁸ Cystic fibrosis lung disease is characterized by chronic bacterial infection of the airways. The pulmonary immune system is not able to clear the resident pathogens, but the underlying causes are not fully understood.

Three studies have demonstrated decreased SP-D levels in the BALF of children with CF.¹²⁻¹⁴ Children with gastroesophageal reflux disease (GERD) have continuous acid airway injury and frequently suffer from chronic lung infections.¹⁵ Similarly to CF patients, decreased SP-D levels are found in BALF of children with GERD compared with control subjects.¹⁶ In the BALF of these children, the high molecular, more active oligomers of SP-D are diminished, while the smaller forms of SP-D are increased compared with BALF of control children. A decrease of SP-D levels and a disturbance of SP-D multimerization in BALF may contribute to the pathogenesis of the chronic lung disease in patients with GERD. Long-term tracheostomy frequently results in chronic airway inflammation.¹⁷ In the BALF of children with tracheostomy, decreased levels of SP-D are found compared with controls, associated with increased neutrophils and bacteria.¹⁸ Thus, low levels of SP-D may support neutrophilic inflammation in the lower respiratory tract of children with tracheostomy. Infection with RSV is a common cause of respiratory disease in infancy. Decreased levels of SP-D are found in the BALF of children with RSV infection compared with control children, suggesting that low pulmonary levels of SP-D may increase the susceptibility to RSV-associated lung disease. Two studies demonstrated that ARDS patients have reduced SP-D levels in BALF compared with control subjects.¹⁹ Furthermore, SP-D levels are lower in deceased ARDS patients compared with survivors and lower SP-D levels are found in ARDS patients with worse oxygenation compared with patients with good oxygenation. In smokers, the levels of SP-D in BALF are reduced to 40% compared with nonsmokers. Therefore, chronic smoking may decrease levels of SP-D in BALF which impairs the pulmonary host defence and may trigger the development of smoking-associated chronic obstructive pulmonary disease.

Kunitake et al. reported increased SP-D levels in patients with sarcoidosis compared with healthy controls. Cheng *et al.* reported increased SP-D levels in the alveolar wash of patients with asthma in compar-

ison with patients with a cough. Increased levels of SP-D are found in the BALF of patients with eosinophilic pneumonia compared with healthy controls.²⁰

Surfactant protein A prevents inhibition of surfactant activity by blood proteins. Leakage of blood components into the alveolar space as a result of lung injury has been implicated in the pathology of respiratory distress syndrome. Plasma proteins have been shown to inhibit surfactant activity both *in vitro* and *in vivo*. SP-A binds the lipopolysaccharide (LPS), or endotoxin, the major component in the cell walls of gram-negative bacteria. LPS consists of lipid A, which is its biologically active component, a core carbohydrate region, and a terminal polysaccharide of variable length and composition, i.e., the O-antigen domain. Lipid A and the core region make up rough LPS, whereas smooth LPS consists of the core region and the O-antigen domain. Human SP-A binds rough LPS via lipid A, and this binding is not inhibited by either the addition of carbohydrates or the removal of N-linked sugars. LPS participates in many events associated with the inflammatory response at the alveolar level, and has been shown to increase the production of colony-stimulating factor (CSF) by cultured alveolar type II cells and macrophages. Human SP-A enhances the binding of LPS to alveolar macrophages, but inhibits the binding of LPS to neutrophils. Human SP-A binds to gram-negative bacteria via LPS and facilitates their aggregation, phagocytosis, and killing by rat macrophages. The interactions of SP-A and LPS suggest that SP-A may influence the pathogenicity of gram-negative bacteria in the pulmonary alveolus. The killing of microorganisms by macrophage phagocytosis can be initiated by a variety of mechanisms, including the secretion of proteolytic enzymes and the production of toxic oxygen metabolites, which together constitute the respiratory burst. Alveolar macrophages are the primary inflammatory cells of the lung capable of phagocytosis, cytokine production, destruction and lung tissue remodelling. These cells can phagocytose bacteria, inert particles, surfactant, and other extracellular components of the alveoli recognized as foreign, thereby playing a role in the metabolic turnover of the lung. Macrophages can also release cytokines, which can recruit other inflammatory cells to the lung. Chemotactic factors of neutrophils, such as leukotriene B₄ or IL-8, are released by macrophages. In addition to these functions, macrophages regulate parenchymal cells by releasing proteases, which can degrade the extracellular matrix of the lung and are important in the repair and remodelling of lung tissue following injury.

Abnormalities in SP-A levels have been detected in several disease states. For example, SP-A levels are decreased in the amniotic fluid of diabetic pregnant

mothers. Pregnancies characterized by low levels of amniotic fluid SP-A before delivery are associated with an increased risk of the infant being born with RDS. SP-A levels are low in tracheal aspirates obtained from infants with RDS.

A decrease in SP-A levels has been observed in the bronchoalveolar lavage (BAL) of adult patients with acute RDS. SP-A levels in patients with cystic fibrosis (CF) and lower airway infections are higher than in CF patients without infection.²¹ This increase may occur in response to infection. However, a decrease in BAL SP-A levels was observed in patients with bacterial and viral pneumonia.²² Decreased BAL SP-A levels have also been reported in bronchopulmonary dysplasia with infection in baboons²³ and in interstitial pneumonia with collagen vascular disease. Decreased SP-A levels are observed in lavage of patients with idiopathic pulmonary fibrosis (IPF). However, another report showed no difference in SP-A levels in BAL of IPF patients when compared to controls. SP-A levels are increased in BAL of patients with pulmonary alveolar proteinosis (PAP), sarcoidosis, and hypersensitivity pneumonitis.^{24,25}

Immune and inflammatory response in the lung

Innate immune mechanisms defend the air space from the array of microbial products that enter the lung on a daily basis and are evident from the nasopharynx to the alveolar membrane. Particles that are carried into the conducting airways sediment onto the mucociliary surface of the airways under the influence of gravity, where they encounter soluble constituents in airway fluids and the upward propulsive force of the mucociliary system. Particles 1 µm in size and smaller, the size of bacteria and viral particles, are carried to the alveolar surface where they interact with soluble components in alveolar fluids (e.g. Ig G, complement, surfactant and surfactant-associated proteins) and alveolar macrophages.

The soluble constituents of airway and alveolar fluids have an important role in innate immunity in the lung. In the conducting airways, constituents of airway aqueous fluids include lysozyme, which is lytic to many bacterial membranes; lactoferrin, which excludes iron from bacterial metabolism; IgA and IgG; and defensins, which are antimicrobial peptides released from leukocytes and respiratory epithelial cells.²⁶ IgG is the most abundant immunoglobulin in alveolar fluids, and complement proteins and surfactant-associated proteins serve as additional microbial opsonins. In particular, SP-A and SP-D are members of the collectin family and promote phagocytosis of particulates by alveolar macrophages. Alveolar surfactant lipids and SP-A and SP-D bind LPS (lipopolysaccharides) and prevent its interaction with LBP (LPS binding protein) in alveolar fluids and the CD14: TLR4 (toll-like receptor 4) com-

plex on alveolar macrophages.^{27,28} Alveolar fluid contain high concentrations of LBP and soluble CD14 (sCD14), which are key molecules in the recognition of LPS by alveolar macrophages and other cells in the alveolar environment.²⁹ Soluble CD14 is released from the surface of alveolar macrophages by proteases, and this is enhanced by IL-6, which is abundant in the bronchoalveolar lavage (BAL) fluids of patients with lung injury.^{30,31} Blood monocytes and newly recruited monocyte-macrophages express considerably more membrane CD14 than mature alveolar macrophages, so newly recruited cells are likely to be an additional source of soluble CD14 in alveolar fluids.³²

The inflammatory cytokines produced by macrophages initiate a localized inflammatory response by recruiting neutrophils from the lung capillary networks into the alveolar space. Another important principle is that acute inflammation alters the set point for the induction of innate immune reactions in the lung. Normally, the airspace environment is a relatively quiet place despite the array of microbial and other products that enter the airspaces by inhalation or subclinical oropharyngeal aspiration. Surfactant lipids and proteins are present in very high concentrations as compared with LBP and sCD14. Surfactant lipids, and SP-A and SP-D, bind LPS and reduce its biological effects. When inflammation occurs, the concentrations of SP-A and SP-D fall, whereas the concentrations of LBP and sCD14 rise markedly, enhancing the effects of LPS in lung fluids.²⁹ Recent studies have defined an important role for airway epithelial cells in innate immune responses in the lung.

SP-A and SP-D as biomarkers in human disease

Because the concentrations of SP-A and SP-D in lavage fluid and serum vary with disease and lung inflammation, these proteins might be useful markers of disease. SP-A or SP-D have been shown to be elevated in the lavage fluid of animals with acute lung injury due to endotoxin instillation or 85% oxygen exposure. Shimura *et al.* measured SP-A in tracheal aspirates and sputum of patients with adult respiratory distress syndrome (ARDS) and cardiogenic pulmonary edema and found it elevated in both conditions. Lavage fluid of patients with idiopathic pulmonary fibrosis (IPF) reveals reduced levels of SP-A and little change in SP-D. The reduction in SP-A in lavage fluid was found in a variety of diffuse interstitial lung diseases. Hamm *et al.* reported an increase in SP-A levels in the lavage fluid of patients with sarcoidosis or hypersensitivity pneumonitis. In patients with ARDS, SP-A is reduced in lavage fluid, and the reduction in SP-A correlates with the severity of ARDS. In addition, Baughman *et al.* reported a decrease in SP-A in the lavage fluid from patients with bacterial pneumonia.

SP-A and SP-D have also been measured in serum and may serve as biomarkers of lung disease, especially when alveolar epithelial integrity is compromised. Both proteins have been identified in serum by sandwich ELISAs that use monoclonal antibodies, and several groups with different reagents have found similar absolute values of serum concentration.²⁵ Serum concentrations of SP-A and SP-D are increased in patients with alveolar proteinosis, IPF, and ARDS. Greene *et al.* have measured SP-A and SP-D in the serum of patients with interstitial lung disease. Measuring SP-A and SP-D in serum might be useful in the diagnosis of alveolar proteinosis because the serum levels are quite high, the image on computed tomography scanning is nearly specific, and a biopsy could be avoided. More clinical correlations are needed between serum values of SP-A and SP-D and the usual clinical variables to judge their value in the care of patients with interstitial lung disease and ARDS. The most promising uses would be to predict the development of ARDS in patients at risk for ARDS and identify a subset of patients with pulmonary fibrosis with a poor prognosis for more aggressive therapy.

Conclusions

The lung is continuously exposed to inhaled pollutants, microbes and allergens. Therefore, the pulmonary immune system has to defend against harmful pathogens, while an inappropriate inflammatory response to harmless particles must be avoided. In the bronchoalveolar space this critical balance is maintained by innate immune proteins, termed surfactant proteins. SP-D plays a central role in the pulmonary host defence by mediating pathogen clearance, modulating allergic responses and facilitating the resolution of lung inflammation. The function of SP-A in the alveolus is to facilitate the surface tension-lowering properties of surfactant phospholipids, regulate surfactant phospholipid synthesis, secretion, and recycling, and counteract the inhibitory effects of plasma proteins released during lung injury on surfactant function. It has also been shown that SP-A modulates host response to microbes and particulates at the level of the alveolus. More recently, several investigators have reported that pulmonary surfactant phospholipids and SP-A are present in nonalveolar pulmonary sites as well as in other organs of the body.

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