

Genetic Abnormalities of Surfactant Metabolism

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Introduction

Pulmonary surfactant is the complex mixture of lipids and proteins needed to reduce alveolar surface tension at the air–liquid interface and prevent alveolar collapse at the end of expiration. It has been recognized for almost 50 years that a deficiency in surfactant production due to pulmonary immaturity is the principal cause of the respiratory distress syndrome (RDS) observed in prematurely born infants.¹ Secondary surfactant deficiency due to injury to the cells involved in its production and functional inactivation of surfactant is also important in the pathophysiology of acute respiratory distress syndrome (ARDS) observed in older children and adults.^{2,3} In the past 15 years, it has been recognized that surfactant deficiency may result from genetic mechanisms involving mutations in genes encoding critical components of the surfactant system or proteins involved in surfactant metabolism.^{4,5} Although rare, these single gene disorders provide important insights into normal surfactant metabolism and into the genes in which frequently occurring allelic variants may be important in more common pulmonary diseases.

Overview of Pulmonary Surfactant

Pulmonary surfactant is synthesized, stored, and secreted by alveolar type II cells. Alveolar type II cells contain a specialized, lysosomally derived organelle, the lamellar body, in which surfactant lipids and proteins are stored.⁶ Surfactant phospholipids, particularly disaturated or dipalmitoyl phosphatidylcholine, are critical for its ability to effectively lower alveolar surface tension.⁷ A large number of enzymes are involved in surfactant lipid synthesis, but in general these enzymes are found in many tissues and are not specific for type II cells. Only a few, such as fatty acid synthase and CTP:phosphocholine cytidyltransferase (CCT α), the rate-limiting enzyme in PC

synthesis, are developmentally regulated.^{8–11} In contrast, member A3 of the adenosine triphosphate (ATP) binding cassette family of proteins (ABCA3) is highly expressed in type II cells, where it is localized to the limiting membrane of the lamellar body, and appears to have an essential role in lamellar body biogenesis and likely in surfactant lipid metabolism.^{12,13} Surfactant is secreted by exocytosis of the lamellar body contents, where it unravels into an intermediate known as tubular myelin before adsorbing to the air–liquid interface. Surfactant lipids and proteins are both recycled by the type II cell through an endocytic pathway, as well as being catabolized by alveolar macrophages, which are dependent on granulocyte-macrophage colony stimulating factor (GM-CSF) for their appropriate maturation.

About 10% of mammalian surfactants by weight is composed of protein, and, while the majority of protein in surfactant is derived from serum, specific proteins found primarily or largely in surfactant have been identified that have important roles in its function and metabolism. Surfactant proteins A and D (SP-A, SP-D) are hydrophilic proteins that are part of the collectin family, having both a collagenous domain and a carbohydrate binding or lectin domain. In their native forms in the airspaces, both are composed of high-order multimers. Both are encoded on chromosome 10, with two genes (*SFTPA1*, *SFTPA2*) contributing to the SP-A protein and a single gene (*SFTPD*) for SP-D.¹⁴ The principal roles for SP-A and SP-D appear to be in innate immunity and regulation of local pulmonary inflammation.^{15,16} Although both are highly expressed in the lung, SP-A and, even more so, SP-D are also expressed in extrapulmonary tissues.^{17–21} Multiple allelic variants of *SFTPA1*, *SFTPA2*, and *SFTPD* that alter their encoded protein sequences have been identified, and genetic association studies have linked certain *SFTPA* and *SFTPD* alleles to susceptibility to a variety of pulmonary diseases, ranging from RDS in premature newborns and viral infection in children to chronic obstructive pulmonary disease and lung cancer in

adults.²²⁻²⁸ However, the functional significance of these variants is uncertain. Currently, no human diseases due to genetic mechanisms disrupting either SP-A or SP-D production or structure have been reported. It may be that deficiencies of these two proteins do not result in human lung disease or that the phenotype associated with deficiencies of these proteins has not yet been determined. Mice genetically engineered to be deficient in SP-A have been generated, and, although more susceptible to a number of different pathogens, they do not have respiratory distress at birth or spontaneously develop lung disease with age.²⁹⁻³⁴ Moreover, as there are two SP-A genes, complete deficiency of SP-A will likely have to result from a major deletion involving their loci. In contrast, SP-D null mice develop a pulmonary lipoidosis and emphysema with age, and thus SP-D remains a candidate gene for pulmonary disease.³⁵⁻³⁷ As both SP-A and SP-D contain collagenous domains and form higher order multimers in the airway, it is possible that mutations introducing structural changes in the collagenous domains or elsewhere could prevent oligomerization and thus result in deficiency of SP-A or SP-D by a dominant negative mechanism.

Surfactant proteins B and C (SP-B, SP-C) are small, hydrophobic proteins that have essential roles in surfactant's ability to lower surface tension. Both SP-B and SP-C when combined with surfactant lipids yield a surfactant that effectively lowers surface tension in vitro and is effective in treating animal models of RDS. Both are found in varying amounts in mammalian derived exogenous surfactant preparations used clinically to treat human infants with RDS.^{38,39} Abnormalities in both SP-B and SP-C expression and structure due to mutations in the genes encoding these proteins have been associated with acute and chronic human lung disease and are discussed in more detail later. While regulation of expression of both SP-B and SP-C is distinct, both are increased with glucocorticoids.⁴⁰⁻⁴² The promoter regions of both genes have been extensively studied, and numerous transcription factors are involved in their expression as well as that of other key components of the surfactant system, with thyroid transcription factor 1 (TTF-1, also known as Nkx2.1), forkhead box A2 (Foxa2), and CCAAT/enhancer binding protein α (C/EBP α) of particular importance in determining tissue specificity and developmentally regulated expression.⁴³⁻⁵¹ Selective inactivation of these transcription factors in the distal respiratory epithelium of experimental animals results in perinatal lethality with decreased production of surfactant proteins and lipids.

Respiratory Distress Syndrome

The primary lung disease related to surfactant is RDS caused by deficient production of surfactant due to pulmonary immaturity. The primary pathologic changes

observed in the lungs of infants dying from RDS are diffuse atelectasis and the formation of hyaline membranes lining small airways. Although a great deal of effort initially focused on the role of the hyaline membranes in the pathophysiology of RDS, the seminal observations of Avery and Mead in 1959 demonstrated the functional absence of surfactant and its ability to lower surface tension as the primary cause of the disease.^{1,52}

Respiratory distress syndrome results not from the selective production of one surfactant component but from global decreases in surfactant lipid and protein production. Immature type II cells do not contain well-developed lamellar bodies but are instead rich in glycogen, which disappears as lamellar bodies appear.^{8,53} The expression of SP-A, SP-B, and SP-C, as well as other proteins involved in surfactant lipid production and homeostasis such as ABCA3, fatty acid synthase, and CCT α , are developmentally regulated, with their expression increasing with advancing gestation.^{11,12,54-61} Decreased expression of SP-A and SP-B has been observed in lung tissue from newborns that died from RDS compared with controls.⁶² Measurements of surfactant lipids in amniotic fluid, including PC (also known as lecithin), disaturated phosphatidylcholine, and phosphatidylglycerol (PG), as well as assessments of lamellar body counts, can be used to predict maturity of the surfactant system in the fetus. Clinical testing for fetal lung maturity introduced in the 1970s resulted in a reduction in iatrogenic RDS.^{63,64}

Along with technical advances in neonatal mechanical ventilation, RDS is now very effectively treated with exogenous surfactant preparations that have substantially reduced mortality from RDS in premature infants.⁶⁵⁻⁶⁷ Respiratory distress syndrome is principally a disease of premature infants, with the risk for RDS primarily dependent on gestational age, although it may also be observed in full-term or near-term infants. As many more infants are born at >35 weeks, RDS in larger infants represents a considerable cause of neonatal morbidity, although mortality in such infants is low.^{68,69} With the effectiveness of modern therapies for RDS in reducing mortality, the phenotype of severe RDS unresponsive to current treatment strategies is one that suggests another etiology for lung disease, particularly a genetic or developmental mechanism disrupting lung development or impairing surfactant metabolism.

Surfactant Protein B

Surfactant protein B is encoded by a single gene (called *SFTPB*) located on the short arm of chromosome 2, spanning approximately 10 kb.^{70,71} The gene contains 11 exons, of which the last is untranslated. The gene is transcribed into an approximate 2 kb mRNA, which is translated into

a 381 amino acid preproprotein.⁷² After cotranslational cleavage of a signal peptide, the proprotein (pro-SP-B) undergoes several proteolytic processing steps at both the amino- and carboxy-terminal ends to yield the 79 amino acid mature SP-B protein that is secreted into the airspaces. Proprotein SP-B has homology to the saposins, proteins that bind to and interact with a number of lipids, and contains three saposin domains. Mature SP-B is encoded in exons 6 and 7 of the gene, which corresponds to the middle domain.^{73,74} Proprotein SP-B contains one potential site for N-linked glycosylation in the carboxy-terminal domain and a possible second site in the amino-terminal domain depending on which variant of a commonly occurring single nucleotide polymorphism (SNP) is present in codon 131.⁷⁵ Alternative splicing at the beginning of exon 8 yields a small percentage of transcripts lacking four amino acids from the carboxy-terminal domain. The functional consequences of this alternative splicing are unknown, although this transcript may be overrepresented in RNA from diseased lung tissues.⁷⁶ Surfactant protein B is expressed in both non-ciliated bronchiolar epithelium in the lung and alveolar type II epithelial cells, although only alveolar type II cells fully process pro-SP-B to mature SP-B.^{60,77} Hereditary SP-B deficiency was the first recognized inborn error of surfactant metabolism, with the first report in 1993.⁷⁸ The index patient was a full-term infant with diffuse lung disease clinically and radiographically suggestive of surfactant deficiency. Unlike premature infants with surfactant deficiency, who generally improve toward the end of the first week of life, this child had persistent hypoxemic respiratory failure and eventually died at age 5 months. The family history was notable for a previous child born to the same parents who also died from neonatal lung disease. Lung biopsy findings included changes similar to those of alveolar proteinosis in adults, with distal airspaces filled with granular eosinophilic material. This pathology had also been observed rarely in newborns with clinically similar lung disease, often with a positive family history.^{79,80} A selective absence of SP-B in lung tissue from this infant was demonstrated by immunologic assays, and SP-B deficiency was established as the basis for the lung disease with the demonstration of a frame shift mutation that precluded SP-B production on both SP-B alleles in affected infants.⁸¹

Surfactant protein B deficiency is an extremely rare disorder. Approximately 50 cases have been reported in the literature, and extrapolations from estimates of the population frequency of the most frequently encountered *SFTPB* mutation yields an expected disease incidence of about 1 in 1 million live births in the United States.^{82–84} Although the disease is almost always fatal, affected infants can survive for months with aggressive support, and it is thus important to establish the diagnosis so as to avoid futile therapy or provide timely referral for lung

transplantation, as well as for proper counseling regarding recurrence risk.

Over 40 different mutations in *SFTPB* have been identified. The first identified mutation consists of a substitution of GAA for C in codon 121 of the SP-B mRNA and has been termed 121ins2.⁸¹ This mutation has accounted for 60%–70% of the mutant alleles in patients of Northern European descent, and the finding of a common mutation likely is the result of a common ancestral origin or “founder” effect.⁸⁵ Other mutations have been found in more than one unrelated family in specific ethnic groups. Although some mutations allow for the production of pro-SP-B, processing to mature SP-B is impaired such that all known mutations lead to an absence or severe reduction in the amount of mature SP-B and can thus be viewed as loss-of-function mutations.⁸⁶ The disease is inherited as an autosomal recessive condition, with mutations needed on both alleles to manifest disease.

The usual clinical presentation is that of a full-term infant without risk factors for lung disease or infection who presents with symptoms of respiratory distress, hypoxemia, and diffuse, homogenous infiltrates on chest radiographs. Although many of the initial reports of infants with this condition involved children with very severe lung disease who often required extracorporeal membrane oxygenation for support, it is clear that some affected infants may have milder disease initially and may not require mechanical ventilation for days to weeks.^{87,88} The disease is progressive, and the diagnosis should thus be considered in full-term infants with a history of neonatal lung disease that is progressive after the first week of life, especially if there is a family history of neonatal lung disease.

The pathophysiology of the lung disease due to SP-B deficiency is incompletely understood. Certainly the lack of mature SP-B could contribute to poorly functioning surfactant and thus accounts for some of the initial clinical symptoms consistent with severe surfactant deficiency. In addition, deficiency of SP-B results in a block in processing of pro-SP-C to mature SP-C. This results in both deficiency of mature SP-C as well as an accumulation of partially processed SP-C-related peptides that are secreted into the airspaces but are not very surface-active and contribute to the pathophysiology of the lung injury.^{89,90} Type II cells in SP-B-deficient lung do not contain normally form lamellar bodies, indicating a fundamental intracellular role for SP-B or pro-SP-B, and the lack of normal lamellar bodies may explain the impaired processing of pro-SP-C, as the final processing steps for pro-SP-C take place in a distal cellular compartment.^{6,91,92}

Genetically engineered SP-B knockout mice have been generated and have a phenotype that recapitulates the human disease, with homozygous null mice dying at birth from respiratory failure.⁹³ The lung pathology of mice dying in the neonatal period is notable principally for

atelectasis and does not have many of the histopathology features observed in lung tissue from human infants with SP-B deficiency (described in detail later). Potential reasons for the differences in histopathology findings include the inherent variations between species, the fact that some changes may take time to develop after birth or are a result of the treatments used to sustain life in human infants with SP-B deficiency, or some combination of these factors. Homozygous SP-B null mice are completely lacking in mature SP-B protein and also have abnormal lamellar bodies and aberrant processing of pro-SP-C to SP-C, indicating that these are due to the primary deficiency of SP-B and are not secondary to other post-natal factors.⁹⁴

Mice that conditionally express SP-B under the control of a tetracycline responsive promoter have also been generated and bred with knockout mice in order to generate animals that can survive the neonatal period and then have SP-B production shut off when the antibiotic is removed from their diet. These animals develop lung disease when SP-B levels fall below 20%–25% of the levels in control mice, indicating that there is a critical level of SP-B needed for proper lung function.⁹⁵ This concept is supported by the observation that human patients with SP-B mutations allowing for some SP-B production survive longer than those with null mutations.^{87,88} Mice heterozygous for one SP-B null allele survive and have only mild abnormalities in lung function but are more susceptible to pulmonary oxygen toxicity.^{96,97} Genetic control of SP-B levels could thus be an important determinant of risk for lung disease, with individuals who have a lower capacity for SP-B production being at risk for lung disease should additional environmental factors (premature birth, inflammation) further reduce SP-B levels.

Multiple polymorphic variants have been identified within the *SFTPB* locus, including an SNP in codon 131 that alters a potential site for N-linked glycosylation, several SNPs in the promoter region that could affect gene transcription, and a variable tandem nucleotide repeat sequence in intron 4.^{26,98–102} This latter variant in intron 4 has been associated with increased risk for several pulmonary diseases, ranging from RDS and bronchopulmonary dysplasia in premature infants to lung cancer in adults.^{101,103–107} The mechanisms by which this variant affects SP-B expression are unknown, although effects on gene transcription based on potential transcription factor binding sites and on mRNA splicing have been proposed.^{104,108} The codon 131 SNP has been associated with both risk for RDS in premature infants as well as acute and chronic lung injury in adults.^{99,109,110} The observed associations have been relatively weak, however, and often in combination with other risk factors or genetic variants at other loci. Additional studies will be needed to fully address the question of whether and which *SFTPB* alleles may predispose to different disease conditions.

Currently there is no specific effective therapy for SP-B deficiency other than lung transplantation. Children with SP-B deficiency have been transplanted in early infancy, with short- and long-term outcomes comparable to those for lung transplantation for other disorders in infancy.¹¹¹ As lung transplantation carries with it significant burdens for the family as well as long-term morbidity and mortality risks, compassionate care is also an appropriate option once the diagnosis is established. Identification of the responsible mutations allows for proper genetic counseling and the option for prenatal or even preimplantation diagnosis for future pregnancies.

Surfactant Protein C

Surfactant protein C is encoded by a single gene (*SFTPC*) on the short arm of chromosome 8. The gene is relatively small, spanning some 3,500 bases, and contains 6 exons, of which the last is untranslated.¹¹² The gene is transcribed into an approximately 0.9kb mRNA, which directs the synthesis of a 191 or 197 proprotein (pro-SP-C), depending on alternative splicing at the beginning of exon 5.^{38,72} Proprotein SP-C does not contain a signal peptide but is a transmembrane protein in which the domain corresponding to mature SP-C acts as the membrane anchoring domain, with the amino-terminus oriented toward the cytoplasm.^{113,114} Proprotein SP-C undergoes a number of posttranslational modifications, including palmitoylation of cysteine residues within the mature peptide domain, such that SP-C is a proteolipid.^{115,116} Like SP-B, pro-SP-C is proteolytically processed at both the carboxy and amino termini, to yield the 34 or 35 amino acid mature SP-C, whose protein sequence is encoded within exon 2 of the gene and is secreted into the airspaces along with SP-B and surfactant lipids. The carboxy-terminal domain of pro-SP-C has homology with a group of proteins linked to forms of familial dementia and cancer (BRICHOS domain), with their common pathogenesis hypothesized as being related to abnormal protein folding and hence conformational diseases.^{117,118} Surfactant protein C expression is confined to type II cells within the lung, and the SP-C human and mouse promoter sequences have been widely used in animal experiments to drive lung-specific gene expression.^{47,119–121}

Lung disease due to *SFTPC* mutations is rare, although the incidence and prevalence are unknown as population-based studies have not been performed. The majority of reported cases have involved single cases or families or small series of cases, and patients have been evaluated primarily by phenotype. The typical presentation in infancy is with symptoms and signs of diffuse lung disease, including tachypnea, retractions, and hypoxemia in room air; digital clubbing and failure to thrive may also occur.^{84,122} Most affected infants do not have symptoms

at birth, although neonatal lung disease similar to that of RDS has been observed and may prove fatal in the neonatal period.¹²³ A family history of interstitial lung disease or pulmonary fibrosis may provide a clue to the diagnosis, although sporadic disease may result from de novo germline mutations, or family members may be asymptomatic.^{124–127} Of reported patients, the majority have presented in the pediatric age group. In one study of adults with idiopathic pulmonary fibrosis (n = 89) or nonspecific interstitial pneumonia (n = 46) evaluated for *SFTPC* mutations, only one patient was found to have an *SFTPC* mutation likely related to lung disease.¹²⁸ Two commonly occurring SNPs that alter the pro-SP-C coding sequence in codons 138 (threonine [T] or asparagine [N]) and 186 (serine [S] or asparagine) have been identified. These two variants are in strong linkage disequilibrium with one another, and the 186N variant was found with increased frequency in patients with RDS compared with controls in one study.¹²⁹ This variant is of particular interest, as 186S has been strongly conserved during evolution, and several *SFTPC* mutations associated with lung disease have been identified in nearby codons. An additional preliminary study noted an association with pulmonary fibrosis in adults.¹³⁰ Further studies are needed to confirm or refute these interesting preliminary observations.

As opposed to SP-B deficiency in which all known mutations would be predicted to preclude or reduce the amount of mature SP-B, all known mutations in *SFTPC* associated with human disease have been missense mutations, small insertions or deletions, splicing mutations that would maintain the reading frame, or frame shifts in the fourth or fifth exons that are likely to be associated with stable transcripts.^{84,122–127,131–133} Almost all of the mutations have mapped to the carboxy-terminal domain of pro-SP-C. Thus the mutations are ones that would be predicted to result in the production of an abnormal form of pro-SP-C. Furthermore, mutations have generally been found on only one allele. When familial, the lung disease associated with *SFTPC* mutations is inherited in an autosomal dominant pattern, with a variable age of onset of lung disease, ranging from early infancy to the fifth or sixth decade of life.^{102,125} Whether there is complete penetrance of the lung disease associated with *SFTPC* mutations is uncertain. Individuals with mutations who do not have lung disease have been reported, but in general these individuals have not been formally evaluated for lung disease and may simply have a later onset of disease.

The exact mechanisms whereby *SFTPC* mutations result in lung disease are unclear. The abnormal pro-SP-C resulting from the mutation may be targeted for degradation, and as pro-SP-C self-associates in the secretory pathway, this may result in degradation of wild-type pro-SP-C as well, leading to SP-C deficiency due to a dominant negative mechanism.^{113,134,135} In support of this,

reduced pro-SP-C and mature SP-C have been demonstrated in lung tissue associated with an SP-C mutation that resulted in the skipping of the fourth exon (Δ exon 4), and expression of this mutation in vitro resulted in its rapid degradation that was prevented by inhibitors of proteasome-mediated degradation.^{131,134,136} Surfactant protein C null mice also develop lung disease in a strain-dependent fashion, with progressive interstitial lung disease and aging.^{137,138} Thus SP-C deficiency may be involved in the pathogenesis of lung disease in some patients with *SFTPC* mutations, although precisely how deficiency of mature SP-C results in chronic lung disease is not known. Although complete SP-C deficiency does not result in RDS at birth, SP-C-deficient surfactant may not be as effective at maintaining low surface tension at low lung volumes and may be particularly important if SP-B levels are also decreased, and thus relative deficiency of SP-C may lead to intermittent alveolar atelectasis over time.^{137,139} In addition, SP-C binds lipopolysaccharide, and SP-C deficiency may therefore lead to an increased inflammatory response.^{140–142}

A second mechanism whereby SP-C mutations may result in lung disease is direct toxicity due to the effects of mutated pro-SP-C. Proprotein SP-C contains the extremely hydrophobic epitopes of mature SP-C, and mutations in pro-SP-C may allow exposure of these epitopes with secondary deleterious effects. Transfection of a construct expressing an SP-C missense mutation (L188Q) into lung epithelial lines in vitro resulted in cytotoxicity as demonstrated by lactate dehydrogenase release.¹⁰² Abnormally folded pro-SP-C due to mutations could also form aggregates, and abnormal accumulation of pro-SP-C containing the SP-C Δ exon 4 mutation has been demonstrated in vitro and in lung tissue from at least one infant with a small in-frame deletion in *SFTPC*.^{126,136} Pro-SP-C molecules containing mutations are likely to be misfolded and hence trigger the unfolded protein response in the endoplasmic reticulum with resultant endoplasmic reticulum stress. Transfection of constructs expressing the SP-C Δ exon 4 mutation identified in index patients has been shown to be associated with induction of the unfolded protein response and with secondary apoptosis of alveolar epithelial cells.^{134,143} In addition, cells stably transfected in vitro with constructs expressing the SP-C Δ exon 4 mutation that induced a state of chronic endoplasmic reticulum stress were more vulnerable to viral infection, thus suggesting a mechanism by which environmental insults could trigger or exacerbate lung disease primarily due to a genetic mechanism.¹⁴⁴ The potential toxic effects of abnormal pro-SP-C have also been demonstrated in vivo in that transgenic mice expressing the human SP-C Δ exon 4 mutation had markedly disrupted lung development that correlated with amount of transgene expression.¹³⁴ Although there are currently no specific treatments for the lung disease

due to *SFTPC* mutations, agents that facilitate trafficking through the secretory pathway that are currently under evaluation for other genetic lung diseases may also be of benefit for SP-C-related lung disease.^{136,145,146}

Member A3 of the Adenosine Triphosphate Binding Cassette Family of Proteins

ABCA3 is a member of the ATP binding cassette family of proteins, transmembrane proteins that hydrolyze ATP in order to translocate a wide variety of substrates across biologic membranes.¹⁴⁷ The gene encoding ABCA3 (*ABCA3*) is located on the short arm of chromosome 16 and spans over 80kb, containing 33 exons.¹⁴⁸ The gene directs the synthesis of a 1,704 amino acid protein and is considered a full transporter with 12 membrane spanning domains and 2 nucleotide binding domains. A number of tissues express ABCA3, but it is highly expressed in lung tissue where it is localized to the limiting membrane of lamellar bodies.^{12,57} As the ABCA subfamily is often involved in transport of lipids, this localization for ABCA3 is consistent with a role for ABCA3 in importing lipids needed for surfactant function into lamellar bodies.^{149,150}

The importance of ABCA3 in surfactant metabolism has been demonstrated by the observation that mutations on both *ABCA3* alleles resulted in severe lung disease in full-term newborns who had clinical and radiographic features of surfactant deficiency.^{13,151–153} In addition, surfactant isolated from bronchoalveolar lavage specimens of children who required lung transplantation for ABCA3 deficiency was shown to have markedly reduced ability to lower surface tension and an abnormal composition, with a particular reduction in phosphatidylcholine content observed.¹⁵² In an in vitro study, downregulation of ABCA3 expression was associated with decreased lipid uptake into lamellar bodies of alveolar type II cells, and cells transfected with constructs expressing forms of ABCA3 containing mutations identified in patients had reduced uptake of lipids into lysosomes compared with cells transfected with wild-type ABCA3.¹⁵⁴ Collectively these observations support a fundamental role for ABCA3 importing surfactant lipids into lamellar bodies, although the exact substrates remain to be determined. Thus quantitative and functional deficiencies of surfactant components likely contribute to the symptoms of surfactant deficiency observed in ABCA3-deficient infants. As lamellar body biogenesis is interfered with, processing of pro-SP-B and pro-SP-C to their mature forms may also be hindered in this condition, leading to deficiencies of these surfactant components as well.¹⁵¹

Although ABCA3 deficiency is the most recently identified inborn error of surfactant metabolism, it is likely a more common cause of disease than *SFTPB* or *SFTPC* mutations. *ABCA3* mutations accounted for a combined 24 of 35 cases of unexplained respiratory failure in two reports^{13,151} and for 8 of 12 infants who underwent lung transplantation in the first year of life for severe lung disease of unknown etiology.¹⁵² Well over 100 different *ABCA3* mutations have been identified^{13,151–153} (and L. Noguee, unpublished observations) and markedly reduced or absent ABCA3 expression has been demonstrated in the lung tissue of affected infants consistent with disease resulting from a loss-of-function mechanism.¹⁵¹ Aside from mutations that completely preclude ABCA3 expression, mutations may also result in abnormal intracellular routing of ABCA3 or decreased functional activity.^{154,155} Although initial studies focused on children with fatal or very severe lung disease, survival with chronic interstitial lung disease is possible.¹⁵³ Because identified patients with interstitial lung disease shared one particular *ABCA3* mutation, this may be a result of partial deficiency, and genotype may thus be important in predicting phenotype in this disease. Additional studies are needed to evaluate this hypothesis.

Detailed studies of the clinical features associated with *ABCA3* mutations have not yet been published. From the initial reports, the phenotype of patients with ABCA3 deficiency often resembles that of infants with SP-B deficiency, with severe neonatal lung disease resembling RDS in premature infants.^{13,151} As with SP-B deficiency, however, some infants may have considerably milder neonatal lung disease, and yet others may not have symptoms in the neonatal period.¹⁵³ The initial lung disease may also improve with time such that affected infants are able to be discharged. The clinical picture associated with ABCA3 deficiency thus overlaps that associated with SP-B deficiency and SP-C mutations.

The incidence and prevalence of lung disease due to ABCA3 deficiency are unknown. Population studies have not yet been done on the frequency of *ABCA3* mutations in the general population. It is likely that the disease is rare, but, particularly if milder variants contribute to chronic lung disease, it may prove to be more common than has initially been appreciated.

Lung Pathology Associated with Inborn Errors of Surfactant Metabolism

The lung pathology changes associated with all three single gene disorders disrupting surfactant metabolism are similar and overlapping (Figure 54.1) These include marked alveolar type II cell hyperplasia, interstitial

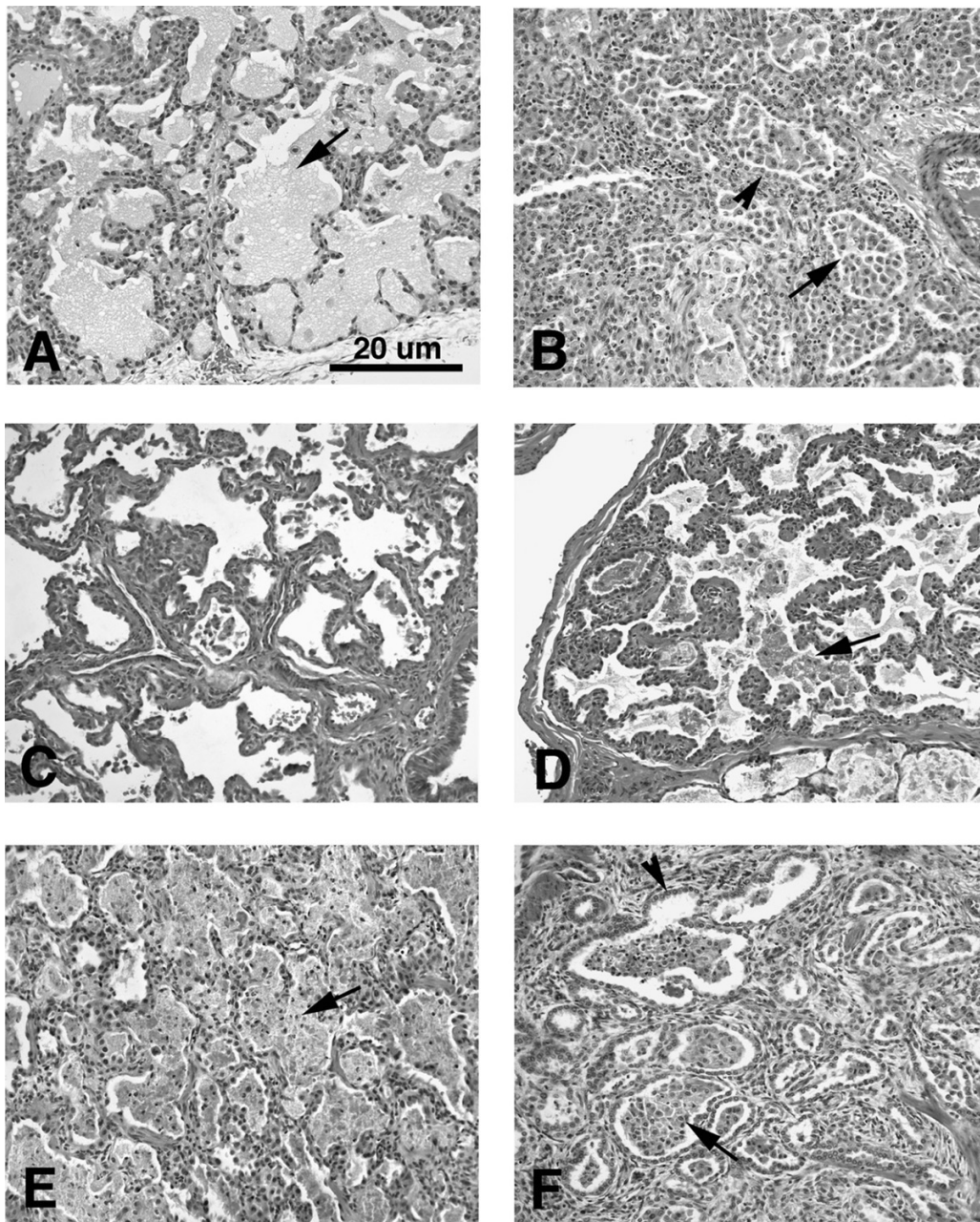


FIGURE 54.1. Lung histopathology of inborn errors of surfactant metabolism. **(A,B)** Representative histopathology of two children with *SFTPB* mutations. Features of pulmonary alveolar proteinosis (PAP) with eosinophilic, proteinaceous material filling the alveoli (arrow) are shown in A. Features of desquamative interstitial pneumonitis with accumulation of foamy alveolar macrophages in the alveoli (arrow) are shown in B. **(C,D)** Representative histopathology of two children with *SFTPC* mutations. Features of nonspecific interstitial pneumonitis with thickened alveolar septa are shown in C. An example of chronic pneumonitis of infancy with both macrophages and

granular, eosinophilic material in the alveoli (arrow) is shown in D. **(E,F)** Representative histopathology of two children with *ABCA3* mutations. Features of PAP (arrow) are shown in E. Features of desquamative interstitial pneumonitis (arrow) are shown in F. Features of alveolar proteinosis with eosinophilic material and/or foamy macrophages and prominent type II cell hyperplasia (arrowheads) are highlighted in B and F, and thickened alveolar septa are variably present in all three types of disorders. (Hematoxylin and eosin stains. All original magnifications, $\times 10$; bar = $20\mu\text{m}$.)

thickening with variable amounts of fibrosis, and numerous foamy macrophages in the airspaces. A prominent feature can be the accumulation of granular, eosinophilic material filling distal airspaces that stains positively with periodic acid–Schiff reagent or alveolar proteinosis material. Although findings of alveolar proteinosis were prominent in the index patient with SP-B deficiency and provided an important clue to the mechanism, similar findings may be seen with other inborn errors of surfactant metabolism due to mutations in the *SFTPC* or *ABCA3* genes. The composition of this material is also likely different in the different disorders and differs from the proteinosis material observed in older children and adults who have alveolar proteinosis due to an immune mechanism.¹⁵⁶ The proteinosis material may be minimal in appearance and the sensitivity and specificity of this finding for each of the disorders has not been critically examined. The term *congenital alveolar proteinosis* is thus probably best avoided in describing these conditions.

In older children with the clinical picture of interstitial lung disease histopathologic diagnoses associated with *SFTPC* and *ABCA3* mutations have included chronic pneumonitis of infancy, nonspecific interstitial pneumonia, and idiopathic pulmonary fibrosis.^{124,125,131,133} Usual interstitial pneumonia has also been reported in older individuals in association with an *SFTPC* mutation.¹²⁷ Desquamative interstitial pneumonia has also been reported as the histologic diagnoses in children with *ABCA3* and *SFTPC* mutations, although the course is much more severe than with desquamative interstitial pneumonia observed in adults.^{153,157,158} The majority of these children were given this diagnosis before the description of chronic pneumonitis of infancy.¹⁵⁹ Although it has not been formally studied, it is likely that histology findings will be unable to discriminate between the three known conditions. Recently the term *surfactant dysfunction mutation* has been used to encompass the changes found in all three disorders, and it is also likely that other genetic mechanisms leading to disruption of surfactant metabolism will yield similar pathology.¹⁶⁰

Specific immunostaining of the lung may be helpful in establishing the specific diagnosis of SP-B deficiency (Figure 54.2) With *SFTPB* mutations, absent or markedly reduced staining for both pro-SP-B and SP-B may be observed, although, depending on the genotype, some staining for both may be detected.⁸⁶ Reduced staining for SP-B may also be seen in association with *ABCA3* mutations, and hence absent staining for SP-B is not sufficient for a specific diagnosis.¹⁵¹ However, because of the presence of large amounts of secreted aberrantly processed SP-C peptides, the extracellular material in SP-B-deficient lung stains intensely with antibodies

directed against pro-SP-C and appear to be a specific marker for this disorder.⁸⁶ Specific staining for the surfactant proteins has not revealed a consistent pattern associated with *SFTPC* mutations. Proprotein SP-C may be readily detected in alveolar epithelial cells or may be markedly reduced or absent.^{125,131} Markedly reduced staining for pro-SP-C has also been observed with familial lung disease in which, however, no *SFTPC* mutations could be identified.¹⁶¹

Ultrastructural studies may be very helpful in establishing a specific diagnosis. Electron microscopy of lung tissue from SP-B-deficient children demonstrates specific changes within the lamellar bodies within type II cells. Instead of normally formed lamellar bodies with well-organized layers and lamellae, the type II cells contain intracellular inclusions with poorly formed lamellae and vesicles of varying size (Figure 54.3).^{162,163} These observations are consistent with a function for SP-B in membrane fusion and indicate a fundamental intracellular role for SP-B in lamellar body biogenesis. These abnormal lamellar bodies appear characteristic for SP-B deficiency.

ABCA3 is localized to the limiting membrane of lamellar bodies, and specific ultrastructural changes have also been observed in the type II cells of *ABCA3*-deficient infants.^{13,151,163–165} Normal-appearing lamellar bodies may appear to be absent, and instead the cytoplasm of alveolar type II cells contains many small, dense bodies that on higher magnification may be seen to contain tightly packed membrane. An eccentrically placed electron-dense core in these small bodies may give them a “fried egg” appearance.¹⁶⁵ Although only a limited number of studies are available, the finding of these bodies has had a very high correlation with the identification of mutations in the *ABCA3* gene, and they have not yet been reported in other conditions. However, the exact sensitivity and specificity of this finding remain unknown. Anecdotal experience indicates that some type II cells in infants with *ABCA3* mutations may have more normal-appearing lamellar bodies, and there are few data on the ultrastructural findings in children with milder lung disease due to *ABCA3* mutations. Currently, no consistent ultrastructural abnormalities in association with *SFTPC* mutations have been identified. Some abnormally formed lamellar bodies were observed both in vitro and in vivo in association with two *SFTPC* mutations, but normal lamellar bodies were also observed^{126,127} and additional study is needed. However, the ultrastructural findings associated with SP-B and *ABCA3* deficiencies are so striking that the preparation of tissue for electron microscopy should be included in autopsies of children dying from neonatal respiratory disease or in biopsies of young infants with diffuse lung disease.¹⁶⁶

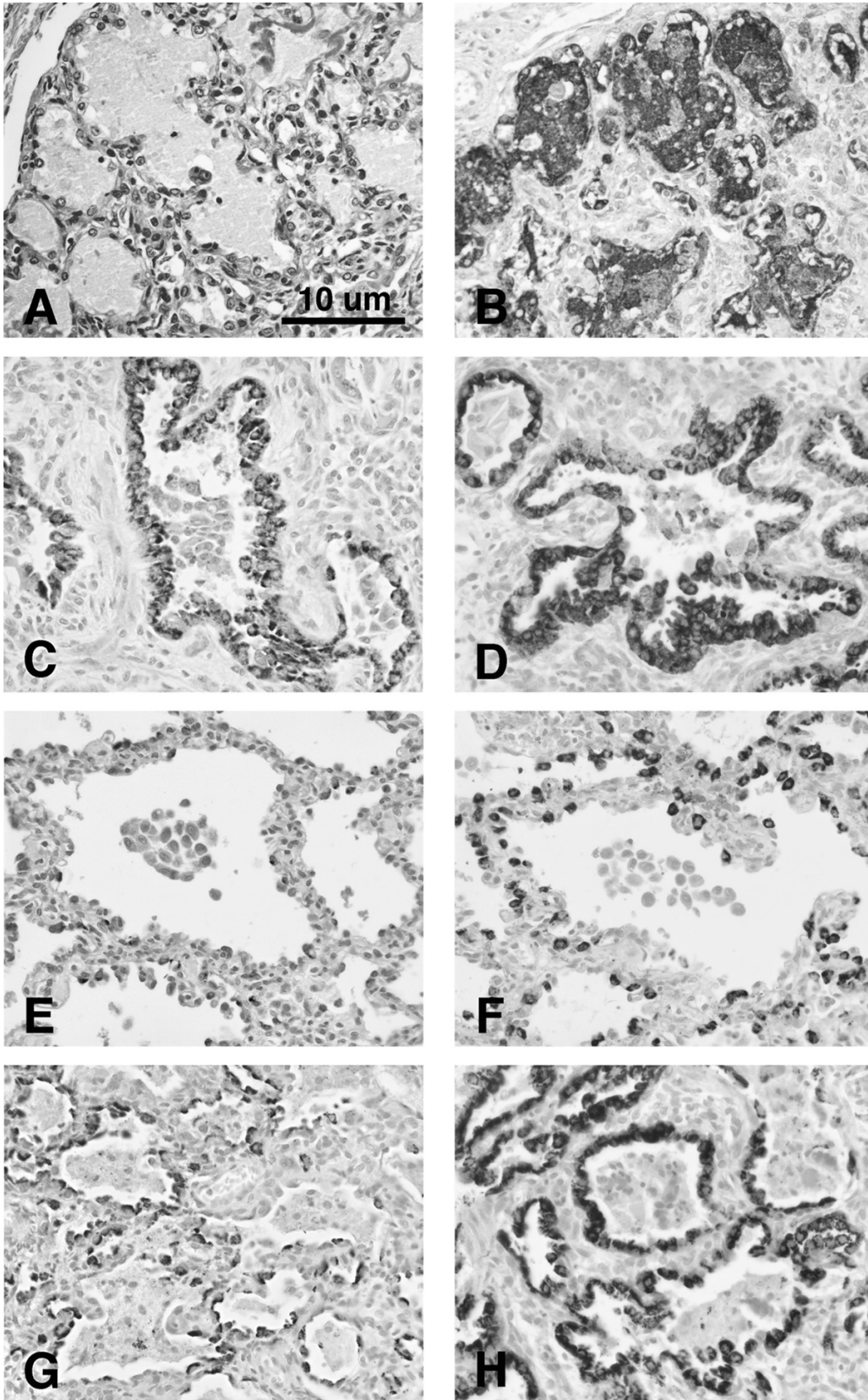


FIGURE 54.2. Immunohistochemical staining for mature surfactant protein B (SP-B) and proprotein surfactant protein C (pro-SP-C). (A,B) Specimens from a child with SP-B deficiency showing absent staining for mature SP-B (A) and intense staining for pro-SP-C (B) of both alveolar epithelium and the intra-alveolar material. (C,D) Specimens from a child with an *SFTPC* mutation showing robust staining for both mature SP-B (C) and

pro-SP-C (D) that is confined to the epithelium. (E-H) Specimens from two subjects with ABCA3 deficiency. In one subject, staining for mature SP-B is severely reduced (E), whereas in the other there is robust staining for mature SP-B (G). Proprotein SP-C staining is robust in both subjects (F and H) and is confined to the alveolar epithelium. (All original magnifications, $\times 20$; bar = $10\mu\text{m}$.)

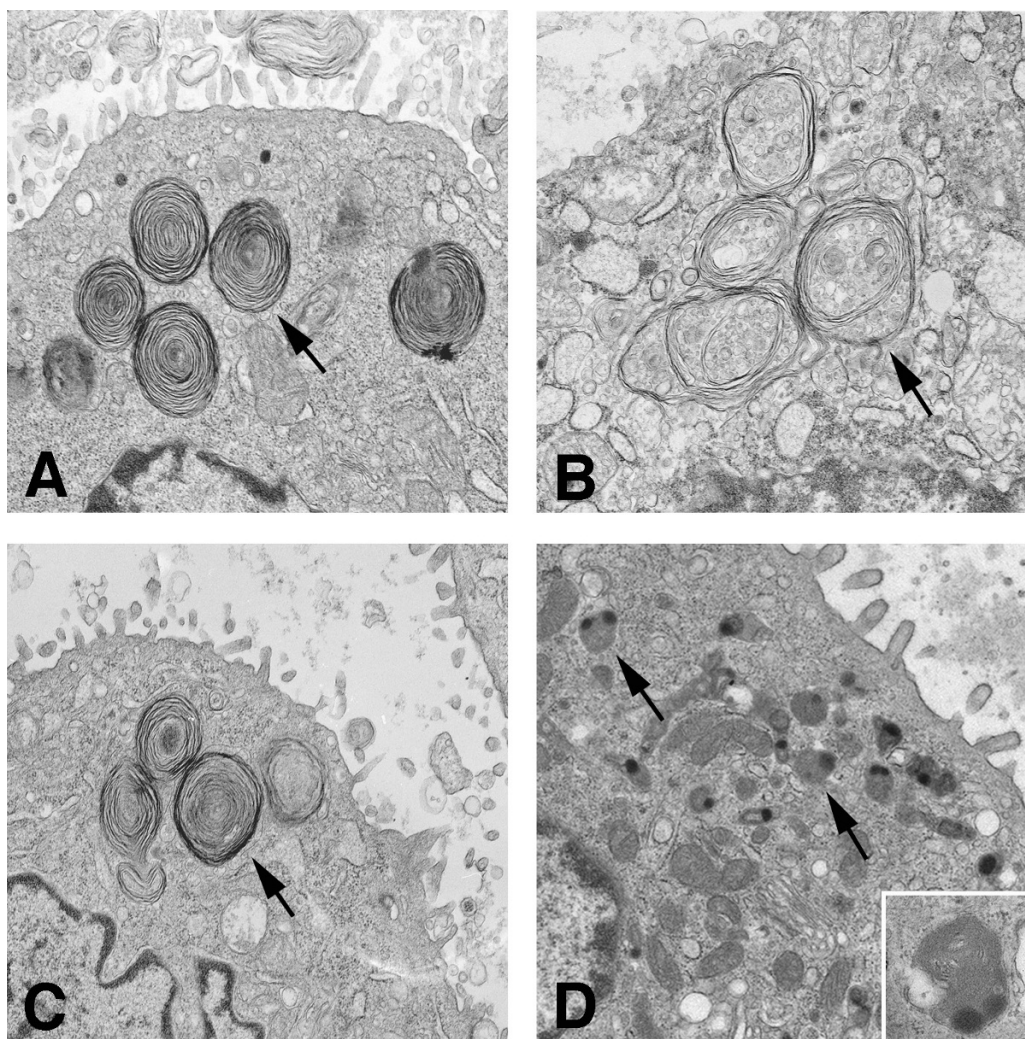


FIGURE 54.3. Electron micrographs of alveolar type II cells. **(A)** Normal lung, showing well-developed lamellar bodies (arrow) within alveolar type II cells. **(B)** Child with surfactant protein B deficiency, demonstrating disorganized, large multivesicular bodies (arrow) in lieu of lamellar bodies. **(C)** Child with an *SFTPC* mutation, showing well-formed lamellar bodies (arrow)

similar to those observed in normal lung **(A)**. **(D)** Child with *ABCA3* deficiency, demonstrating small, dense bodies (arrows) with eccentrically placed electron-dense inclusions and tightly packed phospholipid lamellae (inset). (*A–C*, original magnifications, $\times 5,000$; *D*, original magnification, $\times 10,000$; inset in *D*, $\times 30,000$.)

Genetic Testing

The identification of single gene defects that cause both acute neonatal respiratory failure and chronic interstitial lung disease allows for potential diagnostic testing through analysis of genomic DNA for potential mutations in these genes. Such testing has the advantage that it is noninvasive, potentially obviating the need for biopsy in an unstable patient, and can yield a specific diagnosis. Clinical testing is now available through Clinical Laboratory Improvement Amendments–certified laboratories. The sensitivity of such testing in different clinical situations is unknown, the testing is not inexpensive and costs

may not be covered by insurance, and results may take weeks to months to receive. The interpretation of such testing can be potentially problematic. It may not be possible to distinguish rare yet benign *SFTPC* variants from mutations responsible for disease, and it is apparent that not all *ABCA3* mutations are detected by current methods. In the case of a child with lung disease of unclear etiology in whom only one *ABCA3* mutation is found, it may be difficult to determine whether such an individual is affected with an unknown mutation on the second allele or whether is simply a carrier for an *ABCA3* genetic variant that is unrelated to the cause of the lung disease.

Other Proteins Important in Surfactant Metabolism Linked to Genetic Diseases

Alterations in two other proteins resulting in abnormalities of surfactant expression have been reported in association with human lung disease. A single report associated infantile alveolar proteinosis and interstitial lung disease with abnormalities of the common β -chain of the GM-CSF/IL-3/IL-5 receptors.¹⁶⁷ The rationale for examining this protein was based on observations in genetically engineered mice that targeted disruption of either the ligand (GM-CSF) or common beta chain (β c) of the receptor resulted in a phenotype of alveolar proteinosis as the mice aged.¹⁶⁸⁻¹⁷¹ In four infants examined, defective expression of the receptor was demonstrated on peripheral blood leukocytes, as well as decreased signaling for GM-CSF, but not G-CSF in vitro. Thus, these infants appeared to have a clear functional defect in this receptor. However, in only one infant was an abnormality in the gene encoding β c identified: a substitution of threonine for proline in codon 602, which was found on only one allele and may well have represented a polymorphism. To date, no clear disease-causing mutations in this gene have been identified, and no subsequent reports have appeared to confirm these initial observations.

Thyroid transcription factor 1 (also known as Nkx2.1) is a homeodomain transcription factor that has been shown to be critical for pulmonary development and expression of SP-A, SP-B, and SP-C. Several reports have linked genetic causes of reduced amounts of TTF-1 due to either deletion of a region containing the gene or loss-of-function mutations on one copy of the gene with neonatal respiratory disease with symptoms and signs of surfactant deficiency.¹⁷²⁻¹⁷⁵ As TTF-1 is also expressed in extrapulmonary sites, not surprisingly these patients have had abnormalities in other organ systems, specifically transient neonatal hypothyroidism and central nervous system abnormalities. In general these patients have recovered from the neonatal lung disease, and lung pathology information is not available for these patients. Hypothyroidism and central nervous system abnormalities have also been observed without any respiratory symptoms.¹⁷⁶ Some have had recurrent pulmonary infections, possibly related to the reduced amounts of SP-A, although other explanations are also possible. The incidence and prevalence of this disorder are unknown; only five families have been reported in the literature.

Pulmonary Alveolar Proteinosis

Pulmonary alveolar proteinosis is a lung disease of insidious onset due to an accumulation of surfactant in the airspaces and was first described in 1958.¹⁷⁷ The accumu-

lation of material in the airspaces results in a restrictive lung defect with resultant hypoxemia and pulmonary symptoms. The disease is primarily seen in adults and may occur either in a primary form or secondary to a number of pulmonary insults, including infection and toxic inhalation.^{156,178} The material accumulating in the lungs of patients with pulmonary alveolar proteinosis is rich in surfactant lipids and proteins, and lung lavage material from such patients was often the starting material for the purification of the surfactant proteins.^{179,180} The surfactant material accumulates as the result of the decreased catabolism rather than increased production. Primary pulmonary alveolar proteinosis in adults is now known to largely (if not entirely) be an autoimmune disease due to neutralizing autoantibodies to GM-CSF.¹⁸¹⁻¹⁸³ Such antibodies have been found in both serum and bronchoalveolar lavage fluid of affected patients. The absence of functional GM-CSF interferes with alveolar macrophage function, leading to defective catabolism of surfactant and accumulation of the pulmonary alveolar proteinosis material in the airspaces.

Similar pathology can be seen in newborns and young infants with severe respiratory failure and has been termed *congenital alveolar proteinosis*. Although aspects of the pathology in these infants may be similar to that seen in adults with pulmonary alveolar proteinosis, the underlying causes are different; specifically, in young infants the disease is more likely due to the inborn errors of lung cell metabolism described earlier. As a result, the material accumulating in the lungs of these infants differs from that observed in adults, and the course of the disease differs, with a more rapid downhill course in young infants that is refractory to therapy. Alveolar proteinosis in young infants and children may also occur in children with lysinuric protein intolerance, a disorder of cationic amino acid transport due to mutations in the gene encoding the solute transporter SLC7A7.^{184,185} Children affected with lysinuric protein intolerance usually have other systemic symptoms, such as recurrent vomiting and failure to thrive, but the pulmonary disease may be the most prominent feature and may prove fatal.

Conclusion

An intact pulmonary surfactant system is essential for normal respiratory function. Too little or too much surfactant can lead to profound lung disease, such as surfactant deficiency due to decreased production as a result of developmental immaturity causing RDS in newborn infants, and accumulation of pulmonary surfactant from decreased catabolism as a result of inactivation of GM-CSF due to neutralizing antibodies leading to alveolar proteinosis in adults. The identification of rare genetic variants in genes important in surfactant metabolism and correlation with the resulting phenotypes provides poten-

tial insights into the role of their gene products in surfactant function and support for polymorphic variants in these genes as having a role in more common pulmonary diseases.

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