Review Article

Mechanisms of Disease

Hydrophobic Surfactant Proteins in Lung Function and Disease

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THE hydrophobic surfactant proteins B and C are essential for lung function and pulmonary homeostasis after birth. These proteins enhance the spreading, adsorption, and stability of surfactant lipids required for the reduction of surface tension in the alveolus. Surfactant proteins B and C also participate in the regulation of intracellular and extracellular processes critical for the maintenance of respiratory structure and function. Mutations in the genes encoding surfactant protein B and surfactant protein C (*SFTPB* and *SFTPC,* respectively) are associated with acute respiratory failure and interstitial lung diseases. In this article, we review the current knowledge regarding the structure and functions of surfactant proteins B and C and their roles in the pathogenesis of acute and chronic lung disease in children and adults.

STRUCTURE AND FUNCTION OF ALVEOLAR SURFACTANT

The lung provides an extensive surface area mediating gas exchange between epithelial cells and the capillaries in the alveolus. Since lung cells are in direct contact with environmental gases and particles, an integrated system has evolved to maintain fluid balance and innate functions of host defense, the latter mediated by mucociliary clearance and pathways of innate and acquired immunity. The requirement for an extensive, hydrated surface for gas exchange presents a most interesting bioengineering problem, since surface tension at air–liquid interfaces creates collapsing forces that prevent respiration. The solution to the problem posed by such large surface areas in the lung is a pulmonary surfactant that creates a lipid-rich phase separating alveolar gas and liquid at the surfaces of epithelial cells (Fig. 1). Pulmonary surfactant phospholipids form monolayers and multilayers that reduce surface tension in the alveolus to negligible levels, thereby stabilizing the alveoli and maintaining lung volumes at end-expiration. Lack of pulmonary surfactant, whether caused by premature birth, lung injury, or mutations in genes critical to surfactant production or function, causes respiratory failure.

Pulmonary surfactant is a macromolecular complex composed primarily of lipids and proteins that is present in the alveolus in structurally distinct forms.[1] Surfactant is synthesized by type II epithelial cells lining the alveoli and stored in characteristic intracellular inclusions called lamellar bodies. After secretion, alveolar forms of surfactant include lamellar bodies, highly organized structures termed "tubular myelin," and monolayered and multilayered, phospholipid-rich sheets and vesicles (Fig. 1). The multiplicity of these structural forms is influenced by the stoichiometry of lipids and specific proteins, by mechanical forces exerted on the material during the respiratory cycle, and by the uptake, recycling, and degradation of subgroups of particles by respiratory epithelial cells and alveolar macrophages. Alveolar macrophages regulate catabolism of both lipids and proteins — activities that are under strict control of the signaling of granulocyte–macrophage colony-stimulating factor (GM-CSF). Indeed, deletion of the gene encoding GM-CSF, the gene encoding the GM-CSF receptor, or autoantibodies that block GM-CSF activity causes an accumulation of surfactant that is characteristic of pulmonary alveolar proteinosis in mice and humans.[2-4] Surface tension at the air–liquid interface is reduced by phospholipid films that are stable during the expansion and compression associated with the respiratory cycle. These films are rich in phosphatidylcholine and phosphatidylglycerol, which account for most of the molecular mass of surfactant films. However, at 37°C, pure phospholipids are in a rigid state, resulting in slow formation of films and poor surfactant function. Rapid formation, stability during compression and decompression, and resistance to interference by serum and cellular proteins — properties typical of mammalian surfactants — are critically dependent on the presence of the hydrophobic surfactant proteins, surfactant protein B and surfactant protein C.[5-7] Either protein alone or a mixture of the two proteins confers surfactant-like properties on the phospholipids present in the alveolus.

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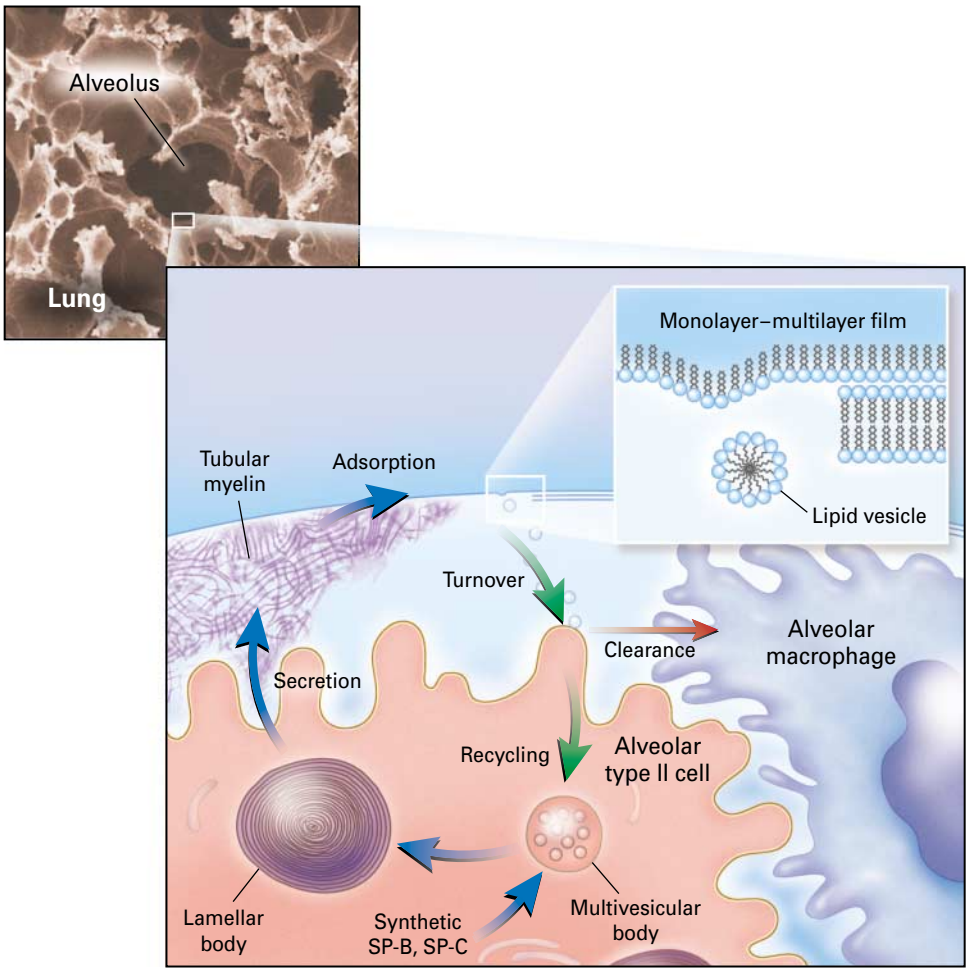


Figure 1. Freeze-Frame View of the Alveolar Space with a Magnified View of the Air–Liquid Interface, with Formation of Pulmonary Surfactant Films.

Surfactant phospholipids and proteins are synthesized by alveolar type II cells lining the alveoli. Surfactant lipids and surfactant protein B (SP-B) precursor protein and surfactant protein C (SP-C) are transported to multivesicular bodies and, after proteolytic processing, stored in lamellar bodies. SP-B, SP-C, and surfactant lipids are secreted into the alveolar subphase and interact with surfactant protein A to form a tubular myelin reservoir from which multilayers and monolayers form a film, thus reducing surface tension at the air–liquid interface. Surfactant remnants are taken up and reutilized or catabolized by type II epithelial cells. Alveolar macrophages play a critical part in the clearance and catabolism of surfactant lipids and proteins. Formation of the active surface film is required to maintain lung volumes, thereby preventing atelectasis and respiratory failure. The freeze-frame view is courtesy of Debra Yager.

SURFACTANT PROTEIN B

Structure and Function

Human surfactant protein B is a relatively small, 79-amino-acid, amphipathic peptide produced by proteolytic processing of a 381-amino-acid precursor in type II epithelial cells lining the alveoli (Fig. 2).[8,9] Surfactant protein B is encoded by a single gene located on chromosome 2 and occurs in various classes of vertebrates, including fish (lungfish), amphibians, reptiles, and mammals. Surfactant protein B is expressed in a highly cell-specific manner. It is synthesized as a precursor protein that is glycosylated and transported from the endoplasmic reticulum to the Golgi apparatus and thence to multivesicular bodies and is ultimately packaged in lamellar bodies. Proteolytic processing of surfactant protein B precursor protein occurs in a pulmonary-cell–specific manner during transit from multivesicular bodies to lamellar bodies, where the active surfactant protein B peptide is stored with surfactant protein C and surfactant phospholipids.[5] The contents of lamellar bodies are secreted into the air space and interact with surfactant protein A to form tubular myelin (Fig. 1) from which the surfactant films that line the alveolus are

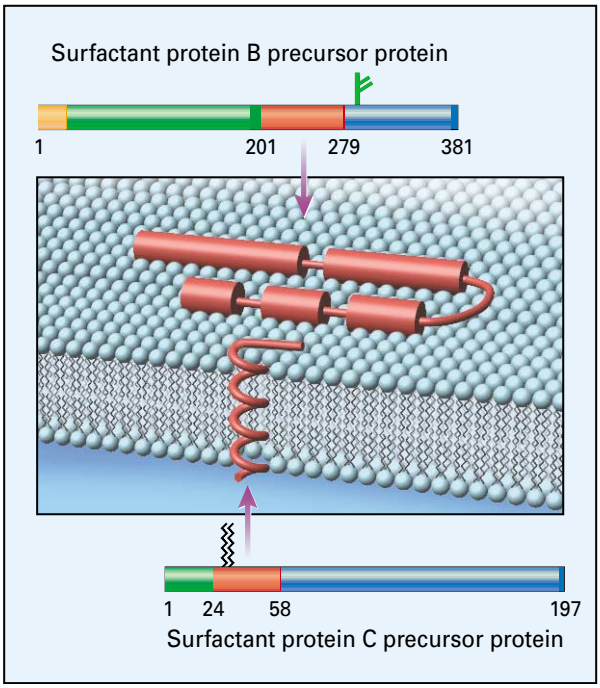


Figure 2. Proteolytic Processing of Surfactant Proteins B and C. Surfactant protein B is a small, amphipathic polypeptide produced by proteolytic processing as a 381-amino-acid precursor, surfactant protein B precursor protein, by type II alveolar epithelial cells in the lung. The active 79-amino-acid surfactant protein B is stored in lamellar bodies and secreted into the alveoli, where it interacts at the surface of surfactant lipids, forming stable monolayers and bilayers that reduce surface tension and enhance the stability and spreading of the lipid film. Surfactant protein C is a small, primarily alpha-helical peptide formed from a 197-amino-acid precursor protein. Surfactant protein C is stored with surfactant protein B and lipids in lamellar bodies. The active, 33-to-34-amino-acid peptide is secreted into the air space, where it is inserted into lipid membranes, enhancing spreading and recruitment of lipids to the surface films.

formed. The active surfactant protein B peptide is present in the alveolus as cysteine-dependent oligomers. Cationic regions of the surfactant protein B molecule are closely associated with the surface head groups of phospholipids through amphipathic helical domains that create hydrophilic and hydrophobic faces, which anchor the surfactant protein B peptide at lipid surfaces (Fig. 2).

Surfactant protein B interacts with lipid vesicles, causing lysis and fusion.[6] This process rapidly generates phospholipid sheets from vesicular lipids — an activity that is most likely required for the packing of lipid vesicles in multivesicular bodies during the formation of lamellar bodies, as well as for the formation of lipid multilayers in the alveolus. Extracellular surfactant protein B plays a critical part in surfactant homeostasis by promoting adsorption of lipid molecules into the expanding surface film and enhancing their stability during the compression and expansion that occur during the respiratory cycle. Because of its surfactant-enhancing properties, surfactant protein B is an active component of surfactant-replacement preparations used in the treatment of respiratory distress syndrome in preterm infants. The level of surfactant protein B is low in preterm infants who are at risk for respiratory distress syndrome, as it is in adults who are at risk for adult respiratory distress syndrome.[10-12]

Critical Role in Perinatal Lung Function

In mice, targeted deletion of the gene encoding surfactant protein B resulted in respiratory failure immediately after birth.[13] This animal model has provided insight into the various roles of surfactant protein B precursor protein and surfactant protein B in the functions of type II cells and surfactants. Although the composition of surfactant phospholipids was normal, pulmonary-function testing demonstrated decreased lung volumes, a lack of hysteresis, and an absence of residual volume at end-expiration in newborn mice without the gene encoding surfactant protein B — findings similar to those in preterm infants with respiratory distress syndrome. Type II epithelial cells in these mice lack typical lamellar bodies and contain enlarged, aberrant multivesicular bodies in which small lipid vesicles have accumulated, indicating the importance of surfactant protein B in the packaging of surfactant phospholipids into lamellar bodies. A deficiency of surfactant protein B also disrupts the proteolytic processing of surfactant protein C precursor protein, producing an abnormal proteolytic fragment containing the mature 35-amino-acid peptide plus an amino-terminal extension. This abnormal polypeptide is secreted into the air space of the gene-knockout mice.[14,15] The phenotype of surfactant protein B–deficient mice indicates that surfactant protein B is critical to the appropriate processing of surfactant protein C precursor protein, the organization of surfactant phospholipids in lamellar bodies, the formation of tubular myelin in the alveolus, the generation of surfactant films capable of reducing surface tension, and lung function during the early postnatal period.

Hereditary Surfactant Protein B Deficiency

Respiratory failure in full-term newborn infants is extremely rare but has been observed repeatedly as an inherited cause of severe respiratory dysfunction. Nogee et al. identified a mutation in the *SFTPB* gene of term siblings among whom homozygosity for the mutation was associated with fatal neonatal respiratory failure that was refractory to standard therapies, including surfactant replacement.[16] More than 22 distinct mutations in the *SFTPB* gene that cause respi-

ratory failure have been identified.[17] Most infants with such a mutation present with progressive respiratory failure in the first 24 to 48 hours of life. Pulmonary-function studies and radiographic findings in these infants are consistent with surfactant deficiency. The disorder is usually inherited as an autosomal recessive condition; a single mutation, termed 121ins2 (a net insertion of two nucleotides in codon 121 that causes a frame shift, unstable surfactant protein B messenger RNA, and a failure to synthesize surfactant protein B precursor protein), accounts for approximately two thirds of mutant *SFTPB* alleles. Mice that are heterozygous, with the deletion of one rather than two alleles encoding surfactant protein B, have abnormal pressure–volume relations during pulmonary-function testing and are susceptible to oxygen-induced lung injury,[18,19] but among humans, heterozygous relatives of surfactant protein B–deficient infants do not have clinically apparent lung disease.[20]

SFTPB mutations have been detected in approximately 10 percent of full-term infants referred to our center with unexplained respiratory failure (unpublished observations). Severe lung disease has been associated with missense mutations, nonsense mutations, point mutations, splice-site mutations, deletions, and insertions in the *SFTPB* gene.[5,17] Some mutations lead to abnormal surfactant protein B proteins that can be detected immunologically, whereas others result in the lack of detectable surfactant protein B in lung tissue or lung-lavage material. Misprocessed surfactant protein C precursor protein accumulates intracellularly, in the airway lumen, and in amniotic fluid. Detection of this surfactant protein C precursor protein fragment is useful in the diagnosis of hereditary surfactant protein B deficiency.[14] The respiratory disease in persons with hereditary surfactant protein B deficiency is refractory to surfactant replacement and usually causes death during the first months of life, despite intensive ventilatory support. Lung transplantation has allowed a number of infants with this disorder to survive.[21]

Association of Chronic Lung Disease with Partial Surfactant Protein B Deficiency

Uncommon mutations that cause a partial deficiency of surfactant protein B have been associated with chronic interstitial lung disease in childhood.[22-24] Some of these mutations allow the production of small amounts of surfactant protein B; others encode a partially functional form of surfactant protein B. Pulmonary disorders associated with partial surfactant protein B deficiency often require intermittent oxygen therapy. Accumulation of extracellular proteins, atypical macrophages, epithelial-cell dysplasia, and pulmonary fibrosis have been associated with mutations in *SFTPB* (Fig. 3). It is unclear whether these pathological findings are caused by chronic abnormalities in surface tension and resultant atelectasis or are mediated by the accumulation of abnormal surfactant protein B or other proteins that cause interstitial lung disease. One patient with partial surfactant protein B deficiency had a response to treatment with corticosteroids, perhaps because of the stimulatory effects of corticosteroids on the expression of surfactant protein B.[22]

SURFACTANT PROTEIN C

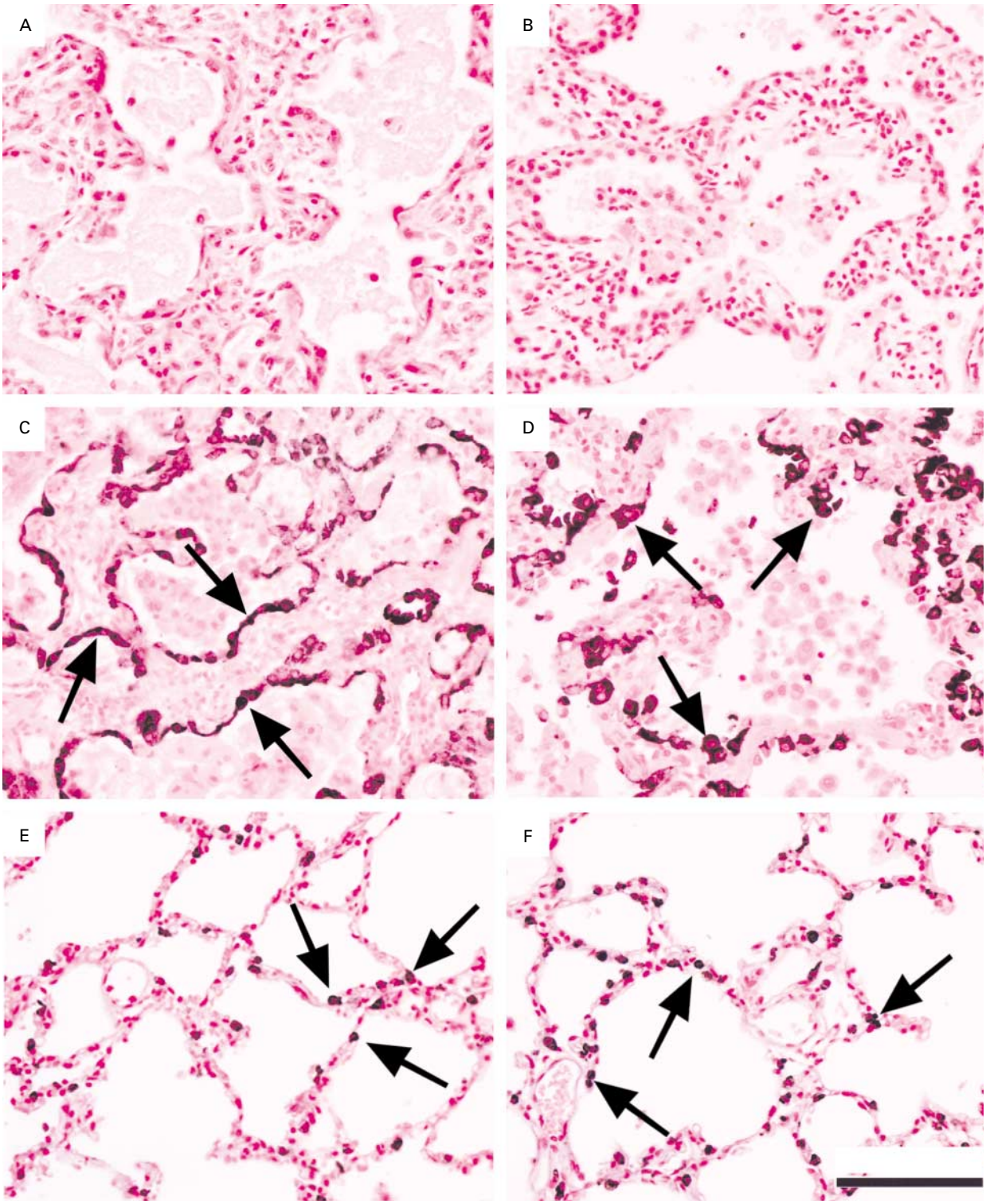
Structure and Function

Surfactant protein C, one of the most hydrophobic proteins in the proteome, consists of a 35-amino-acid polypeptide rich in valine, leucine, and isoleucine. Human surfactant protein C is encoded by a single gene (*SFTPC*) on chromosome 8 whose transcript directs the synthesis of a 197-amino-acid precursor protein. Most of the membrane-spanning domain of this protein consists of the biophysically active mature surfactant protein C peptide (Fig. 2).[5,25,26] Surfactant protein C precursor protein is routed with surfactant protein B precursor protein to multivesicular bodies, where both are processed and packaged into lamellar bodies for secretion into the air space along with phospholipids.[5,27] Surfactant protein C is a relatively abundant surfactant protein, accounting for approximately 4 percent of surfactant by weight. The structure and amino acid sequence of surfactant protein C are selectively expressed in type II epithelial cells in the alveoli of the lung. The structure of surfactant protein C is quite distinct from that of surfactant protein B. The former consists primarily of a membrane-spanning alpha-helical hydrophobic domain, anchored to polar head groups of surfactant lipids by charged residues near the amino terminal (Fig. 2).

Surfactant protein C forms noncovalent oligomers

Figure 3 (facing page). Lung Abnormalities Associated with Defects in Surfactant Protein B and Surfactant Protein C.

Images show immunohistochemical analysis of surfactant protein B in abnormal lung tissue from children with surfactant protein B defects (Panels A and C) and of surfactant protein C precursor protein in abnormal lung tissue from children with surfactant protein C defects (Panels B and D). No mature surfactant protein B was detected in lung tissue from a child who was homozygous for the surfactant protein B mutation *SFTPB* 121ins2 (Panel A). No surfactant protein C precursor protein was detected in the lungs of siblings with chronic interstitial lung disease (example, Panel B). Robust staining for mature surfactant protein B (Panel C, arrows) was detected in the lungs of a child who was homozygous for a splice-site mutation in exon 5 of the *SFTPB* gene. Staining for surfactant protein C precursor protein (Panel D, arrows) was detected in lung tissue from a child who was heterozygous for a point mutation in exon 4 of the *SFTPC* gene. Immunostaining for surfactant protein B (Panel E, arrows) and surfactant protein C precursor protein (Panel F, arrows) was detected in type II alveolar cells in normal lung. The bar, which applies to all the panels, represents 100 μm.



and is palmitoylated at one or two cysteines (depending on the species) located near the surface of the membrane.[28] These palmitoylated groups can move within a lipid layer or between lipid layers and may facilitate the formation of the multilayer films that line the alveolus.[29] Insertion of the surfactant protein C peptide into phospholipid membranes disrupts the packing of lipids, thereby enhancing the movement of lipid molecules between sheets of membrane and vesicles.[30] Dimeric, unfolded, and non–alpha-helical forms of surfactant protein C are present in surfactant.[31] Aggregation of surfactant protein C, particularly nonpalmitoylated forms, produces insoluble, amyloid-like fibrils, which have been detected in surfactant from patients with alveolar proteinosis.[32]

Surfactant protein C also enhances the reuptake of surfactant phospholipids in vitro and may have a role in surfactant catabolism.[33] The addition of surfactant protein C to phospholipid mixtures enhances the spreading and stability of phospholipids in a manner similar to that of surfactant protein B. Surfactant protein C is also an active component of various mammalian surfactant preparations that are used to treat respiratory distress syndrome in preterm infants.

Role in Pulmonary Function and Homeostasis in Vivo

Targeted deletion of the *SFTPC* gene in mice further validated its role in surfactant function and, surprisingly, resulted in a pulmonary syndrome with severe progressive pneumonitis[34] (and unpublished data). The effects of targeting of the *SFTPC* gene in transgenic mice were strongly influenced by genetic background. In outbred Swiss black mice, surfactant protein C deficiency caused defects in surfactant function that were readily discerned but had little physiological consequence at approximately six months of age. In these mice, pneumonitis and emphysema developed after one year of age. In surfactant protein C–deficient mice of the 129J strain, by contrast, severe pneumonitis, emphysema, and airway remodeling developed within two to three months after birth (unpublished data). Morphologic findings included infiltration of foamy macrophages, focal fibrosis, enlargement of the air space, and dysplasia of airway epithelial cells.

Biochemical abnormalities in surfactant protein C–deficient mice included changes in the ratios of surfactant proteins to lipids, activation of macrophage metalloproteinases, fragmentation of elastin fibers, and infiltration of myofibroblasts in the alveolar walls — findings that would be consistent with various forms of interstitial lung disease in humans. Expression of mutant surfactant protein C in transgenic mice also produced severe lung abnormalities, supporting the hypothesis that either the absence of surfactant protein C or the presence of misfolded surfactant protein C peptides may contribute to pulmonary pathology.[35]

Interstitial Pneumonitis Caused by Mutations in *SFTPC*

Several recent studies have associated both deficiency of surfactant protein C and mutations in the *SFTPC* gene with severe familial interstitial lung disease in humans (Fig. 3).[36-38] Amin et al. identified a mother and two similarly affected infants with interstitial lung disease presenting in childhood or adulthood; these patients had decreased levels of surfactant protein C precursor protein in type II cells and no detectable surfactant protein C in the air spaces.[36] Sibships with dominantly inherited mutations in the *SFTPC* gene were recently associated with atypical pulmonary fibrosis, macrophage infiltrates, pulmonary vascular remodeling and fibrosis, and abnormal cuboidal respiratory epithelia with loss of air space and alveolar surface.[37,38] The various pathologic diagnoses associated with a dominantly inherited surfactant protein C mutation can be broadly characterized as interstitial lung disease and include typical interstitial pneumonitis, cellular nonspecific interstitial pneumonitis, and desquamating interstitial pneumonitis. The marked variability in severity of the pathologic features in an extended sibship with an *SFTPC* mutation suggests that modifier genes or environmental factors strongly influence the phenotype of the disease. *SFTPC* mutations produce aberrant surfactant protein C precursor protein peptides that are either degraded or accumulate within type II epithelial cells of the lung, resulting in the absence of the active surfactant protein C peptide in the air space.[37,38] Since both deletion and expression of mutant surfactant protein C are associated with severe interstitial lung disease in transgenic mice and in humans, it is unclear whether the absence of the active surfactant protein C peptide or misfolded surfactant protein C precursors or peptides contribute to the pathogenesis of the disorder.[39] Since surfactant protein C may have other functions, including reuptake and catabolism of surfactant particles and surfactant function in the alveolus, it is unclear whether all or some of these activities contribute to the pathogenesis of lung disease caused by mutations in *SFTPC*.

PROPOSED MODEL FOR THE PATHOGENESIS OF PULMONARY DISEASE CAUSED BY MUTATIONS IN *SFTPB* AND *SFTPC*

Idiopathic interstitial pneumonias include pulmonary disorders with diverse clinical and pathologic features. Although some of these disorders are familial, others are associated with allergy, autoimmune disorders, infection, or exposure to toxic substances.[40] The molecular mechanisms causing this heterogeneous group of lung diseases remain poorly defined. In spite of diverse pathological descriptions and variable clin-

ical outcomes, many of these disorders share common pathologic features, including monocytic infiltration, fibrosis, obliteration of air spaces, alveolar remodeling, and dysplasia of type II epithelial cells. These abnormalities often cause severe pulmonary dysfunction. Pathological findings consistent with usual interstitial pneumonia, nonspecific interstitial pneumonia, or desquamating interstitial pneumonia have been associated with partial mutations in the *SFTPB* gene and dominantly inherited mutations in the *SFTPC* gene. Since synthesis of these extremely hydrophobic proteins is confined to alveolar type II epithelial cells in the lung, these cells are most likely selectively injured by mutations in the genes. Partial processing or misprocessing of surfactant protein B and surfactant protein C polypeptides results in decreased levels of surfactant protein B and surfactant protein C in the alveolus; moreover, the intracellular accumulation of aberrant proteins within type II epithelial cells may perturb the routing and processing of other proteins or cellular processes. The accumulation of misfolded proteins in the endoplasmic reticulum creates stress responses that may injure type II cells. Surfactant proteins B and C share structural features with prions, amyloid, and myelin basic proteins, whose misfolding and accumulation are associated with central nervous system disease.[41] Thus, the various clinical disorders caused by mutations in the genes encoding surfactant protein B and surfactant protein C may serve as examples of the critical role of misrouting and misfolding of proteins in the pathogenesis of pulmonary disease. It is also likely that deficiency of the active surfactant protein B and surfactant protein C peptides in the air space renders the lung susceptible to atelectasis and injury caused by a loss of surfactant function. In keeping with this concept, persons bearing *SFTPC* mutations are susceptible to acute lung failure, and adult respiratory distress syndrome can develop in them at various ages.[37]

In summary, mutations in surfactant proteins B and C cause both acute respiratory distress syndromes and chronic lung disease that may be related to the intracellular accumulation of injurious proteins, extracellular deficiency of the bioactive surfactant peptides, or both. Mutations in other genes that cause protein misfolding and misrouting may contribute to the pathogenesis of chronic interstitial lung disease by similar pathogenic mechanisms.

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