Anita Gupta

43.1 Pulmonary Surfactant

Pulmonary surfactant is a complex mixture of lipids and proteins, and is synthesized and secreted by alveolar type II epithelial cells and bronchiolar Clara cells. It acts to keep alveoli from collapsing during the expiratory phase of the respiratory cycle. After its secretion, lung surfactant forms a lattice structure on the alveolar surface, known as tubular myelin. Surfactant proteins (SP)-A, B, C and D make up to 10% of the total surfactant. SP-B and SPC are relatively small hydrophobic proteins, and are involved in the reduction of surface-tension at the air-liquid interface. SP-A and SP-D, on the other hand, are large oligomeric, hydrophilic proteins that belong to the collagenous Ca^{2+}-dependent C-type lectin family (known as "Collectins"), and play an important role in host defense and in the recycling and transport of lung surfactant (Awasthi 2010) (Fig. 43.1). In particular, there is increasing evidence that surfactant-associated proteins A and -D (SP-A and SP-D, respectively) contribute to the host defense against inhaled microorganisms (see Chaps. 24 and 25). Based on their ability to recognize pathogens and to regulate the host defense, SP-A and SP-D have been recently categorized as "Secretory Pathogen Recognition Receptors". While SP-A and SP-D were first identified in the lung, the expression of these proteins has also been observed at other mucosal surfaces, such as lacrimal glands, gastrointestinal mucosa, genitourinary epithelium and periodontal surfaces. SP-A is the most prominent among four proteins in the pulmonary surfactant-system. The expression of both SP-A and SP-D is complexly regulated on the transcriptional and the chromosomal level. SP-A is a major player in the pulmonary cytokine-network and has been described to act in the pulmonary host defense. This chapter gives an overview on the understanding of role of SP-A and SP-D in for human pulmonary disorders and points out the importance for pathology-oriented research to further elucidate the role of these molecules in adult lung diseases. As an outlook, it

will become an issue of pulmonary pathology which might provide promising perspectives for applications in research, diagnosis and therapy (Awasthi 2010).
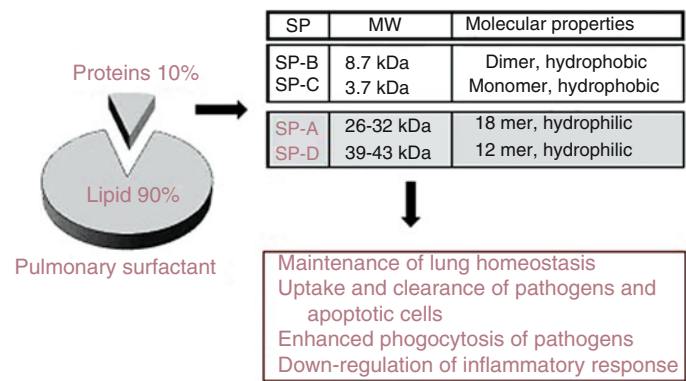
43.2 SP-A and SP-D in Interstitial Lung Disease

SP-A and SP-D appear in the circulation in specific lung diseases. Interstitial lung disease (ILD), also known as diffuse parenchymal lung disease (DPLD), refers to a group of lung diseases affecting the interstitium of lung: alveolar epithelium, pulmonary capillary endothelium, basement membrane, perivascular and perilymphatic tissues. The term ILD is used to distinguish these diseases from obstructive airways diseases. Most types of ILD involve fibrosis, but this is not essential; indeed fibrosis is often a later feature. The phrase "pulmonary fibrosis" is no longer considered a synonym, but the term is still used to denote ILD involving fibrosis. The term is commonly combined with idiopathic in "idiopathic pulmonary fibrosis", denoting fibrotic ILD that cannot be ascribed to a distinct primary cause.

43.2.1 Pneumonitis

Chronic hypersensitivity pneumonitis (HP) eventually ensues to extensive lung fibrosis when exposure to causative antigen continues. Klebs von den Lungen (KL)-6, a mucin-like glycoprotein and SP-D are elevated in most cases. Correct diagnosis in the early stage is crucial, since chronic summer-type HP can result in a fatal outcome after continuous exposure to the causative antigen (Inase et al. 2007). In pulmonary tissues of collagen vascular disease-associated interstitial pneumonia (CVD-IP) and hypersensitivity pneumonitis (HP), SP-D can be a marker for maturity of regenerating epithelial cells. SP-A along with KL-6 is detected in intimate relationship to the stage of regeneration

Fig. 43.1 Presence of surfactant proteins (SP) in lung surfactant, their properties and major functions of SP-A and SP-D

SP	MW	Molecular properties
SP-B	8.7 kDa	Dimer, hydrophobic
SP-C	3.7 kDa	Monomer, hydrophobic
SP-A	26-32 kDa	18 mer, hydrophilic
SP-D	39-43 kDa	12 mer, hydrophilic

Proteins 10%

Lipid 90%

Pulmonary surfactant

Maintenance of lung homeostasis
Uptake and clearance of pathogens and apoptotic cells
Enhanced phogocytosis of pathogens
Down-regulation of inflammatory response

of alveolar epithelial cells and expressed before SP-D (Ohtsuki et al. 2007). Radiation pneumonitis (RP) is most common complication of radiotherapy for thoracic tumors. Both SP-A and SP-D concentrations in sera from patients with RP were significantly higher than those from patients without RP. Serum SP-A and SP-D may be of diagnostic value for detection of RP, even when radiographic change is faint (Takahashi et al. 2001). Despite the rise of SP-D and KL-6 in serum in adult patients with various types of interstitial pneumonia (IP) and collagen diseases with interstitial pneumonia, KL-6 may be superior in sensitivity of IP, whereas SP-D may be more specific for IP than KL-6. Early decrease of SP-D contrasts with the transient increase of KL-6 levels after prednisolone pulse therapy (Arai et al. 2001). High serum KL-6 value is an indicator of ILD of Wilson-Mikity syndrome and better than SP-D and LDH levels (Takami et al. 2003). Thus serum SP-A and SP-D monitoring along with KL-6 is useful indicator for estimating RP (Matsuno et al. 2006).

43.2.2 Interstitial Pneumonia (IP)

SP-A and SP-D in BAL as Indicator of Pneumonia in Children

SP-A and SP-D in serum significantly increase in patients with pulmonary alveolar proteinosis (PAP), idiopathic pulmonary fibrosis (IPF) and interstitial pneumonia with collagen vascular diseases (IPCD) (Kuroki et al. 1998; Takahashi et al. 2006b). The concentrations of SP-A and SP-D in BAL fluids from patients with IPF and IPCD are rather lower than those in healthy controls; and the SP-A/phospholipid ratio may be a useful marker of survival prediction. SP-D-deficient patients have more frequently pneumonias and their long-term outcome is worse than those with detectable SP-D. Among children with recurrent bronchitis and SP-D detectable in bronchoalveolar lavage (BAL), patients with allergic asthma had threefold levels of SP-D compared with controls. In contrast, SP-D deficiency

due to consumption or failure to up-regulate SP-D may be linked to pulmonary morbidity in children (Griese et al. 2008).

SP-A Levels can Differentiate Usual Interstitial Pneumonia with Non-Specific Interstitial Pneumonia (NSIP)

There is a need to use serum markers for differentiating usual interstitial pneumonia (UIP) from other ILD. Serum levels of SP-A and SP-D in patients with UIP and nonspecific interstitial pneumonia (NSIP) are significantly higher than in healthy volunteers. In particular, serum SP-A levels in patients with UIP are significantly higher than in patients with NSIP, where as SP-D in BAL fluid in UIP patients were significantly lower than in patients with NSIP. Thus, serum SP-A level seems useful marker to differentiate UIP from NSIP (Ishii et al. 2003).

Abnormal tracheal aspirate surfactant phospholipids and SP-A are noted in children with bacterial pneumonia, viral pneumonitis, and ARDS, but not in children on cardiopulmonary bypass (Baughman et al. 1993; LeVine et al. 1996). SP-A in pneumonia group is significantly reduced and the reduction was better indicator in the Gm$^+$-pneumonia group than in Gm$^-$-pneumonia group patients (Baughman et al. 1993). Fulminant early-onset neonatal pneumonia is associated with ascending intrauterine infection (IUI) and alveolar M showed significantly less nitric oxide synthase 2 (NOS2) isoform than in the controls. In the airway samples, the infants with fulminant pneumonia after birth had low intracellular NOS2 and significantly low IL-1β and SP-A than noninfected IUI infants (Aikio et al. 2000).

Foster et al. (2002) suggested that signaling of EGF axis and differential regulation of SPs persist during postnatal lung development, and SP-A and SP-D may modulate post-pneumonectomy (PNX) lung growth in dogs. SP-D in patients, hospitalized for community-acquired pneumonia of suspected bacterial origin, indicates significant changes during pulmonary infection (Daimon et al. 2005; Leth-Larsen et al. 2003). The SA-A and SP-D in sera are useful

for identification of the clinical condition of horses with bacterial pneumonia (Hobo et al. 2007).

43.2.3 ILD Due to Inhaled Substances

Cigarette smoke may alter component and function of pulmonary surfactant. Alterations in serum levels of SP-A may reflect smoking habits since serum SP-A was higher in active smokers than in nonsmokers (Nomori et al. 1998). However, SP-A is not a sensitive discriminating factor to separate smokers from nonsmokers. The contents of SP-A and SP-D in BAL fluids were significantly decreased in smokers compared to those in nonsmokers, although there was no significant difference of total phospholipid content between two groups (Honda et al. 1996). SP-A may decrease due to the cumulative effects of long-term smoking and development of emphysema, while SP-D decreases due to long-term smoking (Betsuyaku et al. 2004; Shijubo et al. 1998). Emphysema can be induced in mice by chronic cigarette smoke exposure with increase of SP-D in emphysema lungs. While accumulation of foamy alveolar macrophages may play a key role in the development of smoking-induced emphysema, increased SP-D may play a protective role in the development of smoking-induced emphysema, in part by preventing alveolar cell death (Hirama et al. 2007).

Although effects of maternal smoking on fetal growth and viability are overwhelmingly negative, there is a paradoxical enhancement of lung maturation as evidenced, in part, by a lower incidence of RDS in infants of smoking mothers. Epidemiologic and experimental evidence further support the view that a tobacco smoke constituent, possibly nicotine, affects the development of the lung in utero. The murine embryonic lungs explanted at 11 days gestation showed a 32% increase in branching after 4 days in culture in presence of 1 μM nicotine and 7–15-fold increases in mRNAs encoding SP-A and SP-C after 11 days. The nicotine-induced stimulation of surfactant gene expression could, in part, account for the effect of maternal smoking on the incidence of RDS (Wuenschell et al. 1998).

Intratracheal administration of crystalline silica to rats elicits a marked increase in alveolar accumulation of surfactant lipids and SP-A. The extracellular accumulation of SP-D is markedly increased in silica-induced lipoproteinosis, and that SP-D is associated with amorphous components identified by electron microscopy. SP-D may be useful biomarkers for early diagnosis and serum SP-D concentration may associate with the pathogenesis of silicosis (Barbaro et al. 2002; Wang et al. 2007b). Alcohol consumption at high levels during pregnancy is associated with immuno-modulation and premature birth. Chronic maternal ethanol consumption during the third trimester of pregnancy alters SP-A gene expression in fetal lung. These alterations may underlie increased susceptibility of preterm infants, exposed to ethanol in utero, to RSV and other microbial agents (Lazic et al. 2007). The exposure to moderate and high occupational levels of Diesel exhaust (DE) causes an increase in lung injury and inflammation, and a decrease in host defense molecules, which could result in increased severity of infectious and allergic lung disease. Several inflammatory and immune cytokines are upregulated at various time points and concentrations, in contrast to SP-A and SP-D which were significantly decreased at protein level. (Gowdy et al. 2008).

43.2.4 Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF) is a progressive disease of lung characterized by an inflammatory infiltrate, alveolar type II cell hypertrophy and hyperplasia, and ultimate parenchymal scarring. The phospholipid composition of the surface-active material recovered by BAL is abnormal in this disease. The content of SP-A in lavage was reduced, even when normalized for the total amount of surface-active material (SP-A/total phospholipids (PL)) recovered. The reduction in SP-A was not specific to IPF but also occurred in other interstitial lung diseases. Despite this, SP-A/PL in BAL is a biochemical marker that predicts survival in patients with IPF (McCormack et al. 1995; Phelps et al. 2004).

The serum SP-A and SP-D levels are significantly elevated in patients with IPF and systemic sclerosis compared to sarcoidosis, beryllium disease and normal controls, and correlated with radiographic abnormalities in patients with IPF. Dohmoto et al. (2000) hypothesized that regenerated or premature bronchoepithelial cells may circulate in the blood in patients with IPF. RT-PCR for cytokeratin 19 (CK19) and pulmonary SP-A in peripheral blood in patients with IPF and pulmonary fibrosis (PF) associated with collagen vascular disorders suggests that there were some circulating bronchoepithelial cells expressing mRNA for SP-A in peripheral blood of patients associated with collagen vascular disorders. Thus, both serum SP-A and SP-D levels are highly predictive of survival in patients with IPF (Greene et al. 2002; Takahashi et al. 2006b) and the measurement of SP-D in sera can provide an easily identifiable and useful clinical marker for the diagnosis of IPF, IPCD, and PAP, and can predict the disease activity of IPF and IPCD and the disease severity of PAP (Honda et al. 1995). However, KL-6 is the best serum marker for ILD (Ohnishi et al. 2002). Serum KL-6 and SP-D were also prognostic markers in acute exacerbation of IPF after treatment with Sivelestat (Endo et al. 2006; Nakamura et al. 2007). High levels of SP-D in BAL fluids are associated in patients with PAP, but not with IPF and IPCD.

Selman et al. (2003) examined associations between IPF and genetic polymorphic variants of SP-A1, SP-A2, SP-B, SP-C, and SP-D. One SP-A1 (6A^4) allele and SNPs that characterize the 6A^4 allele and one SP-B (B1580_C) were found with higher in nonsmoker and smoker IPF subgroups, respectively, compared with healthy controls. To explore whether a tryptophan (in 6A^4) or an arginine (in other SP-A1 alleles and in all SP-A2 alleles) at amino acid 219 alters protein behavior, two truncated proteins that varied only at amino acid 219 were oxidized by exposure to ozone. Differences in the absorption spectra (310–350 nm) between the two truncated rSP-A proteins, before and after protein oxidation, suggested allele-specific aggregation attributable to amino acid 2143. The SP-B SNP B1580_C, to be a risk factor for IPF smokers, was also shown to be a risk factor for other pulmonary diseases. The SP-C and SP-D SNPs and SP-B-linked microsatellite markers did not associate with IPF. These findings indicated that surfactant protein variants may serve as markers to identify subgroups of patients at risk. The observed alleles of SP-A and SP-D in association with various diseases are summarized in Table 43.1. Different alleles of these genes seem to predispose the individuals to various diseases. A logical explanation seems to be that different SNPs lead to different alterations in function or expression. However, common SNPs predispose Caucasians to RDS and Mexicans to TB. Similarly, common SNPs predispose the Indian population to ABPA and TB. Furthermore, Met11 SP-D allele is predisposing Mexicans to TB and Finns to RSV infection. It is also interesting to note that some of the alleles of SP-A interact with other alleles of SP-A and SP-B and thus increase the susceptibility of subjects to a disease (Kishore et al. 2005).

43.2.5 Cystic Fibrosis

Cystic fibrosis (CF) is an inherited disorder of CFTR gene, a chloride ion channel. The lack of this channel causes reduced water content of secretions. This affects the mucus secreted as part of the lung's defence and creates sticky, viscous mucus. In patients with CF, neutrophils are recruited in excess to the airways yet pathogens are not cleared and the patients suffer from chronic infections. In CF, the disease-causing gene has been clearly identified as the CF transmembrane conductance regulator gene, but genetic variants of the MBP and SP-A have been associated with disease severity in CF.

Table 43.1 Broad range of pathogens interacting with surfactant protein (SP)-A and SP-D

Type	Name of pathogen	Surfactant protein	Reference(s)
Bacteria	*E. coli*	SP-D	Kuan et al. (1992)
	Salmonela minnesota	SP-D	Kuan et al. (1992)
	H. pylori	SP-D	Appelmelk et al. (2005)
	Klebsiella pneumoniae	SP-D	Keisari et al. (2001), Ofek et al. (2001)
	Mycoplasma pneumoniae and *Histoplasma capsulatum*	SP-A and SP-D	Ernst (1998), Chiba et al. (2002), Gaynor et al. (1995)
	Haemophilus influenzae	Minimal effects of SP-D	Tino and Wright (1996), Restrepo et al. (1999)
	Pseudomonas aeruginosa Stenotrophomonas maltophilia	SP-D, not SP-A	Malloy et al. (2005), Giannoni et al. (2006), Bufler et al. (2004)
	Mycobacterium tuberculosis	Virulent and attenuated M. tuberculosis strains bind best	Ferguson et al. (1999, 2002), Hall-Stoodley et al. (2006)
	Mycobacterium avium	SP-A and SP-D	Kudo et al. (2004)
	Group B streptococcus' (*Streptococcus agalactiae*) and *S. pneumoniae*	SP-A and SP-D	Jounblat et al. (2004), Kuronuma et al. (2004)
	B. bronchiseptica (LPS); Ruminant bronchopneumonia	SP-D	Schaeffer et al. (2004), Grubor et al. (2004)
	Alloiococcus otitidis	SP-A	Konishi et al. (2006)
Yeast and fungi	*Cryptococcus neoformans*	SP-A and SP-D	Schelenz et al. (1995), Walenkamp et al. (1999), van de Wetering et al. (2004)
	Aspergillus fumigatus	SP-A and SP-D	Allen et al. (1999), Madan et al. (1997a, b)
	Coccidioides posadasii	SP-A and SP-D	Awasthi et al. (2004), Awasthi (2010)
	Candida albicans	SP-D	Van Rozendaal et al. (2000)
	Pneumocystis carinii	SP-D	O'Riordan et al. (1995), Vuk-Pavlovic et al. (2001), Atochina et al. (2004a), Yong et al. (2003)
Viruses	Influenza A virus	SP-A and SP-D	Malhotra et al. (1994), Hartshorn et al. (1994, 1997), Levine et al. (2001), Tecle et al. (2007b)
	HIV	SP-D binds HIV – gp120	Meschi et al. (2005)
	Respiratory syncytial virus	SP-A and SP-D	Ghildyal et al. (1999), Hickling et al. (1999), Griese (2002)

Allele associations and allele interaction of surfactant protein genes in relation to RDS have been discussed (Floros and Fan 2001). Studies have shown a deficiency of SP-A in airway fluids from patients with CF and other inflammatory pulmonary conditions. Findings suggest that the neutrophil serine proteases cathepsin G and/or elastase and/or proteinase-3 may contribute to degradation of SP-A and SP-D, thereby diminishing innate pulmonary antimicrobial defence (Rubio et al. 2004; von Bredow et al. 2001, 2003).

The dramatic decrease of SP-A and SP-D in the presence of normal surfactant phospholipid may be a mechanism underlying the relative ineffectiveness of cellular inflammatory response in killing invading bacteria in lungs of patients with CF. In bronchoalveolar lavage fluids (BALFs), although SP-A levels tend to decline in CF patients compared with non-CF, and the decline was only significant in presence of bacterial infection. Among CF patients, SP-A concentrations in BALF were inversely related to inflammation and age (Hull et al. 1997; Noah et al. 2003). Reports suggest that decreasing protease activity and increasing collectin activity may be beneficial in early CF (Alexis et al. 2006; Baker et al. 1999).

However, both, SP-D and TNF-α, are significantly increased in CF patients compared with patients of allergic fungal rhinosinusitis (AFRS), suggesting activation of both innate immunity and Th1-mediated inflammation and potential correlation between SPs and downstream adaptive immune responses (Skinner et al. 2007). Rat SP-D is highly resistant to degradation by a wide range of proteolytic enzymes. Patients with CF and chronic rhinosinusitis (CRS) with nasal polyposis demonstrated elevated SP-A1, -A2, and -D. While in patients with AFS, SP-A1, SP-A2, and SP-D, were not significantly different, these proteins are up-regulated in various forms of CRS, particularly in CF-CRS (Woodworth et al. 2007).

43.2.6 Familial Interstitial Lung Disease

Amin et al. (2001) studied the development of chronic lung injury in a familial form of ILD. An 11-year-old girl, her sister, and their mother who were diagnosed with chronic ILD were negative for SP-C and decreased levels of SP-A and SP-B in BALF. Lung biopsy from both children demonstrated a marked decrease of pro-SP-C in the alveolar epithelial cells but strong staining for pro-SP-B, SP-B, SP-A, and SP-D. The apparent absence of SP-C and a decrease in the levels of SP-A and SP-B were related to familial ILD. Several linkage and association studies have been done using SPs genes as markers to locate pulmonary disease susceptibility genes, but few have studied markers systematically in different ethnic groups.

43.3 Connective Tissue Disorders

43.3.1 Systemic Sclerosis

Significant progress is being made in terms of understanding the pathogenesis and various options for therapy of systemic sclerosis patients whose disease course is complicated by ILD. The significance of serum SP-A, SP-D and KL-6 for diagnosis and treatment of ILD in connective tissue disorders has been evaluated by different workers. Serum KL-6 and SP-D levels are more specific and useful markers for diagnosis and evaluation of ILD compared with serum LDH in connective tissue disorders (Ogawa et al. 2003; Suematsu et al. 2003). Characteristics or disease activity of early ILD has been evaluated in subjects. In abnormal group, curvilinear subpleural lines or thickened interlobular and intralobular lines were observed more frequently in lower lung fields and SP-A and SP-D were higher in true abnormalities group than in control group. True parenchymal abnormalities in posterior subpleural aspect of lung may indicate early ILD activity (Al-Salmi et al. 2005; Kashiwabara 2006). Since higher levels of SP-A and SP-D are associated with more severe lung function impairment at presentation, and better recovery over time, Janssen et al. (2005) suggested that SP-A, SP-D and KL-6 are especial markers of disease activity. Nevertheless, serum pulmonary and activation-regulated chemokine (PARC) levels may be more useful marker for active PF in systemic sclerosis (SSc) (Kodera et al. 2005) since elevated PARC values correlated more sensitively reflecting the PF activity than serum KL-6 or SP-D levels.

In lung fibrosis in patients with SSc and inflammatory myopathies, KL-6, von Willebrandt factor (vWF), soluble E-selectin (sES), SP-D are good surrogate factors of PF but cannot replace conventional diagnostic procedures. However, these markers are suitable for the assessment of progression and severity of PF in systemic autoimmune disorders once the diagnosis is established (Kumánovics et al. 2008). Takahashi et al. (2006b) indicated that elevated levels of serum SP-A and SP-D reflect the presence of ILD and the combination of SP-D and X-ray contributes to reduce the risk of clinicians overlooking ILD complicated by SSc (Highland and Silver 2005; Yanaba et al. 2004).

Maeda et al. (2001) compared serum SP-D in collagen diseases such as systemic scleroderma (SSd), scleroderma spectrum disorders (SSD), systemic lupus erythematodes (SLE), Sjogren syndrome (Sjs), dermatomyositis (DM), rheumatoid arthritis (RA), and dermatitis (DE) as a control. Patients with SSc possess higher levels of SP-D than those with other collagen diseases and dermatitis, which may correspond to severity of pulmonary fibrosis (Maeda et al. 2001). The basic and clinical studies of SSc patients with ILD are yielding promising data that may be translated in to

more effective diagnostic and therapeutic strategies Although the SP-D level in sera of patients with polymyositis/dermatomyositis (PM/DM) is significantly elevated, the serum SP-D in patients with ILD was still higher than those without ILD, suggesting that serum SP-D level is a useful marker for ILD in patients with PM/DM (Ihn et al. 2002). However, there is a need to investigate whether another connective tissue disease has developed when laboratory findings cannot be explained by usual clinical course of an existing connective tissue disease (Ishiguro et al. 2007).

43.3.2 Sarcoidosis

Sarcoidosis also called sarcoid, Besnier-Boeck disease or Besnier-Boeck-Schaumann disease, is a disease in which abnormal collections of chronic inflammatory cells form as nodules in multiple organs. KL-6, SP-A and SP-D levels in BALF were increased in pulmonary sarcoidosis. Since these markers are specifically derived from epithelial cells, it is considered that KL-6 and SP-D levels are reflecting damage or release of these markers from epithelial cells due to the inflammatory response. Among serum Clara cell 16 (CC16), KL-6, and SP-D as markers of ILD, and their ability to reflect pulmonary disease severity and prognosis in sarcoidosis, KL-6 is the best marker in differentiating patients from healthy controls (Günther et al. 1999; Hamm et al. 1994; Janssen et al. 2003; Kunitake et al. 2001). The median amounts of SP-A in BAL fluid in control subjects was 2.82 mg/L (range, 0.92–5.17 mg/L). In comparison to control, SP-A in patients with asthma had a lower value of SP-A, which remained unchanged in patients with pulmonary sarcoidosis (van de Graaf et al. 1992). In contrast, SP-A levels in BAL fluids from patients with sarcoidosis were markedly higher than in control subjects and it was comparable with patients of hypersensitivity pneumonitis (HP). In both conditions, SP-A$^+$ alveolar macrophages were increased (Günther et al. 1999; Hamm et al. 1994).

The serum levels of SP-A in patients with IPF (205 ± 23 ng/mL) and PAP (285 ± 23 ng/mL) were significantly higher than those in healthy controls (45 ± 3 ng/mL). In patients of sarcoidosis, pneumonia, and tuberculosis SP-A values were 52 ± 27 ng/mL, 65 ± 11 ng/mL, and 49 ± 23 ng/mL, respectively. The SP-A appears to circulate in the bloodstream as a complex with Ig in IPF and in PAP (Kuroki et al. 1993).

43.4 Pulmonary Alveolar Proteinosis

A diffuse lung process of unknown etiology is characterized by the presence of alveolar spaces filled with amorphous eosinophilic (but sometimes basophilic) PAS-positive material of predominantly phospholipid nature in alveolar lumina. It is generally regarded as type of response to alveolar injury and results from accumulation of surfactant apoprotein through either: increased secretion by granular pneumocytes, or abnormal uptake and handling by alveolar macrophages. The prominent increase of SP-A and SP-D in BAL fluids and sputum is diagnostic for pulmonary alveolar proteinosis (PAP) (Kuroki et al. 1998; Brasch and Müller 2004; Takahashi et al. 2006a). There are reports about polymorphisms and mutations on the surfactant protein genes, especially SP-B that may be associated with congenital alveolar proteinosis.

43.4.1 Idiopathic Pulmonary Alveolar Proteinosis

SP-A in BALF of PAPs patients is significantly increased in comparison to normal volunteers and hence can be used as a diagnostic tool in the clinical laboratory (Brasch et al. 2004; Honda et al. 1996). PAP is a rare lung disorder and can be caused by inactivation of either granulocyte-macrophage colony-stimulating factor (GM-CSF) or GM receptor common β-chain (βc) genes in mice [GM$^{-/-}$, βc$^{-/-}$], demonstrating a critical role of GM-CSF signaling in surfactant homeostasis. Studies demonstrate abnormal accumulation of SP-A and SP-D in air spaces of patients with PAP (Crouch et al. 1993) and the precursors of SP-B, SP-B and SP-C. Although lung histology in βc$^{-/-}$ and GM$^{-/-}$ mice was indistinguishable, distinct differences were observed in surfactant phospholipid and surfactant protein concentrations in lungs of βc$^{-/-}$ and GM$^{-/-}$ mice. The defect in clearance was significantly more severe in GM$^{-/-}$ than in βc$^{-/-}$ mice. GM-CSF concentrations, increased in BALF but not in serum of βc$^{-/-}$ mice, were consistent with a pulmonary response to the lack of GM-CSF signaling. The observed differences in surfactant metabolism suggest the presence of alternative clearance mechanisms regulating surfactant homeostasis in mice and may provide a molecular basis for the range in severity of PAP symptoms (Reed et al. 2000). In a young patient with idiopathic PAP, the enhanced serum anti-GM-CSF antibody level demonstrated a striking difference in the distribution of SP-A and SP-D in intra-alveolar substance with idiopathic PAP (Ohtsuki et al. 2008; Kobayashi et al. 2008b).

Evidence suggests that not only an impairment of surfactant clearance by alveolar macrophages, but also an abnormal secretion of transport vesicles containing precursors of SP-B (but not SP-C) and an insufficient palmitoylation of SP-C, which may lead to the formation of di- and oligomeric SP-C forms, play a role in the pathogenesis of pulmonary alveolar proteinosis.

43.4.2 Structural Changes in SPs in PAP

The primary structures of human pulmonary SPs isolated from lung lavage of patients with alveolar proteinosis demonstrate significant differences from lung surfactant proteins isolated from lungs of healthy individuals. In contrast to SP-A from normal lungs, PAP-SP-A was shown to contain large amounts of non-reducable cross-linked β chains, where as proteinosis SP-B showed a significantly increased molecular weight by approx. 500 Da for the unreduced protein dimer. In contrast, SP-C from proteinosis patients was modified by (1) partial or even complete removal of palmitate residues and (2) additional N-terminal proteolytic degradation (Voss et al. 1992).

Pathophysiological structural modifications in SP-A seemed to occur in the alveolar space, and may lead to a reduced surfactant function (Voss et al. 1992). Multimerized form of SP-A oligomer (alveolar proteinosis protein-I, APP-I) has been detected besides the normal-sized octadecamer (APP-II) in SP-As isolated from PAP patients. Analysis of APP revealed that it was composed of two proteins. The M_r of APP-I and APP-II were 1.65 MDa and 0.93 MDa, respectively. APP-I and APP-II showed almost identical amino acid compositions. Electron microscopy revealed that APP-II was a hexameric particle, presumably consisting mainly of octadecamers whose diameter was approximately 30 nm. In contrast, APP-I was made of multimerized larger aggregates whose diameter appeared to be about 70–90 nm. Both APP-I and APP-II retained the abilities to bind DPPC. Reconstitution experiments with porcine SP-B and phospholipids revealed that multilamellated membranes in structures formed from APP-I consisted of several layers of doubled unit membranes. APP-I failed to form tubular myelin structures. In contrast, APP-II formed well-formed lattice structures seen in tubular myelin The multimerized form of human SP-A oligomer exhibits the reduced capacity to regulate phospholipid secretion from type II cells, and lower affinity to bind to type II cells. It is to be reminded that the integrity of a flower-bouquet-like octadecameric structure of SP-A oligomer is important for the expression of full activity of this protein, indicating the importance of the oligomeric structure of mammalian lectins with collagenous domains. Thus there exists an abnormal multimerized form of SP-A oligomer in the alveoli of patients with PAP that exhibits abnormal function on phospholipid membrane organization (Hattori et al. 1996a, b).

In alveolar proteinosis, cholesterol/disaturated phospholipid ratios (CHOL/DSP) are invariably elevated, whereas the SP-A/DSP and SP-B/DSP ratios are generally elevated. Because the SP-B/SP-A ratio was normal in all cases, it was suggested that structural changes to the proteins occurred secondarily and that caution must be used in comparing functional data derived using SP-A obtained from patients with PAP (Doyle et al. 1998). The major part of SP-A from a proteinosis patient consisted of SP-A2 gene product while SP-A1 gene product was present in only a small amount. The disulfide bridges in the carbohydrate recognition domain were identified to be in the 1–4, 2–3 pattern common for collectins. Interchain disulfide bridges were discovered between two Cys-48 residues and cysteine residues in the N-terminal region. However, the exact disulfide bridge connections within the bouquet-like ultrastructure could not be established (Berg et al. 2000).

43.5 Respiratory-Distress Syndrome and Acute Lung Injury

43.5.1 ARDS and Acute Lung Injury

Acute respiratory distress syndrome (ARDS), also known as respiratory distress syndrome (RDS) or adult respiratory distress syndrome (in contrast with IRDS) is a serious reaction to various forms of injuries to lung. ARDS is caused by a variety of direct and indirect issues. It is characterized by inflammation of lung parenchyma leading to impaired gas exchange with concomitant systemic release of inflammatory mediators causing inflammation, hypoxemia and frequently resulting in multiple organ failure. A less severe form is called acute lung injury (ALI). Clinical and biochemical evidences suggest that the etiology of RDS is multifactorial with a significant genetic component. There are reports about polymorphisms and mutations on the surfactant protein genes, especially surfactant proteins-B that may be associated with RDS, ARDS, and congenital alveolar proteinosis. The measurement of SP-A and SP-D in amniotic fluids and tracheal aspirates reflects lung maturity and the production level of the lung surfactant in infants with RDS. The SP-A concentrations in BAL fluids are significantly reduced in patients with ARDS and also in patients at risk to develop ARDS (Kuroki et al. 1998; Takahashi et al. 2006a). Patients with low concentrations of SP-A and SP-B in the BAL are at risk for ARDS before onset of clinically defined lung injury, though the SP-D concentrations remain in normal range. Thus, SP abnormalities occur before and after the onset of ARDS, and the responses of SP-A, SP-B, and SP-D differ in important ways. However, plasma SP-D is a valuable biomarker in ALI/ARDS and SP-A increases during the early phase of ARDS, including some molecular alteration followed by decrease during the late phase (Endo et al. 2002; Kuroki et al. 1998; Takahashi et al. 2006b; Zhu et al. 2001).

Elevated level of SP-A has also been reported in the sera of patients with acute cardiogenic pulmonary edema (APE) and in patients with ARDS relative to healthy subjects and ventilated patients with no cardio-respiratory disease. Serum SP-A was inversely related to blood oxygenation and to

static respiratory system compliance both at the time of patient's entry into the study and during the course of admission. Since SP-B is synthesized as a precursor smaller than alveolar SP-A, Doyle et al. (1995, 1997) suggested that immunoreactive SP-B that enters more readily than SP-A, is cleared acutely, and provides a better indicator of lung trauma (Shimura et al. 1996).

Prematurely born infants can develop the neonatal RDS because of a deficiency of pulmonary surfactant. At autopsy RDS lungs lacked tubular myelin and had decreased immunoreactivity for antisera to SP-A, an important component of tubular myelin. Therefore, a role for SP-A in the conversion of lamellar bodies to tubular myelin and in the pathogenesis of RDS was proposed. It was postulated that if SP-A is indeed necessary for the conversion of lamellar bodies to tubular myelin, in RDS either there is a deficiency of adequate amounts of functional SP-A, or some other important component of surfactant is missing (deMello et al. 1993). Mechanical ventilation is the main modality of treatment of ARDS. On mechanical ventilation, there is a progressive increase in SP-A levels in patients with ARDS, and may be one of the contributors for recovery in ARDS. A significant increase within the first 4 days was found in those infants who survived, whereas no such change was found in those infants who died (Balamugesh et al. 2003; Stevens et al. 1992). Intratracheal aerosolization of LPS in rats produces typical features of human ARDS. The SP-D binds inhaled LPS-endotoxin in vivo, which may help to protect the lung from endotoxin-induced disease (van Rozendaal et al. 1999). The SP-D was reduced in lung of young rats following ALI at early stage and early administration of Dex could reverse the SP-D content (Shu et al. 2007). SP-A in sera of cord blood from infants born at gestational ages <32 weeks with RDS was 15.1 ng/mL compared to without RDS (5.8 ng/mL) and significantly related to the non-RDS outcome (Cho et al. 2000). Shimoya et al. (2000) suggested that IL-6 elevation in fetuses with chorioamnionitis promotes fetal lung maturation by inducing SP-A synthesis, thereby decreasing the incidence of RDS in the preterm neonates.

Acute Lung Injury (ALI): Plasma SP-A, but not SP-D, was higher in patients with fewer days of unassisted ventilation and in patients with an absence of intact alveolar fluid clearance. In contrast, pulmonary edema fluid SP-D, but not SP-A, was lower in patients with worse oxygenation. Reduced pulmonary edema fluid SP-D and elevated plasma SP-A concentrations at the onset of ALI may be associated with more severe disease and worse clinical outcome and may serve as valuable biochemical markers of prognosis (Cheng et al. 2003). The BALF proteome analysis showed the presence of several isoforms of SP-A, in which an N-non-glycosylierte form and several proline

hydroxylations were identified (Bai et al. 2007). In the plasma and edema fluid, protein profile of ALI patients showed multiple qualitative changes. Nearly all ALI patients also had protein spots that indicated truncation or other posttranslational modifications (Bowler et al. 2004).

43.5.2 Bronchopulmonary Dysplasia (BPD)

The pathophysiology of bronchopulmonary dysplasia (BPD) as an inflammatory disorder, secondary to neonatal RDS represents a major complication of prematurity. Maximum SP-A and anti-SP-A antibodies (SAS) immune complex values between 2 and 4 weeks after birth correlate with subsequent development of BPD independently and may be useful in analyzing the course and outcome of neonatal RDS, in particular the likelihood of subsequent development of BPD (Strayer et al. 1995). Weber et al. (2000) investigated an association of polymorphisms of SP-A1 and SP-A2 encoding genes and the risk of BPD in Caucasian preterm infants below 32 weeks of gestation matched for immaturity and year of birth. BPD was defined as oxygen dependency or need for mechanical ventilation at day 243. A significantly increased frequency of SP-A1 polymorphism $6A^6$ in infants was associated with BPD compared with controls. In addition to established risk factors for BPD, $6A^6$ polymorphism for SP-A1 gene is an independent co-factor.

BPD_28D (O2 dependency at 28 days of life) and BPD_36W (O2 dependency at 36 week post-menstrual age) are diseases of prematurely born infants exposed to mechanical ventilation and/or oxygen supplementation. Genetic variants of SP-A, B, C, and D and SP-B-linked microsatellite markers are risk factors in BPD. Significant associations were observed for alleles of SP-B and SP-B-linked microsatellite markers, and haplotypes of SP-A, SP-D, and SP-B. Unlike SP-A, SP-D does not contribute to lowering surface tension. SP-D-deficient mice have no respiratory abnormalities at birth, but it causes development of emphysema and predisposition to specific infections. No human infant or child with respiratory distress and mutation in the SP-D gene has been identified (Yurdakök 2004). Studies in larger sample size are warranted to confirm these observations and delineate genetic background of BPD subgroups (Pavlovic et al. 2006).

43.5.2.1 SP-A Deficiency in Primate Model of BPD with Infection

In a baboon model of hyperoxia-induced BPD and superimposed infection, animals constituting a group- pro re nata (PRN) were delivered by hysterotomy at 140 days gestational age and ventilated on clinically appropriate

oxygen for a 16-day experimental period and served as controls. Immunostaining with SP-A, SP-B, and SP-C antibodies showed variable staining patterns. The study demonstrated that a deficiency of SP-A mRNA expression persists in chronic lung injury and variable protein staining patterns are manifested depending upon the underlying pathology (Coalson et al. 1995; King et al. 1995).

Awasthi et al. (1999) measured SP-A and SP-D levels and their mRNAs in three groups of animals: (1) nonventilated premature baboon fetuses; (2) neonatal baboons delivered prematurely at 140 d gestation age (ga) and ventilated with PRN O$_2$; (3) animals of same age ventilated with 100% O$_2$ to induce chronic lung injury. In chronic lung injury, SP-A is significantly reduced in alveolar space. SP-D concentration in lavage was nearly equal to that in normal adults, but the total collectin pool in lavage was still significantly reduced. Because these collectins may bind and opsonize bacteria and viruses, decrements in their amounts may present additional risk to those premature infants who require prolonged periods of ventilatory support (Awasthi et al. 1999). Reduced SP-D expression in BAL fluid was associated with progression of bronchial dysplasia in heavy smokers. SP-D levels in BAL fluid may serve a potential biomarker to identify smokers who are at risk of early lung cancer (Sin et al. 2008b). Cheng et al. (2003) proved the hypothesis that reduced pulmonary edema fluid SP-D and elevated plasma SP-A concentrations at onset of ALI may be associated with more severe disease and worse clinical outcome and may serve as valuable biochemical markers of prognosis (Cheng et al. 2003).

43.6 Chronic Obstructive Pulmonary Disease (COPD)

43.6.1 COPD as a Group of Diseases

Obstructive lung disease is a category of respiratory disease characterized by airway obstruction. Chronic obstructive pulmonary disease (COPD), also known as chronic obstructive airways disease (COAD) or chronic airflow limitation (CAL) is a group of illnesses characterised by airflow limitation that is not fully reversible. The flow of air into and out of the lungs is impaired. The COPD is characterized by chronic inflammation. It is most likely the result of complex interactions of environmental and genetic factors. Term COPD includes the conditions of emphysema and chronic bronchitis although most patients with COPD have characteristics of both conditions to varying degrees. Asthma being a reversible obstruction of airways is often considered separately, but many COPD patients also have some degree of reversibility in their airways. The most common cause of COPD is cigarette smoking. COPD may also be caused by breathing in other particles and gases.

Diagnosis of COPD is established through spirometry and chest X-ray although other pulmonary function tests can be helpful. Emphysema can only be seen on CT scan. COPD is generally irreversible although lung function can partially recover if the patient stops smoking. α1-antitrypsin deficiency is a rare genetic condition that results in COPD (particularly emphysema) due to lack of antitrypsin protein which protects fragile alveolar walls from protease enzymes released by inflammatory processes.

The prevalence of COPD is age-dependent, suggesting an intimate relationship between the pathogenesis of COPD and aging. Genetic polymorphism in SP-A is associated with the development of COPD in Chinese Hans. The genotypes of patients with COPD and healthy smoking subjects as controls for SP-A gene showed that in COPD group, the frequencies of +186 locus genotypes AA, AG and GG were 86.4%, 12.5% and 1.1%i respectively; compared to 66.7%, 27.6% and 5.7% in control group. The frequencies of polymorphic genotypes at +655 locus and +667 loci showed no significant difference between the COPD group and control group (Xie et al. 2005).

43.6.1.1 Serum SP-A in COPD and Its Relation to Smoking

SP-A occurs physiologically in small amounts in blood. Tobacco smoke induces increased alveolo-capillary leakage of SPs into blood and its level in blood may help in the assessment of lung injury caused by smoke. SP-A is occasionally elevated in non-ILD pulmonary patients. Serum SP-A increased in current smokers than in never- or ex-smokers and in COPD and pulmonary thromboembolism than in other diseases. Serum SP-D and KL-6 were unaffected by smoking. Therefore, different baseline levels of serum SP-A need to be established for smokers and non-smokers. Serum SP-A may be a useful marker for predicting COPD in the preclinical stage (Behera et al. 2005; Kobayashi et al. 2008a). Different alleles of SP-A and SP-D associated with various diseases have been summarized by Kishore et al. (2005) and given in Table 43.2. Analysis between COPD and smokers revealed several COPD susceptibility alleles (AA62_A, B1580_C, D2S388_5), based on an odds ratio (OR > 2.5). Results indicate that surfactant protein alleles may be useful in COPD by either predicting the disease in a subgroup and/or by identifying disease subgroups that may be used for therapeutic intervention (Guo et al. 2001).

Proteome research revealed increased levels of SP-A in COPD but not in normal or fibrotic lung. Furthermore, elevated SP-A protein levels were detected from the induced sputum supernatants of COPD patients. The levels of other surfactant proteins (SP-B, SP-C, SP-D) were not altered. It is suggested that SP-A is linked to the pathogenesis of COPD and can be considered as a potential COPD biomarker (Ohlmeier et al. 2008). Toxic metals and transition elements

are detectable in exhaled breath condensate (EBC) of studied subjects (Mutti et al. 2006).

43.6.1.2 SP-D Is an Ideal Biomarker in COPD

In COPD, SP-D is an ideal biomarker that is produced mostly in lungs and can be measured in the peripheral circulation. It changes with the clinical status of the patient and has inherent functional attributes that suggest a possible causal role in pathogenesis of disease (Sin et al. 2008b, c).

In a multivariable linear regression model, COPD was independently associated with lower SP-D levels. Given the importance of this molecule in lung, low levels may play a role in the pathogenesis and/or progression of COPD (Sims et al. 2008). Inhaled corticosteroids alone or in combination exhibited partial systemic anti-inflammatory effects, reducing significantly only SP-D serum levels. ICS in conjunction with long-acting β2-adrenergic agonist significantly reduced serum SP-D levels. These drugs reduce lung-specific but not generalized biomarkers of systemic inflammation in COPD. Hydrofluoroalkane-beclomethasone dipropionate (HFA-BDP) controls eosinophilic inflammation, including in distal airways, more effectively than fluticasone propionate (FP) Diskus (Ohbayashi and Adachi 2008; Sin et al. 2008a).

43.6.2 Emphysema

Emphysema is a chronic pulmonary disease marked by an abnormal increase in size of air spaces. Pulmonary emphysema, a major component of COPD, is pathologically characterized by destructive alterations in pulmonary architectures as a result of persistent inflammation. Emphysema may be a dynamic disease process in which alveolar wall cell death and proliferation are repeated. The decrease of surfactant protein secreted by the alveolar type II cell is one of the important causes of limiting air of pulmonary emphysema and the changes of SP-A may be related to emphysematous changes in the lung. Cigarette smoke and LPS alter lung SP-A gene activity and protein homeostasis (Hu et al. 2008). Mice deficient in SP-D$^{-/-}$ develop progressive emphysema with age. SP-D gene-targeted mice develop severe pulmonary lipidosis, and foamy macrophage infiltrations. By lowering surface tension at the air-water interface in the surfactant deficient premature lung, exogenous surfactant replacement therapy for neonatal RDS has been highly successful in decreasing mortality after preterm birth. It has emerged that SP-A and SP-D have additional roles in host defence distinct from the surface tension lowering effects of surfactant. Recombinant forms of SP-D could be useful therapeutically in attenuating inflammatory processes in neonatal chronic lung disease, cystic fibrosis, and emphysema (Clark and Reid 2003).

43.6.3 Allergic Disorders

43.6.3.1 Allergic Inflammation in Asthma

Asthma is an obstructive lung disease where the bronchial tubes (airways) are extra sensitive (hyperresponsive). The airways become inflamed and produce excess mucus and muscles around the airways tighten making the airways narrower. Asthma is usually triggered by breathing in things present in air such as dust or pollen that produces an allergic reaction. It may be triggered by other things such as an upper respiratory tract infection, cold air, exercise or smoke. Asthma is diagnosed by the characteristic pattern of symptoms. A peak flow meter can record variations in the severity of asthma over time. Spirometry can provide an assessment of the severity, reversibility, and variability of airflow limitation, and help confirm the diagnosis of asthma. Significant changes occur in levels of SP-A and SP-D during the asthmatic response in animal models as well as in asthmatic patients. The impact of the SP-A and SP-D on asthmatic allergic inflammation and vice versa has been reviewed (Hohlfeld et al. 2002). Serum SP-D concentrations are affected in allergic patients and correlate with changes in allergic airway inflammation. Serum SP-D levels may give additional information, beside bronchial hyperresponsiveness (BHR) and sputum eosinophils, about the degree of bronchial inflammation in allergic patients (Koopmans et al. 2004).

Immunoregulatory Roles of SP-A and SP-D

Studies on allergen-sensitized murine models and asthmatic patients show that SP-A and SP-D can: specifically bind to aero-allergens; inhibit mast cell degranulation and histamine release; and modulate the activation of alveolar macrophages and DCs during the acute hypersensitive phase of allergic response (Erpenbeck et al. 2005; Wang et al. 1998). They also can alleviate chronic allergic inflammation by inhibiting T-lymphocyte proliferation as well as increasing phagocytosis of DNA fragments and clearance of apoptotic cell debris. Furthermore, it has emerged, from the studies on SP-D-deficient mice, that, when these mice are challenged with allergen, they develop increased eosinophil infiltration, and abnormal activation of lymphocytes, leading to the production of Th2 cytokines. Intranasal administration of SP-D significantly attenuated the asthmatic-like symptoms seen in allergen-sensitized wild-type, and SP-D-deficient, mice. These findings provide a new insight of role that surfactant proteins play in handling environmental stimuli and in their immunoregulation of airway inflammatory disease (Wang and Reid 2007).

Both SP-A and SP-D can inhibit histamine release in the early phase of allergen provocation and suppress lymphocyte proliferation in the late phase of bronchial inflammation, the two essential steps in the development of asthmatic

symptoms (Wang et al. 1998). Studies suggest that the increased levels of SP-A and D may play a protective role in an allergic inflammation in the pathogenesis of bronchial asthma. Structural remodelling of airways in asthma that follows inflammation may be affected by SP-D-mediated effects on immune response. SP-D accumulation is increased in this model of allergen-induced eosinophilia, both in upper and lower airways (Cheng et al. 2000; Kasper et al. 2002). SP-D gene-deficient mice ($Sftpd^{-/-}$) have an impaired systemic Th-2 response at baseline and reduced inflammation and airway responses after allergen exposure. Translational studies revealed that a polymorphism in *SFTPD* gene was associated with lower atopy and possibly asthma susceptibility. Thus, SP-D-dependent innate immunity influences atopy and asthma (Brandt et al. 2008). Dex significantly down-regulates SP-D in allergic airways and lavage fluid. In addition, Dex promoted airway expression of vitamin D-binding protein, heptoglobin and α1-antitrypsin (Zhao et al. 2007).

Serum SP-D is increased in acute and chronic inflammation in mice. Profiles of SP-A and SP-D in acute and chronic inflammation indicated that serum SP-D can serve as a biomarker of lung inflammation in both acute and chronic lung injury in mice (Fujita et al. 2005). Because of their capability to directly inhibit T-cell activation and T-cell-dependent allergic inflammatory events, SP-A and SP-D may be significant contributors to the local control of Th-2 type inflammation in the airways. SP-D is able to reduce the immediate allergen-induced mediator release and the early bronchial obstruction in addition to its effects on airway inflammation and bronchial hyperresponsiveness in an *A. fumigatus* mouse asthma model. Thus, SP-D not only reduces allergen-induced eosinophilic inflammation and airway hyper-responsiveness but also provides protection against early airway obstruction by inhibition of early mediator release (Erpenbeck et al. 2006; Takeda et al. 2003). However, mice sensitized and challenged with either *A. fumigatus* or OVA increased SP-D levels in their lung. Allergen exposure induced elevation in SP-D protein levels in an IL-4/IL-13-dependent manner, which in turn, prevents further activation of sensitized T cells. This negative feedback regulatory circuit could be essential in protecting the airways from inflammatory damage after allergen inhalation (Haczku et al. 2006). Haczku (2006) support the hypothesis that SP-A and SP-D have a role in regulation of allergic airway sensitization.

Murine Model of Asthma

Dust mite allergens can directly activate alveolar macrophages (AΦs), induce inflammatory cytokines, and enhance T-helper type 2 cytokine production. The SP-D is able to bind mite allergens and alleviates allergen-induced airway inflammation and may be an important modulator of allergen-induced pulmonary inflammation (Liu et al. 2005a). There is marked reduction in SP-A and SP-D levels in the BALF of dust mite (*Dermatophagoides pteronyssinus*, Der p)-sensitized BALB/c mice after allergen challenge. Both SP-A and SP-D were able to suppress Der p-stimulated intrapulmonary lymphocyte proliferation of naïve mice with saline or allergen challenge, or of Der p-sensitized mice with saline challenge. On the contrary, this suppressive effect was mild on lymphocytes from sensitized mice after allergen challenge. These results indicated the involvement of pulmonary surfactant proteins in the allergic bronchial inflammation of sensitized mice (Wang et al. 1996, 2001). Both SP-A and SP-D down-regulate the eosinophilic inflammation in murine asthma models and shift the cytokine profile towards a T helper cell type 1 response. In addition, they are effective at alleviating bronchial hyperresponsiveness. There is evidence of activation of innate immune system in asthma which results in the production of pro-inflammatory cytokines and may contribute to the pathogenesis of neutrophilic asthma (Simpson et al. 2007).

43.6.3.2 Chronic Sialadenitis and Chronic Rhinosinusitis

SP-A and mRNA and protein were detected in glands of patients with chronic sialadenitis. The expression in salivary glands of patients with chronic sialadenitis was significantly higher than from healthy salivary glands. SP-A immunoreactivity, localized in the epithelial cells and submucosal glands of paranasal sinus mucosa in normal and chronic sinusitis patients, was enhanced in chronic rhinosinusitis mucosa as compared with normal paranasal sinus mucosa (Lee et al. 2004, 2006). SP-A expression in human nasal tissue was correlated with symptoms suggestive of allergic rhinitis. (Wootten et al. 2006).

43.6.4 Interactions of SP-A and SP-D with Pathogens and Infectious Diseases

Microbial targets for SP-D include both Gram-positive and Gram-negative respiratory pathogens, influenza, and respiratory syncytial viruses, *Cryptococcus neoformans, Pneumocystis carinii*, and *Aspergillus fumigatus*. Both monocytes/macrophages and neutrophils express surface receptors that can interact with SP-D. The interactions between SP-D and microorganisms and in many instances immune cells promote both microbial aggregation and enhanced phagocytosis. SP-D has been shown to bind to a variety of bacteria, including rough strains of *Salmonella Minnesota* and *E. coli* as well as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Lim et al. 1994). SP-D also stimulates the phagocytosis of *Pseudomonas aeruginosa* (Restrepo et al. 1999). The interaction of SP-D with bacteria

often results in CRD-dependent bacterial aggregation or agglutination. Unlike SP-A (van Iwaarden et al. 1994), SP-D does not bind to lipid A. It interacts with *E. coli* through the core polysaccharides and/or the O-specific antigens. The core region of the LPS of other gram-negative bacteria is broadly recognized by SP-D as well (Kuan et al. 1992). SP-D can be used as a biomarker for chronic periodontitis. As no significant associations of SFTPD gene polymorphisms could be detected, other mechanisms influencing SP-D serum/plasma expression might exist (Glas et al. 2008).

SP-D has been shown to bind to the influenza A virus, resulting in aggregation of the target (Hartshorn et al. 1996a). The binding and inhibition of hemagglutination was inhibited by chelation of calcium and by carbohydrates, suggesting that the interaction of SP-D with the virus was mediated via the CRD. SP-D also enhances the neutrophil uptake of the virus in a calcium-dependent manner (Hartshorn et al. 1997). Further enhanced antiviral and opsonic activity for influenza A virus was obtained by making a human MBP and SP-D chimera (White et al. 2000) (Table 43.1). The degree of multimerization of SP-D also appears to be important for its interactions with viruses (Brown-Augsburger et al. 1996; Hartshorn et al. 1996b). SP-D induces massive aggregation of influenza A virus particles (Hartshorn et al. 1996a). This massive agglutination of organisms could contribute to lung host defence by promoting airway mucociliary clearance, but it could also promote internalization by phagocytic cells. Recombinant SP-D inhibited RSV infectivity both in vitro and in vivo (Hickling et al. 1999; Le Vine et al. 2004), and reduced SP-D protein levels have been detected in RSV infection (Kerr and Paton 1999). A direct interaction between the yeast *Candida albicans* and SP-D confirms the importance of SP-D in innate immunity (van Rozendaal et al. 2000).

43.6.4.1 Distinct Effects of SP-A or -D Deficiency During Bacterial Infection

Surfactant proteins A and D expressed in respiratory tract bind bacterial, fungal and viral pathogens, enhancing their opsonization and killing by phagocytic cells. Clearance of bacterial pathogens including group *B streptococci, Haemophilus influenza, Pseudomonas aeruginosa* and viral pathogens, *respiratory syncytial virus, adenovirus and influenza A virus*, was deficient in SP-A$^{-/-}$ mice (Table 43.1). Mice lacking SP-A (SP-A$^{-/-}$) or SP-D (SP-D$^{-/-}$) and wild-type mice, infected with group *B streptococcus* or *Haemophilus influenzae*, are associated with increased inflammation and inflammatory cell recruitment in lung after infection. Although, decreased killing of group B *streptococcus* and *H. influenzae* was observed only in SP-A$^{-/-}$ mice but not in SP-D$^{-/-}$ mice, bacterial uptake by alveolar macrophages was reduced in both SP-A- and SP-D-deficient mice. Isolated alveolar macrophages from SP-A$^{-/-}$ mice

generated significantly less, whereas those from SP-D$^{-/-}$ mice generated significantly greater superoxide and H$_2$O$_2$ compared with wild-type alveolar macrophages.

In SP-D$^{-/-}$ mice, bacterial killing was associated with increased lung inflammation and increased oxidant production. Where as, bacterial killing was decreased and associated with increased lung inflammation and decreased oxidant production in SP-A$^{-/-}$, macrophage phagocytosis was decreased in both SP-A and SP-D deficient mice. SP-A deficiency was associated with enhanced inflammation and synthesis of pro-inflammatory cytokines. SP-D$^{-/-}$ mice cleared these bacteria as efficiently as wild-type mice; however, clearance of viral pathogens was deficient in SP-D$^{-/-}$ mice and associated with increased inflammation. Study suggests that SP-A and SP-D play distinct roles during bacterial infection of lung (LeVine et al. 2000, 2001).

Alloiococcus otitidis has been found to be associated with otitis media with effusion. SP-A and MBL interact with *A. otitidis* in Ca^{2+}-dependent manner. Results demonstrate that *A. otitidis* is a ligand for SP-A and TLR2, and that the collectins enhance the phagocytosis of *A. otitidis* by macrophages, suggesting important roles of collectins and TLR2 in the innate immunity of the middle ear against *A. otitidis* infection (Konishi et al. 2006). Meningococcal disease occurs after colonization of nasopharynx with *Neisseria meningitidis*. Variation in genes of surfactant proteins affects the expression and function of SPs. Gene polymorphism resulting in substitution of glutamine with lysine at residue 223 in the CRD of SP-A2 increases susceptibility to meningococcal disease, as well as the risk of death (Jack et al. 2006). In contrast to defensive function, SP-D in BALF binds β-glucan onB. *Dermatitidis and*, blocks BAM access to β-glucan, thereby inhibiting TNF-α production. Thus, whereas BALF constituents commonly mediate antimicrobial activity, *B. dermatitidis* may utilize BALF constituents, such as SP-D, to blunt the host defensive reaction; this effect could reduce inflammation and tissue destruction but could also promote disease (Lekkala et al. 2006)

43.7　Pulmonary Tuberculosis

43.7.1 Enhanced Phagocytosis of *M. tuberculosis* by SP-A

During initial infection with *M. tuberculosis*, bacteria that reach the distal airspaces of lung are phagocytosed by AMΦs in presence of pulmonary surfactant. Studies indicated a direct interaction between SP-A and macrophage in mediating enhanced adherence of *M. tuberculosis* (Gaynor et al. 1995). Since, SP-A binds mannose, it was hypothesized that SP-A attaches to *M. tuberculosis* and serves as a ligand

between *M. tuberculosis* and AΦs. Stokes et al. (1998) demonstrated that explanted alveolar AΦs do not efficiently bind *M. tuberculosis* in a serum-free system, although a small subpopulation of these AΦs could bind mycobacteria. In contrast, almost 100% of peritoneal AΦs bind mycobacteria under similar conditions. Evidence suggests that opsonic binding of *M. tuberculosis* by differentiated alveolar Ms is mediated by complement and CR3, and that the poor binding by resident alveolar AΦs is due to their poor expression of CR3. Thus, attachment of *M. tuberculosis* to AΦs is an essential early event in primary pulmonary tuberculosis and SP-A helps in early capture and phagocytosis of *M. tuberculosis* by AΦs. Ferguson et al. (2002) provided evidence for specific binding of SP-D to *M. tuberculosis* and indicated that SP-D and SP-A serve different roles in the innate host response to this pathogen in lung.

43.7.1.1 Lipomannan and ManLAM are Major Mycobacterial Lipoglycans as Potential Ligands

The SP-A binds to *M. bovis Bacillus Calmette-Guerin* (BCG), the vaccinating strain of pathogenic mycobacteria, and also to a lesser extent to *M. smegmatis*, which indicates that SP-A does not discriminate virulent from nonpathogenic strains. Lipomannan and mannosylated lipoarabinomannan (ManLAM) are two major mycobacterial cell-wall lipoglycans, which act as potential ligands for binding of SP-A. Both the terminal mannose residues and the fatty acids are critical for binding. It appears that recognition of carbohydrate epitopes on lipoglycans by SP-A is dependent on the presence of fatty acids (Sidobre et al. 2000, 2002).

Rivière et al, (2004) claim that the hydrophobic aglycon part of ManLAM is associated to a supra-molecular organization of these complex molecules. Furthermore, the deacylated ManLAMs or the lipid-free mannosylated arabinomannans, which do not exhibit characteristic ManLAM activities, do not display this supra-molecularorganization. These observations suggest that the ManLAMs immunomodulatory activities might be associated to their particular organization. The critical micellar concentration of ManLAMs obviously supports the notion that this supra-molecularorganization may be responsible for the specific biological activities of these complex molecules (Rivière et al. 2004).

As indicated, the molecular recognition of ManLAM terminal mannose units by CRDs of SP-A depends on the presence of lipid moiety of ManLAMs associated to a characteristic supra-molecular organization of ManLAM complex. On the other hand, the deacylated ManLAM or the lipid-free mannosylated arabinomannans, which do not exhibit characteristic ManLAM activities, do not display this supra-molecular organization. Therefore the ManLAM immunomodulatory activities might be associated to their particular organization. The critical micellar concentration

of ManLAM supports the notion that this supra-molecular organization is responsible for specific biological activities of these complex molecules.

Apa Glycoprotein on *M. tuberculosis*: A Potential Adhesion to SP-A: Although lipoglycan ManLAM is considered as the major C-type lectin target on mycobacterial surface, Ragas et al. (2007) identified Apa (alanine- and proline-rich antigenic) glycoprotein as new potential target for SP-A, which binds to purified Apa. Apa is associated to the cell wall for a long time to aid in the attachment of SP-A. Because, Apa seems to be restricted to the *M. tuberculosis* complex strains, it was proposed that it may account for selective recognition of complex strains by SP-A containing homologous functional domains.

SP-A Enhances *M. avium* Ingestion by Macrophages: Tuberculosis leads to immune activation and increased HIV-1 replication in lung. SP-A promotes attachment of *M. tuberculosis* to AΦs during infection with HIV. SP-A levels and attachment of *M. tuberculosis* to AΦs inversely correlate with peripheral blood CD4 lymphocyte counts (Downing et al. 1995). *M. avium* complex (MAC) is a significant cause of opportunistic infection in patients with AIDS. Once in lung, MAC can interact with SP-A. Work on pulmonary pathogens including *M. bovis* BCG suggests that SP-A participates in promoting efficient clearance of these organisms by AMs. Lopez et al. (2003) reported that SP-A can bind to and enhance the uptake of MAC by AΦs, similar to BCG and *M. tuberculosis*. However, unlike BCG and other pulmonary pathogens that are cleared in presence of SP-A via a NO-dependent pathway, macrophage-mediated clearance of MAC is not enhanced by SP-A.

Suppression of Reactive Nitrogen Intermediates by SP-A in AMs in Response to *M. tuberculosis*: Reactive nitrogen intermediates (RNIs) play a significant role in the killing of *mycobacteria*. RNI levels generated by AΦs were significantly increased when IFNγ-primed AΦs were incubated with *M. tuberculosis*. However, the RNI levels were significantly suppressed in presence of SP-A. Furthermore, incubation of deglycosylated SP-A with *M. tuberculosis* failed to suppress RNI by AΦs, suggesting that the oligosaccharide of SP-A, which binds to *M. tuberculosis*, is necessary for this effect. Pasula et al. (1999) showed that SP-A-mediated binding of *M. tuberculosis* to AΦs and decreased RNI levels may be one mechanism by which *M. tuberculosis* diminishes the cytotoxic response of activated AΦs.

43.7.2 SP-A Modulates Inflammatory Response in AΦs During Tuberculosis

There is a severe reduction in SP-A levels in BAL during tuberculosis only in the radiographically involved lung

segments, and the levels returned to normal after 1 month of treatment. The SP-A levels were inversely correlated with the percentage of neutrophils in BAL fluid, suggesting that low SP-A levels were associated with increased inflammation in the lung. SP-A has pleiotropic effects even at low concentrations found in tuberculosis patients. This protein augments inflammation in presence of infection and inhibits inflammation in uninfected macrophages, protecting uninvolved lung segments from the deleterious effects of inflammation (Gold et al. 2004).

SP-A modulates phenotypic and functional properties of cells of adaptive immune response such as DCs and lymphocytes. Bone marrow-derived DCs generated in presence of SP-A fail to increase LPS-induced up-regulation of MHC class II and CD86 co-stimulatory molecule on DCs surface and behaves like "tolerogenic DCs". SP-A may also induce tolerance by suppressing the proliferation of activated T lymphocytes (Hussain 2004). SP-A suppresses lymphocyte proliferation and IL-2 secretion, in part, by binding to its receptor, SP-R210. However, the mechanisms underlying this effect are not well understood. The effects of antibodies against the SP-A-binding (neck) domain (α-SP-R210n) or nonbinding C-terminal domain (α-SP-R210ct) of SP-R210 on human peripheral blood T cell immune responses against *M. tuberculosis* support the hypothesis that SP-A, via SP-R210, suppresses cell-mediated immunity against *M. tuberculosis* via a mechanism that up-regulates secretion of IL-10 and TGF-β1 (Samten et al. 2008). Role of SP-A and SP-D in linking innate and adaptive immunity to regulate host defense has been suggested by Wright (2005). Although both SP-A and SP-D can bind to T cells and directly inhibit proliferation, SP-A can also indirectly inhibit T-cell proliferation via suppression of dendritic cell (DC) maturation. SP-D has been shown to enhance antigen uptake and presentation. Taken together, these in vitro results suggest that the combined role of SP-A and SP-D is to modulate the immunologic environment of the lung so as to protect the host, yet thwart an overzealous inflammatory response that could potentially damage the lung and impair gas exchange (Wright 2005)

43.7.3 Marker Alleles in *M. tuberculosis*

Regression analyses of tuberculosis and tuberculin-skin test positive groups, on the basis of odds ratios, revealed tuberculosis susceptibility (DA11_C and GATA_3) and protective (AAGG_2) marker alleles. Similarly, between tuberculosis patients and general population control subjects, susceptibility $1A^3$, $6A^4$, and B1013_A and protective

AAGG_1, and AAGG_7 marker alleles were observed. Moreover, interactions were seen between alleles $6A^2$ and $1A^3$ and between $1A^3$ and B1013_A. Studies indicate a possible involvement of SP alleles in tuberculosis pathogenesis (Floros et al. 2000). Malik et al. (2006) investigated polymorphisms in the *SFTPA1* and *SFTPA2* genes for association with tuberculosis in 181 Ethiopian families comprising 226 tuberculosis cases. Four polymorphisms, SFTPA1 307A, SFTPA1 776T, SFTPA2 355C, and SFTPA2 751C, were associated with tuberculosis. Additional subgroup analysis in male, female and more severely affected patients provided evidence for SFTPA1/2-covariate interaction. Among five intragenic haplotypes identified in SFTPA1 gene and nine identified in SFTPA2 gene, $1A^3$ was most significantly associated with tuberculosis susceptibility (Table 43.2).

SNPs in Collagen Region of SP-A2 as a Contributing Factor: Relation exists between polymorphisms in the collagen regions of SP-A2 genes and pulmonary tuberculosis. Seven SNPs (4 exonic and 3 intronic) were identified in collagen regions of SP-A1 and SP-A2 genes in Indian population. Two intronic polymorphisms, SP-A1C1416T and SP-A2C1382G showed significant association with pulmonary tuberculosis. A redundant SNPA1660G of SP-A2 gene showed significant association with pulmonary tuberculosis. This polymorphism, when existing along with a nonredundant polymorphism, SP-A2G1649C (Ala91Pro) resulted in a stronger association with pulmonary tuberculosis. The SNPs in collagen region of SP-A2 may be one of the contributing factors to the genetic predisposition to pulmonary tuberculosis (Madan et al. 2002).

43.7.4 Interaction of SP-D with *M. tuberculosis*

Since many mycobacteria are facultative intracellular pathogens, their ability to cause disease involves entry, survival and replication within host cells. Although much progress has been made in our understanding of entry by mycobacteria, we anticipate that clarification of role of entry in pathogenesis will require further application of newly developed molecular tools to dissect each of the proposed mechanisms.

SP-D is known to bind *M. tuberculosis*. Binding of SP-D to *M. tuberculosis* is calcium dependent, and carbohydrate inhibitable. The binding of SP-D to Erdman lipoarabinomannan is mediated by terminal mannosyl oligosaccharides of this lipoglycan. Incubation of *M. tuberculosis* with sub-agglutinating concentrations of SP-D leads to reduced adherence of bacteria to macrophages, whereas incubation of

Table 43.2 SP-A and SP-D alleles associated with various diseases (Kishore et al. 2005).

Polymorphism		Disease association, population, type of study
Gene	Allele	
SP-A2	1A0	Susceptibility, RDS, Caucasian
SP-A1	6A2	Susceptibility, RDS, Caucasian
SP-A1	6A3	Protection, RDS, Caucasian
SP-A2	1A2	Protection, RDS, Caucasian
SP-A1	6A3	Protection, RDS, Negroids
SP-A1, SP-A2	6A2-1A0	Susceptibility, RDS, Caucasian, family
SP-A1	6A2	Protection, RDS, Caucasian, twins
SP-A1, SP-A2	6A2-1A0	Protection, RDS, Caucasian, twins
SP-A1	6A6	Susceptibility, BPD, Caucasian
SP-A	AA62_A	Susceptibility, COPD, Mexican
SP-D	D2S388_5	Susceptibility, COPD, Mexican
SP-A1	6A1	Susceptibility, IPF, Mexican
SP-D	DA11_C	Susceptibility, TB, Mexican
SP-A2	1A3	Susceptibility, TB, Mexican
SP-A1	6A4	Susceptibility, TB, Mexican
SP-A1, SP-A2	6A2-1A0	Susceptibility, TB, Mexican
SP-A1	C1416T	Susceptibility, TB, Indian
SP-A2	C1382G	Susceptibility, TB, Indian
SP-A2	A1660G-G1649C	Susceptibility, TB, Indian
SP-D	G459A	Susceptibility, TB, Indian
SP-D	T3130G	Susceptibility, TB, Indian
SP-D	Met 11	Susceptibility, RSV, Finnish
SP-A2	A1660G-G1649C	Susceptibility, ABPA, Indian

RDS respiratory distress syndrome, *BPD* bronchopulmonary dysplasia, *COPD* chronic obstructive pulmonary disease, *IPF* idiopathic pulmonary fibrosis, *TB* tuberculosis, *RSV* respiratory syncy–tial virus, *ABPA* allergic bronchopulmonary aspergillosis (Adapted with permission from Kishore et al. 2005 © Springer)

bacteria with SP-A leads to significantly increased adherence to monocyte-derived macrophages. Ferguson et al. (2002) provided evidence for specific binding of SP-D to *M. tuberculosis* and indicated that SP-D and SP-A serve different roles in the innate host response to this pathogen in lung. Further studies provide direct evidence that inhibition of phagocytosis of *M. tuberculosis* affected by SP-D occurs independently of aggregation process. SP-D limits the intracellular growth of bacilli in macrophages by increasing phagosome-lysosome fusion but not by generating a respiratory burst (Ferguson et al. 2006). Results also provide evidence that SP-A and SP-D enhance mannose receptor-mediated phagocytosis of *M. avium* by macrophages (Kudo et al. 2004). Virulent and attenuated *M. tuberculosis* strains bind best to immobilized SP-A (Hall-Stoodley et al. 2006). *Mycobacterium avium* has developed numerous mechanisms for entering mononuclear phagocytes. The SP-A, and SP-D, exhibit a concentration-

dependent binding to *M. avium*. Studies provide evidence that SP-A and SP-D enhance mannose receptor-mediated phagocytosis of *M. avium* by macrophages (Kudo et al. 2004).

43.7.5 Association of SPs with Diabetes

Insulin decreased SP-A gene transcription in human lung epithelial cells (Miakotina et al. 2002). Alveolar type II cells and nonciliated bronchiolar epithelial (Clara) cells in lungs of rats with diabetes have decreased SP-A, but increased mRNA. This is on account of differential expression in the level of SP-A, SP-B, and SP-C mRNAs in both alveolar and bronchiolar epithelial cells from diabetic lungs in comparison to control lungs (Sugahara et al. 1994). Nonetheless, Fernández-Real et al. (2008) reported circulating SP-A significantly higher among patients with glucose intolerance and type 2 diabetes than in subjects with normal glucose tolerance, even after adjustment for BMI, age, and smoking status. In amniotic fluid from diabetic women, SP-A levels were significantly less than in nondiabetic pregnancies. Hypertension did not modify SP-A in diabetic women. Although Snyder et al. (1988) suggested that the concentration of amniotic fluid SP-A is decreased in diabetic pregnancies, McMahan et al. (1987) concluded that in well controlled diabetic pregnancies fetal lung maturation is not adversely affected. SP-A and SP-B were significantly elevated in amniotic fluid from black mothers and in amniotic fluid from mothers who smoked during pregnancy (Pryhuber et al. 1991).

43.8 Expression of SPs in Lung Cancer

43.8.1 Non-Small-Cell Lung Carcinoma (NSCLC)

Molecular mechanisms underlying carcinogenesis of non-small cell lung cancer(NSCLC) may provide gene targets in critical pathways valuable for improving the efficacy of therapy and survival of patients with NSCLC (Chong et al. 2006). SP-A is described for a portion of NSCLC facilitating a diagnostic marker for these carcinomas (Goldmann et al. 2009). Studies in human lung carcinoma reported positive staining of tumor cells for SP-A, especially in peripheral airway cell carcinoma, which include bronchioloalveolar carcinoma and in some reports also papillary subtypes. The SP-A gene is expressed at higher levels in hyperplastic cells; the expression occurs predominantly, but not exclusively, in adenocarcinomas (Broers et al. 1992; Linnoila et al. 1992). The determination of SP-A in malignant effusions may help in distinguishing primary lung adenocarcinoma from adenocarcinomas of

miscellaneous origin. Analysis of SP-A gene transcript in pleural effusion is useful for diagnosis of primary lung adenocarcinoma (Saitoh et al. 1997; Shijubo et al. 1992). Gene expression of SP-A and SP-C was restricted to metastatic pulmonary adenocarcinomas (Betz et al. 1995). Camilo et al. (2006) suggested that all adenocarcinomas were negative for p63 where as 4 (26.6%) of 15 were positive for SP-A.

Uzaslan et al. (2005) studied 169 primary adenocarcinomas of lung (109 acinar, 32 solid with mucin, 24 papillary and 4 mucinous) for SP-A expression. Twenty-five percent of acinar, 38% of papillary and 3% of solid adenocarcinoma with mucin showed a positive intracytoplasmic SP-A reaction of the tumor cells. Results support the theory that SP-A-producing cells may generate not only bronchioloalveolar and papillary carcinoma, but also other subtypes of lung adenocarcinoma (Stoffers et al. 2004; Uzaslan et al. 2005). Tsutsumida et al. (2007) advocate that high MUC1 expression on the surface is an important characteristic of a micropapillary pattern, where as reduced surfactant apoprotein A expression in the micropapillary pattern may be an excellent indicator for poor prognosis in small-size lung adenocarcinoma.

43.8.1.1 Genetic Factors as Lung Cancer Risk

Deletions of the SP-A gene are specific genomic aberrations in bronchial epithelial cells adjacent to and within NSCLC, and are associated with tumor progression and a history of smoking. SP-A deletions might be a useful biomarker to identify poor prognoses in patients with NSCLC who might therefore benefit from adjuvant treatment (Jiang et al. 2005). Seifart et al. (2005) genotyped for SP-A1, -A2, -B, and -D marker alleles in lung cancer subgroups, which included 99 patients with small cell lung carcinoma (SCLC), or non-SCLC (NSCLC, n = 68) consisting of squamous cell carcinoma (SCC), and adenocarcinoma (AC); controls and healthy individuals (population control). Seifart et al. (2005) found (a) no significant marker associations with SCLC, (b) rare SP-A2 ($1A^9$) and SP-A1 ($6A^{11}$) alleles associate with NSCLC risk when compared with population control, (c) the same alleles ($1A9, 6A^{11}$) associate with risk for AC when compared with population ($6A^{11}$) or clinical control ($1A^9$), and (d) the SP-A1-$6A^4$ allele (found in ~10% of the population) associates with SCC, when compared with control. A correlation between SP-A variants and lung cancer susceptibility appears to exist, indicating that SP-A alleles may be useful markers of lung cancer risk.

The SP mRNAs with SP-A, B, and C were coexpressed in 10/12 (83%) of adenomas and 4/5 (80%) of carcinomas in both solid and tubulopapillary areas. SP-D mRNA signals were not noted in normal or neoplastic lung. ISH for SP A, B, or C mRNA was a helpful aid in the diagnosis of proliferative lesions of the murine lung (Pilling et al. 1999). In ovine pulmonary adenocarcinoma, caused by

jaagsiekte sheep retrovirus, SP-A and C were expressed in 70% and 80% of tumor cells, respectively, whereas Clara cell 10-kDa protein was expressed in 17% of tumor cells (Platt et al. 2002).

43.8.1.2 TTF-1 and SP-A in Differential Diagnosis

Results suggest that TTF-1 can play an important role for the maintenance and/or differentiation process in bronchiolar and alveolar cells (Nakamura et al. 2002). TTF-1 is frequently expressed in human lung cancer, especially in adenocarcinoma and small cell lung cancer, and TTF-1 expression is closely related to the expression of surfactant protein. Zamecnik and Kodet (2002) described positive results for TTF-1 and SP-A in 75% and 46% of pulmonary adenocarcinomas and in 50% and 25% of pulmonary non-neuroendocrine large cell carcinomas (LCCs), respectively. Small cell lung carcinomas were TTF-1 positive in 89% of cases and completely negative for SP-A. Squamous cell carcinomas and carcinoid tumors were negative for both proteins. The frequency of TTF-1 expression in the nucleus was very low in human lung cancer cell lines; however, their cytoplasmic positivities should be further investigated (Fujita et al. 2003. Rossi et al. (2003) (1) support the metaplastic histogenetic theory for pulmonary carcinomas group of tumors; (2) show that cytokeratin 7 and TTF-1, but not SP-A, are useful immunohistochemical markers in this setting, and (3) suggest that this group of tumors has a worse prognosis than conventional NSCL carcinoma at surgically curable stages I, justifying their segregation as an independent histologic type. Lu et al. (2006) suggested that nuclear inclusions positive for SP-A antibody staining in adenocarcinomas of lung were derived from accumulated content in the perinuclear cistern resembling pseudoinclusion processes and composed of proteins antigenically cross-reactive with SP-A. Because of its diagnostic utility TTF-1 should be added to a panel of antibodies used for assessing tumors of unknown origin. The combination of anti-TTF-1 with anti-SP-A does not increase the diagnostic usefulness of TTF-1 alone (Lu et al. 2006). Suzuki et al. (2005) and Ueno et al. (2003) reported that Napsin is better marker than SP-A for diagnosis of lung adenocarcinoma. Napsin A is an aspartic proteinase expressed in lung and kidney. Napsin A is expressed in type II pneumocytes and in adenocarcinomas of lung.

43.8.1.3 SPs as a Tool for Diagnosis of Lung Tumors

Most bronchioloalveolar carcinomas of lung react positively for SP-A. Positive SP-A staining of large cell carcinoma of the lung could indicate that at least part of these tumors have the same cellular origin or differentiation as bronchioloalveolar carcinoma. Twenty of 63 (32%) tumors stained positive for SP-A. This may imply that about one third of large cell

carcinomas of lung have a similar cellular origin or differentiation as bronchioloalveolar carcinoma (Uzaslan et al. 2006).

SP-A, a marker for lung adenocarcinomas, can be used to differentiate lung adenocarcinomas from other types and metastatic cancers of other origins (Kuroki et al. 1998; Takahashi et al. 2006b). RT-PCR and primers specific for SP-A, SP-B, SP-C and SP-D genes were used to detect nodal metastases and occult tumor spread of pulmonary adenocarcinomas. A combination of SP-A and SP-D may help to establish a differential prognosis in patients with gefitinib-induced ILD (Kitajima et al. 2006). ILD is a serious adverse event in lung cancer patients treated with gefitinib, an epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI). Pretreatment with gefitinib exacerbated LPS-induced lung EGFR-TKI by reducing SP-A expression in lung. EGFR- TKI may reduce SP-A expression in lungs of lung cancer patients and thus patients treated with EGFR tyrosine kinase inhibitor may be susceptible to pathogens (Inoue et al. 2008).

SP mRNAs were present in all lung tumors, with SPs A, B, and C being co-expressed in 83% of adenomas and 80% of carcinomas in both solid and tubulopapillary areas. No signals for SP D mRNA were noted in normal or neoplastic lung. Additionally, no staining for any SP transcript was observed in the hepatocellular carcinoma metastases. In situ hybridization for SP A, B, or C mRNA was helpful in diagnosis of proliferative lesions of the murine lung, enabling differentiation from hepatocellular metastases (Pilling et al. 1999; Qi et al. 2002).

43.9   Other Inflammatory Disorders

43.9.1 Airway Inflammation in Children with Tracheostomy

The long-term tracheostomy in infants and children may perpetuate chronic airway inflammation and airway remodeling due to easier access to the lungs for microorganisms. The SP-A and SP-D may directly interact with invading microorganisms and also modulate the activity of local immune cells. Children with tracheostomy had an increased total number of cells, increased neutrophils, and more frequently bacteria, but no viruses were recovered. SP-D concentration was reduced to half, though SP-A, SP-B, and SP-C were not different from controls. SP-D was inversely correlated to neutrophils, and high numbers of bacteria were associated with lower SP-D concentrations. It was suggested that bacteria and low SP-D support neutrophilic inflammation in the lower respiratory tract of nonsymptomatic with children with tracheostomy (Griese et al. 2004). BAL fluids from patients carrying a chronic tracheostoma agglutinated *P. aeruginosa*, which was completely inhibited by maltose. The agglutination of

P. aeruginosa by BAL fluid was related in part to the concentration of SP-D. Additional factors, such as the multimeric organization of SP-D, are likely to contribute to the agglutination of microorganisms by BAL or other body fluids (Griese and Starosta 2005).

Pulmonary Alveolar Microlithiasis (PAM): Pulmonary alveolar microlithiasis (PAM) is an uncommon chronic disease characterized by calcifications within the alveoli and a paucity of symptoms in contrast to image findings. PAM occurs in the absence of any known disorder of calcium metabolism. Takahashi et al. (2006a) reported two cases of PAM, with markedly elevated sera concentrations of SP-A and SP-D, which showed a tendency to increase as the disease progressed. Therefore, SP-A and SP-D may function as serum markers to monitor the disease activity and progression of PAM.

Gastroesophageal Reflux Disease: Children with gastroesophageal reflux often suffer from chronic, severe lung damage and recurrent infections. The mechanisms may involve reflux induced lung injury with alterations of the SP-A and SP-D, which bind specifically to various microbes and increase their elimination by granular leukocytes and macrophages. In children with gastroesophageal reflux disease (GERD), the macromolecular organization of SP-A and SP-D were significantly reduced. The more active SP-A and especially those of SP-D were diminished, whereas the smaller sized forms of SP-D were markedly increased. Reduced amounts of SP-A and SP-D and an altered structural organization of the surfactant proteins may contribute to pathogenesis of chronic lung disease commonly observed in these children (Griese et al. 2002).

43.9.2 Surfactant Proteins in Non-ILD Pulmonary Conditions

Infants with increased pulmonary blood flow secondary to congenital heart disease suffer from tachypnea, dyspnea, and recurrent pulmonary infections. In congenital heart disease with pulmonary hypertension secondary to increased pulmonary blood flow, there is a decrease in SP-A gene expression as well as a decrease in SP-A and SP-B protein contents (Gutierrez et al. 2001). In an experiment involving 4-week-old lambs with pulmonary hypertension secondary to increased pulmonary blood flow following an in utero placement of an aortopulmonary vascular graft, Lee et al. (2004) found a decrease in SP-A gene expression as well as a decrease in SP-A and SP-B protein contents. But in a lamb model of congenital heart disease with pulmonary hypertension and increased pulmonary blood flow, the effect of the shunt on SP gene expression and protein content was not

apparent within first week of life (Lee et al. 2004). No significant association between the common genetic variants of SP-A and SP-D and victims of sudden infant death syndrome (SIDS) was disclosed by Stray-Pedersen et al. (2009). However, low SP-A protein expression may possibly be determined by the 6A2/1A0 SP-A haplotype, which should be a subject for further investigation.

The SP-A level decreases significantly in acute pulmonary embolism, which may play an important role in hypoxemia in pulmonary embolism (Xie et al. 2005). Although an immunohistochemical investigation of pulmonary SP-A suggested a characteristic increase in fatal asphyxiation, no particular change was observed in the total amount of SP-A mRNA. The analysis of the SP-A1/A2 ratio may assist interpretation of the molecular alterations of SP-A related to acute asphyxial death (Ishida et al. 2002).

In hyperpnea there is a significant increase in lamellar bodies (LB) SP-A, lysozyme, and phospholipid (PL) but no change in the protein-to-prolonged hyperpnea ratios. It was suggested that (1) surfactant-associated lysozyme is secreted with LB, (2) the majority of SP-A is linked to lipid secretion but not necessarily with LB, and (3) the majority of SP-B secretion is independent of PL secretion. (4) Hyperpnea did not alter the mRNA expression of SP-A, SP-B, SP-C, or lysozyme in alveolar type II cells, but expression of SP-A and SP-B mRNA was significantly increased in lung tissue (Yogalingam et al. 1996).

43.10 DNA Polymorphisms in SPs and Pulmonary Diseases

Though the genes underlying susceptibility to RDS are insufficiently known, genes coding for SP-A and B have been assigned as the most likely genes in the etiology of RDS. Acute-RDS (ARDS) develops in association with many serious medical disorders. Mortality is at least 40%, and there is no specific therapy. The deficiency in SP-A level has been implicated in the pathophysiology of ARDS. Associations between single nucleotide polymorphisms (SNPs) of human gene coding *SFTPA1, SFTPA2,* and *SFTPD* and infectious pulmonary diseases have been established by several groups.

43.10.1 Association Between SP-A Gene Polymorphisms and RDS

Evidences suggest that the etiology of RDS is multifactorial with a significant genetic component. There are reports about polymorphisms and mutations on the surfactant protein genes, especially surfactant proteins-B, that may be associated with RDS, ARDS, and congenital alveolar proteinosis. The human SP-A gene locus includes two functional genes, *SFTPA1* and *SFTPA2* which are expressed independently, and a pseudo gene. SP-A polymorphisms play a role in respiratory distress syndrome, allergic bronchopulmonary aspergillosis and idiopathic pulmonary fibrosis. The levels of SP-A are decreased in lungs of patients with CF, RDS and chronic lung diseases (Heinrich et al. 2006).

Both low levels of SP-A and SP-A alleles have been associated with RDS. Floros et al. characterized four allelic variants of SP-A1 gene (6A, $6A^2$, $6A^3$, and $6A^4$) and five allelic variants of the SP-A2 gene (1A, $1A^0$, $1A^1$, $1A^2$, and $1A^3$) and hypothesized that specific SP-A alleles/genotypes are associated with increased risk of RDS. Because race, gestational age (**GA**), and sex are risk factors for RDS, Kala et al. (1998) studied the distribution and frequencies of SP-A alleles/genotypes while adjusting for these factors as confounders or effect modifiers in control and RDS populations with GAs ranging from 24 week to term. Although the odds ratios of several alleles and genotypes were in opposite directions for black and white subjects, the homogeneity of odds ratio reached statistical significance only in case of $6A^3/6A^3$. Although differences were observed in subgroups with different GAs of RDS white population, definitive conclusions could not be made regarding the effect of modification by GA or as a function of sex. Study suggested that (1) the genetic analyses of RDS and SP-A locus should be performed separately for black and white populations and (2) SP-A alleles/genotypes and SP-B variant may contribute to the etiology of RDS and/or may serve as markers for disease subgroups. In a genetically homogeneous Finnish population, Rämet et al. (2000) showed that certain SP-A1 alleles ($6A^2$ and $6A^3$) and an SP-A1/SP-A2 haplotype ($6A^2/1A^0$) were associated with RDS. The $6A^2$ allele was over-represented and the $6A^3$ allele was under-represented in infants with RDS. According to results, diseases associated with premature birth did not explain the association between the odds of a particular homozygous SP-A1 genotype ($6A^2/6A^2$ and $6A^3/6A^3$) and RDS. In the population evaluated, SP-B intron 4 variant frequencies were low and had no association with RDS. Thus, SP-A gene locus is an important determinant for predisposition to RDS in premature infants.

Floros et al. (2001b), in family-based linkage studies to discern linkage of SP-A to RDS, showed a link between SP-A and RDS; certain SP-A alleles/haplotypes are susceptibil ($1A^0$, $6A^2$, $1A^0/6A^2$) or protective ($1A^5$, $6A^4$, $1A^5/6A^4$) for RDS. Some differences between blacks and whites with regard to SP-A alleles may exist. In a 107 father-mother-offspring trios, divided into two sets according to proband's phenotype, Haataja et al. (2001) evaluated familial segregation of candidate gene polymorphisms by the transmission disequilibrium test. A set of 76 trios were analyzed for transmission disequilibrium from parents to affected offspring. Another set of

31 trios were studied for allele transmission from parents to hypernormal offspring born very prematurely before GA of 32 weeks. SP-A1-A2 haplotype $6A^2$-$1A^0$ showed significant excess transmission to affected infants and SP-A1 allele $6A^2$ decreased transmission to the hypernormals. Study provides a support for a role of SP-A alleles as genetic predisposers to RDS in premature infants.

43.10.2   SP-A and SP-B as Interactive Genetic Determinants of Neonatal RDS

Haataja et al. (2000) investigated if SP-B gene or interaction between SP-A and SP-B genes has a role in genetic susceptibility to RDS. Of the two SP-B polymorphisms genotyped, the Ile131Thr variation, a putative N-terminal N-linked glycosylation site of proSP-B and length variation of intron 4 have been suggested to associate with RDS. Neither of the two SP-B polymorphisms associated directly with RDS or with prematurity. Instead, results showed that known association between SP-A alleles and RDS was dependent on the SP-B Ile131Thr genotype. Hence, the SP-B Ile131Thr polymorphism is a determinant for certain SP-A alleles as factors causing genetic susceptibility to RDS ($6A^2$, $1A^0$) or protection against it ($6A^3$, $1A^2$).

Floros et al. (2001a) studied genotypes for SP-B intron 4 size variants and for four SNPs [−18 (A/C), 1013 (A/C), 1580 (C/T), 9306 (A/G)] in SP-B in black and white subjects. Based on odds ratio: (1) the SP-B intron 4 deletion variant in white subjects is more of an RDS risk factor for males and for subjects of 28 weeks < gestational age (GA) <33 weeks; (2) the SP-B intron 4 insertion variant in black subjects is more of an RDS risk factor in females; (3) in white subjects, SP-A1 ($6A^2$/$6A^2$) or SP-A2 ($1A^0$/$1A^0$ or $1A^0$/*) genotypes in subjects of certain GA and with a specific SP-B genotype (9306 (A/G) or deletion/*) are associated with an enhanced risk for RDS; (4) in black subjects, SP-A1 ($6A3$/$6A^3$ or $6A^3$/*) genotypes in subjects of 31 weeks < or = GA < or = 35 weeks and with the SP-B (1580 (T/T)) genotype are associated with a reduced risk for RDS. The SP-B polymorphisms are important determinants for RDS. These may identify differences between black and white subjects, as well as, between males and females regarding the risk for RDS. Moreover, SP-A susceptibility or protective alleles, in specific SP-B background, are associated with an increased or reduced risk for RDS.

43.10.3   RDS in Premature Infants

DNA samples from 441 premature singleton infants and 480 twin or multiple infants were genotyped for SP-A1, SP-A2, and SP-B exon 4 polymorphisms and intron 4 size variants in a homogeneous white population. Distribution of SP-A and SP-B gene variants between RDS and no-RDS infants were determined alone and in combination. The SP-A1 allele 6A2 and homozygous genotype 6A2/6A2 are over-represented in RDS of singletons when SP-B exon 4 genotype was Thr/Thr, and under represented in RDS of multiples when the SP-B genotype was Ile/Thr. The SP-A 6A2 allele in SP-B Thr131 background predisposed the smallest singleton infants to RDS, whereas near-term multiples were protected from RDS. There was a continuous association between fetal mass and risk of RDS, defined by SP-A and SP-B variants. Labeled lung explants with the Thr/Thr genotype showed proSP-B amino-terminal glycosylation, which was absent in Ile/Ile samples. Hence, Genetic and environmental variation may influence intracellular processing of surfactant complex and the susceptibility to RDS (Marttila et al. 2003b). However, the association between SP-A polymorphisms and RDS may not be applicable to entire population of premature infants. In twins, the association between SP-A polymorphism and RDS is different from that seen in premature singleton infants. The factor associated with SP-A genotype-specific susceptibility to RDS appears to be related to the size of uterus and length of gestation at birth (Marttila et al. 2003a). Zhai et al. (2008) reported that the frequency of SP-A1 allele $6A^2$ and $6A^3$ expression of SP-A in Chinese premature infants was low in neonatal RDS. In contrast, the frequency of SP-A2 allele $1A^0$ and $1A^1$ was high in normal Chinese premature infants. It supports that SP-A1 allele $6A^2$ may be a susceptible gene for RDS.

43.10.4   Gene Polymorphism in Patients of High-altitude Pulmonary Edema

A pathogenetic cofactor for development of high-altitude pulmonary edema (HAPE) is an increase in capillary permeability, which could occur as a result of an inflammatory reaction and/or free-radical-mediated injury to lung. Pulmonary SP-A has potent antioxidant properties and protects unsaturated phospholipids and growing cells from oxidative injury (Swenson et al. 2002). In view of protective role of SP-A against oxidative damage, Saxena et al. (2005) examined the association of constitutional susceptibility to HAPE with polymorphisms in SP-A1 and SP-A2. Allele frequencies of three loci in SP-A1 and one in SP-A2 were significantly different between low-altitude native (LAN) HAPE patients and LAN control subjects. Heterozygous individuals, with respect to SP-A1 C1101T and SP-A2 A3265C, showed less severity in oxidative damage in comparison with homozygous subjects (SP-A1 T1101 and SP-A2 C3265). The polymorphisms in SP-A1 might be one of the genetic factors contributing to susceptibility to HAPE (Saxena et al. 2005).

43.10.5 SNPs in Pulmonary Diseases

Four validated SNPs were genotyped with sequence-specific probes (TaqMan 7000) in 284 newborn infants below 32 weeks of GA. The finding of an association of a variant of the *Sftpd* gene, that has previously been shown to be associated with increased SP-D serum levels in adult patients with RDS in preterm infants, may provide a basis for the initial risk assessment of RDS and modification of surfactant treatment strategies. A role for SP-D in neonatal pulmonary adaptation has to be postulated. Genotyping for three SNP altering amino acids in the mature protein in codon 11 (Met[11]Thr), 160 (Ala[160]Thr), and 270 (Ser[270]Thr) of the SP-D gene was performed and related to the SP-D levels in serum. Individuals with Thr/Thr-11-encoding genotype had significantly lower SP-D serum levels than individuals with Met/Met (11) genotype. Polymorphic variation in the N-terminal domain of the SP-D molecule influences oligomerization, function, and the concentration of the molecule in serum (Hilgendorff et al. 2009; Leth-Larsen et al. 2005; Sorensen et al. 2007).

Studies on twins indicated very strong genetic dependence for serum levels of SP-D. Sequencing of 5′ untranslated region (5′UTR), the coding region and the 3′ region of *Sftpd* gene of 32 randomly selected blood donors indicated one single *Sftpd* haplotype (allele frequency 13.53%) that showed a negative association with serum SP-D levels. The discovery of a frequent negative variant of *Sftpd* gene provides a basis for genetic analysis of function of SP-D in resistance against pulmonary infections and inflammatory disorders in humans (Heidinger et al. 2005). The presence of SP-D in non-pulmonary tissues, such as gastrointestinal tract and genital organs, suggest additional functions located to other mucosal surfaces. Sorensen et al. (2007) summarized studies on genetic polymorphisms, structural variants, and serum levels of human SP-A and SP-D and their associations with human pulmonary disease.

Polymorphisms of genes are transmitted together in haplotypes, which can be used in study of development of complex diseases such as RDS. Genetic haplotypes of these SP genes are associated with the development of RDS. Studies identify protective haplotypes against RDS and support findings related to SP genetic differences in children who develop RDS. An allele association study of 19 polymorphisms in SP-A1, SP-A2, SP-B, and SP-D genes in ARDS was carried out. Analysis revealed differences in frequency of alleles for some of the microsatellite markers flanking SP-B, and for one polymorphism (C/T) at nucleotide 1580 [C/T (1580)], within codon 131 (Thr[131]Ile) of the SP-B gene. The latter determines the presence or absence of a potential N-linked glycosylation site. Based on the odds ratio, the C allele may be viewed as a susceptibility factor for ARDS. These data suggest that SP-B or a linked gene contributes to susceptibility to ARDS (Lin et al. 2000; Thomas et al. 2007).

Amino Acid Variants in SP-D Are Not Associated with Bronchial Asthma: As SP-D binds and neutralizes common allergens like house dust mites it is especially important in allergic asthma. Levels of SP-D are elevated in serum and alveolar lavage of asthmatic patients. Three common amino acid variants have been identified in SP-D and association of first variant has been described to severe infection with respiratory syncytial virus. The three polymorphisms leading to amino acid exchanges (Met[11]Thr, Ala[160]Thr, and Ser[270]Thr) were typed in 322 asthmatic children and none of these polymorphisms was associated with bronchial asthma. Haplotype analyses revealed four major haplotypes all of which were evenly distributed between the populations. Functional amino acid variants in SP-D do not seem to play a major role in the genetic pre-disposition to bronchial asthma in children (Krueger et al. 2006).

Following allergen exposure in vivo, SP-D$^{-/-}$ mice expressed higher bronchoalveolar lavage (BAL) eosinophils and IL-13 and lower FN-γ expression at early time points compared with wild mice. IL-10 expression was increased at early time points in SP-D$^{-/-}$ compared with wild mice. SP-D may be critical for the modulation of early stages of allergic inflammation in vivo (Schaub et al. 2004).

Pettigrew et al. (2006, 2007) evaluated gene polymorphisms in loci encoding SP-A and risk of otitis media during first year of life among a cohort of infants at risk for developing asthma in white infants. Polymorphisms at codons 19, 62, and 133 in SP-A1, and 223 in SP-A2 were associated with race/ethnicity. In regression models incorporating estimates of uncertainty in haplotype assignment, the 6A[4]/1A[5] haplotype was protective for otitis media among white infants. On similar line, analyses suggested that polymorphisms within *SFTPA* loci may be associated with wheeze and persistent cough in white infants at risk for asthma. These associations require replication and exploration in other ethnic/racial groups.

43.10.6 Allergic Bronchopulmonary Aspergillosis and Chronic Cavitary Pulmonary Aspergillosis (CCPA)

Individuals with any structural or functional defects in SP-A and SP-D due to genetic variations might be susceptible to aspergillosis. Single nucleotide polymorphism in genes of collagen region of SP-A1 and SP-A2 has been associated with allergic bronchopulmonary aspergillosis (ABPA) and its clinical markers. SP-A2 G1649C and SP-A2 A1660G, polymorphisms in the collagen region of SP-A2, might be one of the contributing factors to genetic predisposition and

severity of clinical markers of ABPA. SNPs in SP-A2 and MBL genes showed significant associations with patients of ABPA in an Indian population. Patients carrying either one or both of GCT and AGG alleles of SP-A2 and patients with A allele at position 1011 of MBL had markedly higher eosinophilia, total IgE antibodies and lower FEV1. Therapeutic administration of SP-D and MBL proteins in a murine model of pulmonary invasive aspergillosis rescued mice from death. In mice mimicking human ABPA, SP-A and SP-D suppressed IgE levels, eosinophilia, pulmonary cellular infiltration and cause a marked shift from a pathogenic Th2 to a protective Th1 cytokine profile. Thus, collectins play an important role in Aspergillus mediated allergies and infections (Madan et al. 2005; Saxena et al. 2003).

Patients with CCPA or ABPA of Caucasian origin were screened for SNPs in collagen region of SP-A1 and SP-A2 and MBL. The T allele at T1492C and G allele at G1649C of SP-A2 were observed at slightly higher frequencies in ABPA patients (86% and 93%) than in controls (63% and 83%), and the C alleles at position 1492 and 1649 were found in higher frequencies in CCPA patients (33% and 25%) than in ABPA patients (14% and 7%). However, the CC genotype at position 1649 of SP-A2 was significantly associated with CCPA. Similarly, ABPA patients showed a higher frequency of TT genotype (71%) at 1492 of SP-A2 than controls (43%) and CCPA patients (41%). In case of MBL, the T allele and CT genotype at position 868 (codon 52) were significantly associated with CCPA, but not with ABPA. Further analysis of genotype combinations at position 1649 of SP-A2 and at 868 of MBL between patient groups showed that both CC/CC and CC/CT SP-A2/MBL were found only in CCPA patients, while GG/CT SP-A2/MBL was significantly higher in CCPA patients in comparison to ABPA patients. SNPs in SP-A1 did not differ between patients and controls. Distinct alleles, genotypes and genotype combinations of SP-A2 and MBL may contribute to differential susceptibility of the host to CCPA or ABPA (Vaid et al. 2007).

Allergic Airway Inflammation: The SP-A has potent immunomodulatory activities. SP-A protein levels in the BAL fluid showed a rapid, transient decline that reached the lowest values (25% of controls) 12 h after intranasal Af provocation of sensitized mice. It was speculated that a transient lack of SP-A following allergen exposure of airways may significantly contribute to the development of a T-cell dependent allergic immune response (Scanlon et al. 2005). After acute ovalbumin-induced allergic airway inflammation (1) alveolar epithelial type II cells (AEII) but not Clara cells show a significantly higher expression of SP-A and SP-D in rats leading also to higher amounts of both SPs in BALF and (2) macrophages gather predominantly SP-A (Schmiedl et al. 2008).

43.10.7 Autoreactivity Against SP-A and Rheumatoid Arthritis

Circulating SP-D is decreased in early rheumatoid arthritis and SP-A and SP-D levels in synovial fluid from patients correlated with rheumatoid factor, CRP, IgA, IgM, and IgG, and total lipid content. SP-A and SP-D seem to participate in initiation of immune system and joint inflammation within the joint (Kankavi 2006) and may be an additional RA disease modifier like MBL. The Met[11]Thr polymorphism in the N-terminal part of SP-D is important determinant in serum SP-D. But this polymorphism is also essential to the function and assembly into oligomers. SP-D levels did not correlate with traditional disease activity measures. The Thr[11]/Thr[11] genotype and the Thr[11] allele tended to be more frequent in RA patients. Therefore, the low serum level of SP-D and the lack of correlation with traditional disease activity measures indicate that SP-D reflects a distinctive aspect in the RA pathogenesis (Hoegh et al. 2008; Miyata et al. 2002). Trinder et al. (2000) were able to show autoreactivity to SP-A, as expressed by IgG and IgM autoantibodies, and present in synovial fluid (SF) from patients with RA. There was no cross-reactivity between autoantibodies reactive with type II collagen (CII) and those reactive with SP-A or C1q; However, autoantibodies reacted with polymeric (dimers and larger) SP-A, but not with monomeric SP-A subunits, indicating that a degree of quaternary structure is required for antibody binding.

43.11 Inhibition of SP-A Function by Oxidation Intermediates of Nitrite

43.11.1 Protein Oxidation by Chronic Pulmonary Diseases

The oxidation of proteins may play an important role in the pathogenesis of chronic inflammatory lung diseases, and may contribute to lung damage. Higher levels of protein oxidation than in healthy controls were observed in patients with interstitial lung disease, gastro-esophageal reflux disease, and PAP. The proteins most sensitive to oxidation were serum albumin, SP-A, and α1-antitrypsin. Abundance of reactive oxygen species produced during neutrophilic inflammation may be a deleterious factor that leads to pulmonary damage in these patients (Starosta and Griese 2006). Primary chain and quaternary structure of SP-D in BALFs

showed significant changes under oxidative conditions in vitro and in vivo and functional capacity to agglutinate bacteria was impaired by oxidation. Free radicals generated in lungs resulting in oxidation of SP-D may impair host defense and may contribute to the suppurative lung diseases like cystic fibrosis (Starosta and Griese 2006).

43.11.2 Oxidation Intermediates of Nitrite

Nitration of protein tyrosine residues by peroxynitrite ($ONOO^-$) has been implicated in a variety of inflammatory diseases such as ARDS. A mixture of hypochlorous acid (HOCl) and nitrite (NO_2^-) induces nitration, oxidation, and chlorination of tyrosine residues in human SP-A, and inhibits SP-A's ability to aggregate lipids and bind mannose. Nitration and oxidation of SP-A was not altered by the presence of lipids, suggesting that proteins are preferred targets in lipid-rich mixtures such as pulmonary surfactant. Moreover, both horseradish peroxidase and myeloperoxidase (MPO) can utilize NO_2^- and H_2O_2 as substrates to catalyze tyrosine nitration in SP-A, and inhibit its lipid aggregation function. SP-A nitration and oxidation by MPO is markedly enhanced in presence of Cl^- and the lipid aggregation function of SP-A is completely abolished. Studies suggest that MPO released by activated neutrophils during inflammation utilizes physiological or pathological levels of NO_2^- to nitrate proteins, and may provide an additional mechanism in addition to ONOO – formation, for tissue injury in ARDS and other inflammatory diseases associated with upregulated NO* and oxidant production. The oxidant-mediated tissue injury is likely to be important in the pathogenesis of ARDS/ALI (Davis et al. 2002; Lang et al. 2002; Narasaraju et al. 2003).

In vitro and in vivo data suggest that NO alters surfactant protein gene expression. The role of NO in ALI remains controversial. Although inhaled NO increases oxygenation in clinical trials, inhibiting NOS can be protective. However, inhalation of NO may not be indicated in sepsis because of excessive NO production. Aikio et al. (2003) indicated that inhaled NO is effective in a select group of small premature infants and that the responsiveness to NO is associated with low NOS2 enzyme. Very low birth-weight infants (birthweight <1,500 g), infants with progressive respiratory failure and infection at birth have deficient pulmonary NOS2 and cytokine response. After surfactant therapy, these infants responded strikingly to inhaled NO. An acute pulmonary inflammatory response may contribute to respiratory adaptation in early-onset pneumonia. In intact lambs inhaled NO increased SP-A and SP-B mRNA and protein content with no change in DNA content. The mechanisms and physiological effects of these findings warrant further investigation (Stuart et al. 2003; Hu et al. 2007). Exposure of rats to

NO2 showed impairment of SP-A and a higher alveolar pool size after in vivo exposure. The NO2-induced alterations of SP-A may contribute to the pulmonary toxicity of this oxidant (Müller et al. 1992). NO production from NOS2 expressed in lung parenchymal cells in a murine model of ARDS correlates with abnormal surfactant function and reduced SP-B expression. $NOS2^{-/-}$ null mice exhibit significantly less physiologic lung dysfunction and loss of SP-B expression. Study indicated that the expression of NOS2 in lung epithelial cells is critical for the development of lung injury and mediates surfactant dysfunction independent of NOS2 inflammatory cell expression and cytokine production (Baron et al. 2004).

43.11.3 BPD Treatment with Inhaled NO

Inhaled NO is used to treat a number of disease processes. BPD is characterized by arrested alveolar and vascular development of immature lung. The increased expression of SP-A mRNA under hyperoxia can be attributed, at least in part, to an induction of mRNA and protein expression in bronchial Clara cells. The expanded role of Clara cells in the defence against hyperoxic injury suggests that they support alveolar type 2 cell function and may play an important role in the supply of surfactant proteins to the lower airways (ter Horst et al. 2006). The inhaled nitric oxide treatment of premature infants at risk for bronchopulmonary dysplasia does not adversely affect endogenous surfactant function or composition and may improve surfactant function transiently (Ballard et al. 2007). Chorioamnionitis is a risk factor for the development of bronchopulmonary dysplasia. Endotoxin-induced oxidative stress to the fetus in the uniquely hypoxic intrauterine environment has been reported. SP-A and B mRNAs were highest at Day 2, suggesting that oxidative stress did not contribute to the lung maturation response. A modest lung oxidative stress in chorioamnionitis could contribute to bronchopulmonary dysplasia (Cheah et al. 2008).

43.12 Congenital Diaphragmatic Hernia

Pulmonary hypoplasia is one of the main causes for high mortality rate in patients with congenital diaphragmatic hernia (CDH). The expression of SP-A in hypoplastic CDH lung is reduced, and its concentration is decreased in amniotic fluid of pregnancies complicated by CDH. In animal models, surfactant deficiency contributes to the pathophysiology of the disease. In humans surfactant disaturated phosphatidylcholine (DSPC) synthesis and SP-A were significantly lower in infants with CDH than in control subjects (Cogo et al. 2002).

SP-A is altered in developing lungs from rat fetuses with CDH induced by maternal ingestion of Nitrofen on Day 9 of

gestation. There is decreased expression of SP-A in rat fetuses with CDH secondary to Nitrofen exposure (Mysore et al. 1998). In rat CDH model, induced in pregnant rats following administration of nitrofen, SP-A, SP-B, and SP-D mRNA expression in CDH lung were significantly decreased compared to controls at birth and 6 h after ventilation. The inability of O2 to increase SP mRNA expression in hypoplastic CDH lung suggests that the hypoplastic lung is not responsive to increased oxygenation for synthesis of SP (Shima et al. 2000). Though, SP's deficiency appears to be a common feature among various CDH models, TTF-1 expression was not altered in surgical model in contrast to nitrofen model, indicating different molecular mechanisms in two models (Benachi et al. 2002).

43.13 Protective Effects of SP-A and SP-D on Transplants

Surfactant treatment has been shown to improve lung transplant function, but the effect is variable. Erasmus et al. (2002) indicated that SP-A enrichment of surfactant improves the efficacy of surfactant in lung transplantation. After instillation of SP-A-enriched surfactant, P_{O2} values were reached to control values, whereas after SP-A-deficient surfactant treatment, the P_{O2} values did not improve (Erasmus et al. 2002). The impairment of surfactant adsorption from transplanted lungs may be correlated with decreased levels of SP-A, and increased levels of serum acute-phase protein C-reactive protein (CRP). The elevated levels of CRP in BAL can be a very sensitive marker of lung injury (Casals et al. 1998).

Bronchiolitis obliterans syndrome (BOS) affects long-term survival of lung transplant recipients (LTRs). Among 11 differentially expressed proteins in BALF, peroxiredoxin 2 (Prdx2) exclusively expressed in BOS; and SP-A expressed consistently less in BOS patients than in stable LTRs. The reduction of SP-A in BALF was detectable early after lung transplant, preceding BOS onset in four of five patients and indicated that SP-A levels in BALF could predict LTR patients who are at higher risk of BOS development (Meloni et al. 2007) BOS and IPS cause high mortality and impaired survival after allogeneic hematopoietic stem-cell transplantation (allo-HSCT). The pretransplant serum SP-D levels but not SP-A, KL-6 in BOS/IPS patients were lower than those in non-BOS/IPS patients. However, the patients with lower pretransplant serum SP-D level had a trend toward frequent development of BOS/IPS. Constitutive serum SP-D level before allo-HSCT may be a useful, noninvasive predictor for the development of BOS/IPS (Nakane et al. 2008).

Keratinocyte growth factor (KGF) given before bone marrow transplantation (BMT) can prevent allogeneic T cell-dependent lung inflammation, but the antiinflammatory effects of KGF were impaired in mice injected with both T cells and conditioning regimen of cyclophosphamide. Yang et al. (2000, 2002) demonstrated that addition of cyclophosphamide interferes with the ability of KGF to enhance SP-A production. The systemic pre-BMT injection of KGF in recipients of allogeneic T cells up-regulates SP-A, which may contribute to the early antiinflammatory effects of KGF. Exogenous and basal endogenous SP-A can suppress donor T-cell-dependent inflammation that occurs during the generation of idiopathic pneumonia syndrome after BMT. Wild-type and SP-A-deficient mice, given allogeneic donor bone marrow plus inflammation-inducing spleen T cells, showed that basal endogenous SP-A, and enhanced alveolar SP-A level modulate donor T-cell-dependent immune responses and prolong survival after allogeneic BMT.

43.14 Therapeutic Effects of SP-A, SP-D and Their Chimeras

43.14.1 SP-A Effects on Inflammation of Mite-sensitized Mice

SP-A and SP-D interact with a wide range of inhaled allergens, competing for their binding to cell-sequestered IgE resulting in inhibition of mast cell degranulation. SP-D interacts with glycoprotein allergens of house dust mite (*Dermatophagoides pteronyssinus*, Derp) via its CRDs and thus inhibits specific IgE, isolated from mite-sensitive asthmatic patients, from binding these allergens, and blocking subsequent histamine release from sensitized basophils. Exogenous administration of SP-A and SP-D diminishes allergic hypersensitivity in vivo. A fragment of recombinant human SP-D (rfh SP-D) has a therapeutic effect on allergen-induced bronchial inflammation through its inhibitory effect on NO and TNF-α production by AΦs, and thus preventing the development of Th-2 type cytokine response (Liu et al. 2005b; Singh et al. 2003). The rfhSP-D that is effective in diminishing allergic hypersensitivity in mouse models of dust mite allergy was more susceptible to degradation than the native full-length protein. The degradation and consequent inactivation of SP-A and SP-D may be a mechanism to account for the potent allergenicity of these common dust mite allergens (Deb et al. 2007). Evidence suggests for an antiinflammatory role for SP-D in response to noninfectious, subacute lung injury via modulation of oxidative-nitrative stress (Casey et al. 2005).

43.14.2 SP-D Increases Apoptosis in Eosinophils of Asthmatics

The effect of exogenous rfhSP-D on protection of adult mouse lung from LPS-induced and lipoteichoic acid (LTA)-induced injury was assessed in $Sftpd^{+/+}$ and $Sftpd^{-/-}$

mice. Intratracheal rhSP-D inhibited inflammation induced by intratracheal LPS and LTA instillation in lung. The antiinflammatory effects of rhSP-D were enhanced by addition of pulmonary surfactant, providing a potential therapy for the treatment of lung inflammation (Ikegami et al. 2007). In view of therapeutic effects of exogenous SP-D or rfhSP-D (composed of eight Gly-X-Y collagen repeat sequences, homotrimeric neck and lectin domains) in murine models of lung allergy and hypereosinophilic SP-D gene-deficient mice, Mahajan et al. (2008) suggested that rfhSP-D mediated preferential increase of apoptosis of primed eosinophils while not affecting the normal eosinophils. The increased phagocytosis of apoptotic eosinophils may be important mechanisms of rfhSP-D and SP-D-mediated resolution of allergic eosinophilic inflammation in vivo.

43.14.3 Targeting of Pathogens to Neutrophils Via Chimeric SP-D/Anti-CD89 Protein

Intratracheal rfhSP-D prevents shock caused by endotoxin released from the lung during ventilation in the premature newborn (Ikegami et al. 2006). In lambs, preterm infants experience enhanced susceptibility and severity to respiratory syncytial virus (RSV) infection. This was observed when SP-A, -D and TLR4 mRNA expression increased from late gestation to term birth, where as in preterm lungs, studies showed reduced SP-A, -D, and TLR4 expression and enhanced RSV susceptibility (Meyerholz et al. 2006).

A chimeric protein, consisting of a recombinant fragment of human SP-D coupled to a Fab' fragment directed against human Fc α receptor (CD89) (chimeric rfSP-D/anti-Fc), effectively targets pathogens recognized by SP-D to human neutrophils. A recombinant trimeric fragment of SP-D (rfSP-D), consisting of CRD and neck domain of human SP-D, cross-linked to the Fab' of an Ab directed against the human Fc α RI (CD89) (chimeric rfSP-D/anti-CD89 protein) enhanced uptake of *E. coli*, *C. albicans*, and *influenza A virus* by human neutrophils (Tacken et al. 2004). Both chimeric rfSP-D/anti-Fc receptor proteins increased internalization of *E. coli* by human promonocytic cell line U937, but only after induction of monocytic differentiation. Both CD64 and CD89 on U937 cells proved suitable for targeting by rfSP-D/anti-Fc receptor proteins (Tacken and Batenburg 2006). Collectin-based chimeric proteins may thus offer promise for therapy of infectious disease.

43.14.4 Anti-IAV and Opsonic Activity of Multimerized Chimeras of rSP-D

A recombinant human SP-D, consisting of a short collagen region (two repeats of Gly-Xaa-Yaa amino acid sequences),

the neck domain and CRD can form a trimeric structure owing to neck domain and exhibits sugar-binding activity and specificity similar to those of native human SP-D. Though the truncated SP-D could bind to IAV, like native SP-D, but the truncated human SP-D was less effective in agglutinating bacteria than the native structure and failed to inhibit haemagglutination by IAV (Eda et al. 1997). On the other hand, chimeric collectin containing N-terminus and collagen domain of human SP-D and CRD of MBL showed greater anti-IAV activity than similarly multimerized preparations of SP-D or incompletely oligomerized preparations of the chimera. Highly multimerized preparations of chimera also caused greater increases in uptake of IAV by neutrophils. These studies may be useful for development of collectins as therapeutic agents against IAV infection (Hartshorn et al. 2000b; White et al. 2000).

Bovine serum conglutinin has greater ability to inhibit IAV infectivity than other collectins. Altering the carbohydrate binding properties of SP-D [e.g., by replacing its CRD with that of either MBL or conglutinin] can increase its activity against IAV. Hence, recombinant conglutinin and a chimeric protein containing NH_2 terminus and collagen domain of rat SP-D (rSP-D) fused to neck region and CRD of conglutinin (termed SP-D/Cong(neck + CRD)) have markedly greater ability to inhibit infectivity of IAV than wild-type recombinant rSP-D, confirming that potent IAV-neutralizing activity of conglutinin resides in its neck region and CRD. Furthermore, SP-D/Cong(neck + CRD) also caused substantially greater enhancement of neutrophil binding and H_2O_2 responses to IAV than r-conglutinin or rSP-D. Hence, chimeric SP-D/Cong(neck + CRD) protein showed favorable antiviral and opsonic properties of conglutinin and SP-D (Hartshorn et al. 2000a). Thus, the SP-D N-terminal and/or collagen domains contribute to the enhanced bacterial binding and aggregating activities of SP-D. Although replacement of neck recognition domains and CRDs of SP-D with those of MBL and conglutinin confer increased viral binding activity, it does not favorably affect bacterial binding activity, suggesting that requirements for optimal collectin binding to influenza virus and bacteria differ (Hartshorn et al. 2007).

Chimera of Trimeric Neck + CRDs of Human SP-D: The recombinant trimeric neck + CRDs of human SP-D (NCRD) retains binding activity for some ligands and mediates some functional activities. In comparison to strong neutralizing activity of lung SP-D for IAVs in vitro and in vivo, the NCRD derived from SP-D has weak viral-binding ability and lacks neutralizing activity. Using a panel of mAbs against NCRD, Tecle et al. (2008) showed that antiviral activities of SP-D can be reproduced without the N-terminal and collagen domains and that cross-linking of NCRDs is

essential for antiviral activity of SP-D with respect to IAV (Tecle et al. 2008).

Incubation of native SP-D or NCRDs with peroxynitrite results into nitration and nondisulfide cross-linking. Modifications could be blocked by peroxynitrite scavengers or pH inactivation of peroxynitrite. Abnormal cross-linking leads to defective aggregation. Thus, modification of SP-D by reactive oxygen-nitrogen species could contribute to alterations in the structure and function of SP-D at sites of inflammation in vivo (Matalon et al. 2009). In contrast, a trimeric neck and CRD construct of bovine serum collectin CL-46 induces aggregation of IAV and potently increases IAV uptake by neutrophils. CL-46-NCRD showed calcium-dependent and sugar-sensitive binding to both neutrophils and IAV. Results indicate that collectins can act as opsonins for IAV even in the absence of the collagen domain or higher order multimerization. This may involve increased affinity of individual CRDs for glycoconjugates displayed on host cells or the viral envelope (Hartshorn et al. 2010).

Insertion of Arg-Ala-Lys in NCRD Increases Inhibitory Activity: Arg-Ala-Lys (RAK) (immediately N-terminal to the first motif) in CL-43 contributes to differences in saccharide selectivity and host defense function. Insertion of CL-43 RAK sequence or a control Ala-Ala-Ala sequence (AAA) into corresponding position in NCRD increased the efficiency of binding to mannan and changed the inhibitory potencies of competing saccharides to more closely resemble those of CL-43. In addition, RAK resembled CL-43 in its greater capacity to inhibit infectivity of IAV and to increase uptake of IAV by neutrophils (Crouch et al. 2005).

43.15 Lessons from SP-A and SP-D Deficient Mice

SP-D deficient (SP-D$^{-/-}$) mice exhibit an increase in the number and size of airway macrophages, peribronchiolar inflammation, increases in metalloproteinase activity, and development of emphysema. Mice deficient in SP-D$^{-/-}$ develop progressive emphysema with age, associated with loss of parenchymal tissue, subpleural fibrosis, and accumulation of abnormal elastin fibers. The changes in lung structure in SP-D$^{-/-}$ mice are reflected in the mechanical properties of both airway and lung parenchyma measured in vivo (Yoshida and Whitsett 2006).

Gene-targeted mice deficient in SP-D develop abnormalities in surfactant homeostasis, hyperplasia of alveolar epithelial type II cells, and emphysema-like pathology. Alveolar and tissue phosphatidylcholine pool sizes are markedly increased in SP-D$^{-/-}$ mice. The pulmonary lipoidosis in SP-D$^{-/-}$ mice was not associated with accumulation of SP-B or C, or their mRNAs, distinguishing the disorder from alveolar proteinosis syndromes. Surfactant protein A mRNA was reduced and, SP-A protein appeared to be reduced in SP-D$^{-/-}$ compared with wild type mice. Targeting of mouse SP-D gene caused accumulation of surfactant lipid and altered phospholipid structures, demonstrating a unsuspected role for SP-D in surfactant lipid homeostasis in vivo (Botas et al. 1998; Korfhagen et al. 1998; Ikegami et al. 2005). HDL cholesterol was significantly elevated in SP-D$^{-/-}$ mice while treatment of SP-D$^{-/-}$ mice with rhSP-D resulted in decreases of HDL-cholesterol as well as total cholesterol, and LDL cholesterol along with reduced plasma TNF-α in SP-D$^{-/-}$ mice. It shows that SP-D regulates atherogenesis in mouse model (Sorensen et al. 2006). SP-D plays a critical role in the suppression of alveolar macrophage activation, which may contribute to the pathogenesis of chronic inflammation and emphysema (Wert et al. 2000). Oxidant production and reactive oxygen species were increased in lungs of SP-D$^{-/-}$ mice, in turn activate NF-kB and MMP expression. SP-D plays an unexpected inhibitory role in the regulation of NF-kB in AΦs (Yoshida et al. 2001).

Studies indicate that GM-CSF-dependent macrophage activity is not necessary for emphysema development in SP-D-deficient mice, but that type II cell metabolism and proliferation are, either directly or indirectly, regulated by GM-CSF in this model (Hawgood et al. 2001; Ochs et al. 2004). SP-D and GM-CSF play distinct roles in the regulation of surfactant homeostasis and lung structure (Ikegami et al. 2001).

SP-A and SP-D Double Deficient Mice SP-A and SP-D proteins have overlapping as well as distinct functions. Mice singly deficient in SP-A and SP-D have distinct phenotypes and produce altered inflammatory responses to microbial challenges. Adult mice deficient in both SP-A and SP-D (A$^-$D$^-$) show fewer and larger alveoli, an increase in the number and size of type II cells, as well as more numerous and larger alveolar macrophages. Chronic deficiency of SP-A and SP-D in mice leads to parenchymal remodeling, type II cell hyperplasia and hypertrophy, and disturbed intracellular surfactant metabolism (Jung et al. 2005) In double deficient mice, there is a progressive increase in bronchoalveolar lavage phospholipid, protein, and macrophage content through 24 week of age. The macrophages from doubly deficient mice express high levels of the MMP-12 and develop intense but patchy lung inflammation. Qualitative changes resemble the lung pathology seen in SP-D-deficient mice (Hawgood et al. 2002).

Treatment of SP-D deficient mice with a truncated recombinant fragment of human SP-D (rfhSP-D) decreased lipidosis and alveolar macrophage accumulation as well as

production of proinflammatory chemokines. The rfhSP-D treatment reduced the structural abnormalities in parenchymal architecture and type II cells characteristic of SP-D deficiency and reduced degree of emphysema and a corrected type II cell hyperplasia and hypertrophy. This suggests that rfhSP-D might become a therapeutic option in diseases that are characterized by decreased SP-D levels in the lung (Knudsen et al. 2007; Zhang et al. 2002).

Treatment with a recombinant fragment of human SP-D consisting of a short collagen-like stalk (but not the entire collagen-like domain of native SP-D), neck, and CRD inhibited development of emphysema-like pathology in SP-D deficient mice. On the other hand, the entire collagen-like domain was necessary for preventing SP-D knockout mice from pulmonary emphysema development. The fragment of SP-D lacking the short collagen-like stalk failed to correct pulmonary emphysematous alterations demonstrating the importance of the short collagen-like stalk for the biological activity of the recombinant fragment of human SP-D (Knudsen et al. 2009; Breij and Batenburg 2008).

NO Production and S-Nitrosylation of SP-D Controls Inflammatory Function SP-D$^{-/-}$ mice exhibit an increase in the number and size of airway macrophages, peribronchiolar inflammation, increases in metalloproteinase activity, and development of emphysema. SP-A inhibited production of NO and inducible nitric oxide synthase (iNOS) in rat AΦ stimulated with smooth LPS. In contrast, SP-A enhanced production of NO and iNOS in cells stimulated with IFN-γ or IFN-γ plus LPS. SP-A contributes to the lung inflammatory response by exerting differential effects on the responses of immune cells, depending on their state and mechanism of activation (Stamme et al. 2000). NO is involved in a variety of signaling processes, and because altered NO metabolism has been observed in inflammation, it is predicted that alterations in its metabolism would underlie the proinflammatory state observed in SP-D deficiency (Atochina et al. 2004a, c). Treatment with the iNOS inhibitor 1,400 W can inhibit inflammatory phenotype and can attenuate inflammatory processes within SP-D deficiency. Mice treated with 1,400 W reduced total lung NO synthase activity (Atochina-Vasserman et al. 2007). Guo et al. (2008) suggest that NO controls the dichotomous nature of SP-D and that posttranslational modification by S-nitrosylation causes quaternary structural alterations in SP-D causing it to switch its inflammatory signaling role. This represents new insight into both the regulation of protein function by S-nitrosylation and NO's role in innate immunity (Guo et al. 2008). Thus, inflammation that occurs in SP-D deficiency is due to an increase in NO production and a shift in the chemistry and targets of NO from a disruption of NO-

mediated signaling within the innate immune system. However, purified preparations of SPs often contain endotoxin and the functions of SP-A and SP-D are affected by endotoxin. Therefore, the monitoring of SP preparations for endotoxin contamination is important (Wright et al. 1999).

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