Progressive Lung Disease and Surfactant Dysfunction with a Deletion in Surfactant Protein C Gene

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Mutations in the surfactant protein (SP)-C gene are responsible for familial and sporadic interstitial lung disease (ILD). The consequences of such mutations on pulmonary surfactant composition and function are poorly understood. To determine the effects of a mutation in the SP-C gene on surfactant, we obtained lung tissue at the time of transplantation from a 14-mo-old infant with progressive ILD. An in-frame 9-bp deletion spanning codons 91–93 in Exon 3 of the SP-C gene was present on one allele; neither parent carried this deletion. SP-C mRNA was present in normal size and amount. By immunofluorescence, proSP-C was aggregated within alveolar Type II cells in a compartment separate from SP-B. In airway surfactant, there was little or no mature SP-B or SP-C; SP-A content was increased. Minimum surface tension was increased (20 mN/m, normal < 5 mN/m). Type II cells contained normal and disorganized appearing lamellar bodies by electron microscopy. This spontaneous deletion on one allele of the SP-C gene was associated with sporadic ILD and abnormalities in surfactant composition and function. We propose that a dominant negative effect on surfactant protein metabolism and function results from aggregation of misfolded proSP-C and subsequent cell injury and inflammation.

Pulmonary surfactant is a phospholipid–protein complex that is synthesized by alveolar Type II cells and maintains alveolar expansion at end expiration. Of the four surfactant-associated proteins (surfactant protein [SP]-A, SP-B, SP-C, and SP-D), SP-B and -C interact with the phospholipid components in a tightly coordinated itinerary of synthesis, secretion, film formation, and recycling (1).

SP-C is a hydrophobic peptide encoded by an ~3-kb, 6-exon gene on the short arm of human chromosome 8. The synthesis and post-translational processing of SP-C have been recently reviewed (2). Alternative splicing results in a 191– or 197–amino acid, 21-kD propeptide that is proteolytically cleaved to a 35–amino acid ~4-kD peptide. The mature peptide is encoded within Exon 2 of the SP-C gene and is palmitoylated at cysteine residues at positions 5 and 6. Complete biosynthesis requires sequential endoproteolytic cleavages of the SP-C propeptide and depends upon oligomeric sorting and targeting to subcellular processing compartments distal to the Golgi. The mature SP-C domain contained within the propeptide (residues 24–58) functions as a signal-anchor sequence effecting endoplasmic reticulum (ER) translocation, establishing a type II ($NH2_{cytoplasm}$/$COOH_{lumen}$) integral membrane orientation, and facilitating homomeric association during sorting (3). The NH_2 flanking propeptide contains a functional targeting motif necessary for directing proSP-C from the ER to distal processing compartments, the multivesicular and lamellar bodies (4, 5). Deletion or alteration of this region results in ER retention. Conversely, although the COOH flanking propeptide is not required for targeting or post-translational processing, removal or alteration of one or more cysteine residues from the COOH flanking propeptide results in mutant protein accumulation in a novel juxtanuclear compartment, the aggresome (6). SP-B is integrally involved in this metabolic itinerary in that infants and mice genetically deficient in SP-B exhibit a 6- to 12-kD incompletely processed proSP-C peptide in both intracellular and extracellular surfactant (7, 8).

Mice genetically deficient in SP-C exhibit strain dependent pulmonary pathology. Swiss black SP-C null mice have no pulmonary pathology, but have surfactant that is unstable at low volumes (9). In contrast, 129/Sv SP-C null mice develop significant lung remodeling and increases in phospholipid pools, but no alteration in the expression of SP-B (10). Recently, mutations in the SP-C gene corresponding to the COOH terminus of proSP-C have been associated with familial and sporadic interstitial lung disease in adults and children (11–13). In contrast to the recessive null mutations seen in SP-B–deficient infants, all affected patients to date possess a mutation on only one allele of the SP-C gene, suggesting a dominant negative effect of the mutant SP-C, which has been recently demonstrated *in vitro* (14). The histologic and clinical expressions of these mutations have been variable with children exhibiting chronic pneumonitis of infancy or nonspecific interstitial lung disease (ILD) and adults exhibiting usual interstitial pneumonitis, desquamative interstitial pneumonitis, or idiopathic pulmonary fibrosis. The age of onset has ranged from 6 wk to over 50 yr. The mechanisms of cell injury and lung disease are thought to result from accumulation of incompletely processed/misfolded SP-C protein that is toxic to cells *in vitro* (12, 14) .

Lung transplantation has been successful for infants with inherited SP-B deficiency and has provided the opportunity to elucidate pulmonary surfactant expression, composition, and function (15, 16). This opportunity has not previously been available for subjects with mutations in the SP-C gene. Therefore, to address the hypothesis that pulmonary surfactant expression, composition, and function are disrupted in patients with mutations in the SP-C gene, we analyzed lung tissue and BAL fluid

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Abbreviations: bronchoalveolar lavage, BAL; bronchopulmonary dysplasia, BPD; electron microscopy, EM; endoplasmic reticulum, ER; electrospray ionization, ESI; interstitial lung disease, ILD; pulmonary alveolar proteinosis, PAP; phosphatidylcholine, PC; polymerase chain reaction, PCR; phosphatidylethanolamine, PE; phosphatidylglycerol, PG; phosphatidylinositol, PI; surfactant protein, SP.

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obtained from an infant with a novel mutation who underwent lung transplantation for progressive ILD.

Case

This 4.1-kg female was the full-term product of an uncomplicated pregnancy. She is the first child of these two parents. She was healthy until 3 mo of age when she presented with growth failure, difficulty feeding, and diffuse interstitial infiltrates on chest radiograph. Open lung biopsy at 6 mo of age revealed interstitial pneumonitis without a defined etiology (Figure 1). Genetic analysis at 9 mo of age revealed a 9-bp deletion in Exon 3 of the SP-C gene. Progressive decline in pulmonary function prompted bilateral lung transplantation at 14 mo of age. At 30 mo of age, she was breathing ambient air, gaining weight, and had a developmental stage of $\sim$ 24 mo. Both parents were healthy and there was no family history of pulmonary disease, specifically interstitial lung disease or idiopathic pulmonary fibrosis.

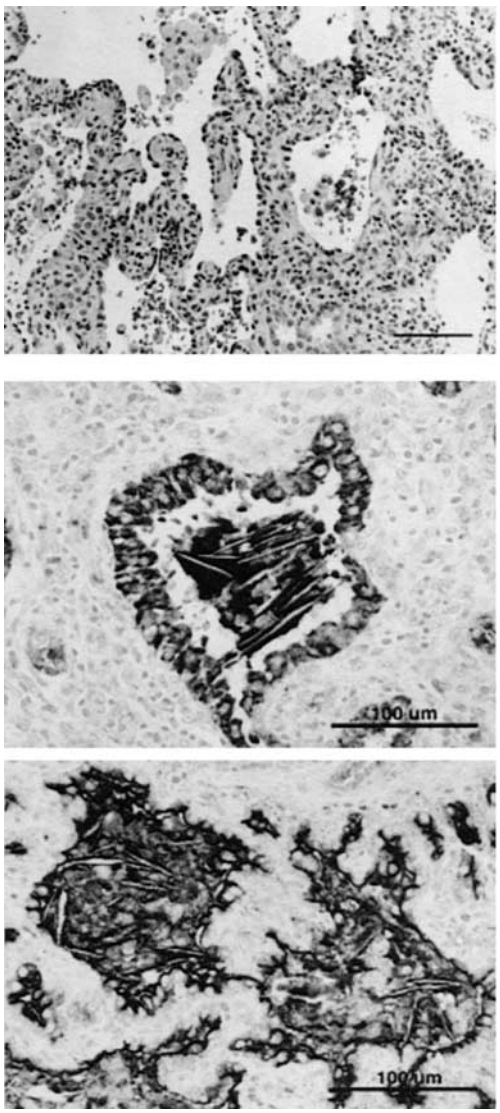

Figure 1. Photomicrographs of lung biopsy obtained at 6 mo of age. Hematoxylin and eosin staining (H&E) shows prominent type II pneumocyte hyperplasia and alveolar septal widening with a mild lymphoplasmacytic infiltrate along with smooth muscle extension and fibroblast proliferation. In this field, foamy macrophages and granular material are focally present in alveolar spaces. SP-A and SP-D expression is robust in Type II cells and alveolar material, as seen by staining with polyclonal rabbit anti-human SP-A antibody and polyclonal rabbit anti-mouse SP-D antibody. Pneumocyte hyperplasia, alveolar macrophage accumulation, and cholesterol clefts are prominent in these fields. *Scale bars* equal 100 μm.

Materials and Methods

Genetic Analysis

Genomic DNA was prepared from blood leukocytes using a commercially available kit (Puregene; Gentra Systems, Minneapolis, MN). Amplimers spanning exons 1 to 2 (genomic positions −143–996) and exons 3 to 6 (genomic positions 1,212–2,522) of the SP-C gene were generated by polymerase chain reaction (PCR) from genomic DNA of the child and parents and analyzed by direct sequencing of the PCR products. The SP-B gene was amplified using primers and conditions as previously described (17). PCR conditions were the same for both the SP-B and SP-C genes, with an annealing temperature of 60°C, and cycle sequencing was performed as previously described. Patient SP-C gene sequence was compared with previously published SP-C sequence. DNA from 50 adults without known lung disease were analyzed anonymously for the mutation found in the patient by direct analysis of PCR products on 3% metaphor agarose gels.

RNA Preparation, Northern Blot Hybridization, and *In Situ* Hybridization

Total RNA was extracted from frozen lung tissue obtained at transplantation by the acidic guanidinium thiocyanate method (18). OD_{260}/OD_{280} ratios were 1.80 to 1.94 for all samples. Contents of specific mRNAs were analyzed by Northern blot hybridization and scanning densitometry using [^{32}P]-labeled human cDNA probes for SP-A (900 bp), SP-C (875 bp), and β-actin (1.9 kb), prepared by random primer labeling with [^{32}P]dCTP and the Ready-To-Go kit (Amersham Pharmacia Biotech, Piscataway, NJ), as described (19). For Northern analyses, total RNA (10 μg applied per lane) was run on 1.2% agarose-formaldehyde gels, and RNA species were transferred to nitrocellulose membranes (Duralose-UV; Stratagene, La Jolla, CA) which were baked, and hybridized with ^{32}P-labeled cDNA probes. Autoradiography films were scanned with a densitometer and data expressed as density units per μg RNA applied.

In situ hybridization was performed on paraffin-embedded lung tissue as described previously (20). Briefly, antisense and sense [^{35}S]cRNA probes to SP-A, SP-B, and SP-C were synthesized as described using ^{35}S-labeled UTP and ^{35}S-labeled CTP. After deparaffinization, sections were hybridized with antisense and sense ^{35}S-labeled cRNA probes at a specific activity of 4×10^7 counts/min/ml of hybridization solution. After exposure for 7–10 d at 4°C, slides were developed, counterstained with hematoxylin and eosin, and examined under bright-field and darkfield illumination.

Surfactant Composition and Function

At the time of lung transplantation, sections of the explanted lung were frozen immediately upon removal and bronchoalveolar lavage (BAL) was performed upon one lobe of the explanted lung. Tissue, BAL, and tracheal aspirate fluid used as controls came from transplant donor lungs, premature infants requiring intubation beyond 7 d of life, and infants who required lung transplantation for refractory lung disease of undetermined etiology. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and protein blotting were performed on homogenates of lung tissue and on the BAL fluid as previously described (7, 16). Surface tension measurements and immunodot assays for SP-A, SP-B, and SP-C were performed on serial dilutions of large aggregate surfactant-rich fraction isolated by centrifugation of the BAL (27,000 × *g* for 60 min) (21). Surface properties were assessed at 37°C in humidified air with a pulsating bubble surfactometer (Electronetics Corp., Buffalo, NY) after adjusting the phospholipid concentration to 1.5 mg/ml.

After chloroform methanol extraction of BAL fluid with addition of 14:0/14:0 phosphatidylcholine (PC, 25 μmol), 14:0/14:0 phosphatidylglycerol (PG, 3 μmol), and phosphatidylethanolamine (PE, 1.5 μmol) as the internal standards, electrospray ionization (ESI) mass spectrometric analyses were performed to assess phospholipid composition using a Finnigan (San Jose, CA) TSQ-7000 triple stage quadrupole mass spectrometer.

PC was quantified as sodiated ion species in the positive-ion mode using constant neutral loss scanning of 183 (22). To quantify PG, PE, and phosphatidylinositol (PI) species in the negative-ion mode by ESI/MS, 0.1% ammonium hydroxide in methanol was added to the lipid mixture, which was re-extracted with chloroform, dried under nitrogen, re-dissolved in chloroform/methanol (1/3, vol/vol), and subjected to ESI analysis. The structures of the molecular species of the phospholipids were identified by the product-ion spectra of the $[M + Na]^+$ ions for PC, and of the $[M - H]^-$ ions for PG, PE and PI as previously described (23, 24).

Antibodies

Production and characterization of polyclonal antisera directed against SP-A, SP-B, proSP-B, and SP-D have been described (25–28). Monospecific polyclonal rat and rabbit proSP-C antisera produced against synthetic peptide proSP-C peptides have been previously characterized (29, 30). Anti–NPROSP-C (Met^{10} to Glu^{23}) recognizes proSP-C_{21} and all major intermediates but does not recognize mature SP-C. Antibody to mature SP-C was provided by ALTANA Pharma AG (Konstanz, Germany), and was generated against recombinant human SP-C containing Phe substituted for Cys residues 3 and 4 and Ile for Met at residue 32. On protein blotting, this antibody recognizes mature SP-C of $\sim$ 4 kD and has very weak immunoreactivity for precursor forms of SP-C (31).

Pulmonary Histology, Immunohistology, and Ultrastructure

Immunohistochemical analyses using polyclonal antisera directed against SP-A, SP-B, proSP-C, and SP-D were performed on formalin-fixed, paraffin-embedded tissue as previously described (11, 32). Fluorescence immunocytochemistry of fixed lung sections was done using anti–NPROSP-C as previously described (29). Lung tissue obtained at transplantation was fixed in 3% glutaraldehyde and prepared for electron microscopy (EM) as previously described (33).

Informed consent was obtained from the family and the study was approved by the institutional review boards of the respective institutions.

Results

Genetic Analysis

Sequence analysis demonstrated a 9-bp deletion (sequence ACT GCC ACC) on one allele in Exon 3 of the SP-C gene corresponding to codons 91–93 in the proSP-C peptide. This in-frame deletion would result in the deletion of three amino acids (Thr Ala Thr) in the proximal COOH flanking domain of the SP-C proprotein. This mutation was not present in either parent, suggesting that this was a *de novo* mutation (Figure 2). The deletion was also not observed in 100 control chromosomes, indicating that it is not a common polymorphism.

No variations from the known SP-B gene sequences or single nucleotide polymorphisms were identified.

Transcript Expression

Northern blot analysis of RNA from the explanted lung tissue showed a single SP-C band of the expected size with a staining intensity similar to comparison samples from infants with lung disease (inherited SP-B deficiency and bronchopulmonary dysplasia, not shown). This technique could not resolve transcripts differing by 9 bp; however, a band corresponding to each allele was observed with RT-PCR using primers spanning the site of the deletion (not shown). SP-A transcript in the patient was the expected size with somewhat stronger staining intensity compared with other samples tested. *In situ* hybridization revealed the expected amount and distribution of SP-A, SP-B, and SP-C mRNA expression (data not shown).

SP Expression

By Western blot analysis, mature SP-C was not detected in large aggregate surfactant prepared from the patient's BAL, but was present in control samples (Figure 3). A strong band for SP-A was observed in the patient's surfactant and was increased relative to premature infant controls by immunodot analysis (Table 1) and immunohistochemistry (Figures 1 and 4). By Western blot analysis, no immunoreactive SP-B bands were seen in BAL. However, mature SP-B was present in the patient's lung homogenate (data not shown), was low in large aggregate surfactant by immunodot analysis, and was weakly detected in Type II cells by immunohistochemistry (Figure 4). ProSP-B staining was found both within Type II cells and in alveolar spaces of the patient; however, it was not significantly different from that of lung injury controls (data not shown). Staining with anti–NPROSP-C showed a stronger and more localized signal in the perinuclear region of the patient's Type II cells than in normal and lung disease control subjects (Figure 5). In the control subjects, SP-B and proSP-C had similar distribution in cytosolic vesicles, suggesting colocalization in multivesicular or lamellar bodies. In contrast, in the patient, proSP-C and SP-B had distinctly different distributions, suggesting localization in different compartments in the alveolar type II cell. These findings were supported by routine

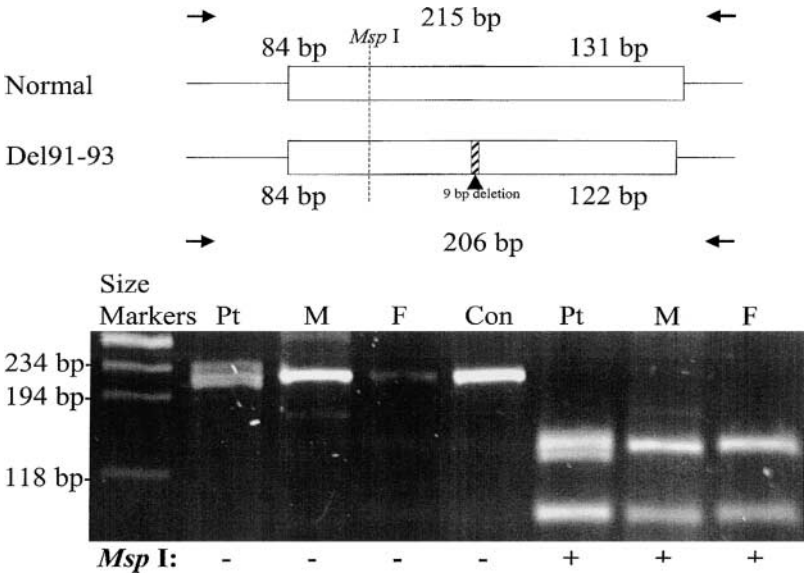

Figure 2. PCR amplification of SP-C exon 3. In the lane corresponding to the patient's DNA (Pt), there is a band at 206 bp, corresponding to the allele with the 9-bp deletion, along with a band migrating at the expected size of 215 bp, corresponding to the normal allele. The lanes corresponding to the mother's (M), the father's (F), and an unaffected control subject's (Con) DNA contain only the 215-bp fragment band corresponding to the normal allele. The smaller PCR product corresponding to the mutated allele in the patient's DNA was better resolved after digestion of the PCR products with the restriction enzyme *MspI* (*right*).

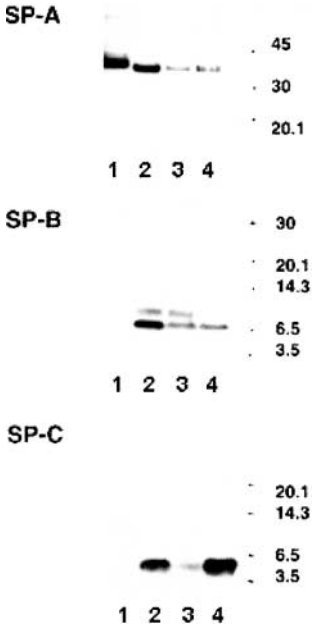

Figure 3. Protein immunoblots for SP-A, SP-B, and SP-C. Large aggregate surfactant was prepared from BAL of the patient (*lane 1*), and tracheal aspirate samples from three premature infants (*lanes 2–4*). The amount of total protein applied was 7 μg for all samples. Mature SP-A is observed at ~ 35 kD. The gels for SP-B and SP-C were run under reducing conditions. Mature SP-B migrates at ~ 8 kD with a doublet band for two tracheal aspirate samples. Mature SP-C is seen at ~ 4 kD. In the patient's sample, SP-A was detected, but mature SP-B and SP-C were not.

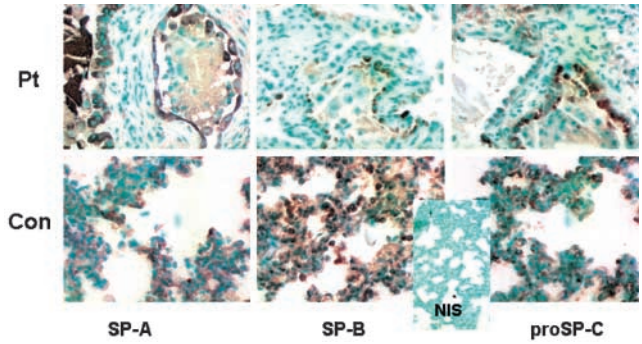

Figure 4. Immunohistochemical stains for SP-A, SP-B, and proSP-C in lung tissue of the case patient (*top row*) and a transplant donor (*bottom row*). The case patient exhibits decreased staining for SP-B and normal to increased staining for proSP-C in Type II cells. SP-A is normal in the Type II cells but increased in the material in the airspaces. *Inset:* negative control with nonimmune serum (NIS). *Original magnification:* ×400 for all images except the NIS (×100).

immunostaining of formalin-fixed, paraffin-embedded lung tissue for mature SP-B and proSP-C (data not shown).

Function and Phospholipid Composition of Large Aggregate Surfactant

The surfactant fraction of the patient was compared with that of six intubated premature infants (samples selected for normal surface tension properties) and adults with pulmonary alveolar proteinosis (PAP). As assessed in a pulsating bubble surfactometer, the minimum surface tension of the patient's sample was elevated, indicating *in vitro* surfactant dysfunction (Table 1). The content of the total protein relative to phospholipid was increased in the patient's surfactant compared with infant control subjects. PC comprised 78% of the phospholipid (controls 84 ± 1%), 46% of which was dipalmitoyl PC, which was not significantly different from control subjects (43 ± 2% of total PC). The relative proportion of PI was increased (8% versus 2 ± 1%, patient and control subjects, respectively), the significance of which remains to be determined.

Histology and Ultrastructure

The histologic appearance of the explanted lungs resembled that of the biopsy obtained 8 mo earlier, with increased interstitial fibrosis and focal acute pneumonia. There were also focal areas with weakly periodic acid-Schiff positive granular material in the airspaces suggestive of alveolar proteinosis (not shown).

TABLE 1. Function and surfactant profile of large aggregate surfactant pellet

	Patient BAL	Infant Tracheal Aspirate	Adult PAP BAL
Minimum surface tension, mN/m	20.0	3.0 (0–6.1)	12.0 (7.8–15.0)
Total protein, μg/μgPL	2.5	0.7 (0.2–1.8)	6.0 (3.1–9.1)
SP-A, μg/100 μgPL	15.0 (10.8–21.0)	4.0 (0.3–10.0)	647.0 (506.9–727.0)
SP-B, μg/100 μgPL	0.6 (0.4–1.1)	1.9 (0.6–4.6)	9.4 (4.0–16.6)

Data are mean and range for four replicate determinations of the patient sample, six samples of premature infant tracheal aspirate, and four samples from adults with PAP. Data for SP-A and SP-B are presented as μg per 100 μg total phospholipid (PL) by weight (by phosphorous assay of large aggregate surfactant pellet).

EM of the explanted lung revealed type II pneumocytes with abundant lamellar bodies in apical cytoplasm, some of which were disorganized (Figure 6). In addition, a minority of type II cells contained large disorganized perinuclear membranous structures. A rare cell contained cytoplasmic dense membrane bound and multivesicular structures; however, pneumocytes with numerous multivesicular bodies characteristic of SP-B deficiency were not seen (34).

Discussion

In this report of an infant with progressive and severe ILD associated with a spontaneous 9-bp deletion on one allele of the SP-C gene, proSP-C accumulated in an intracellular compartment separate from SP-B and mature SP-C was not detected in large aggregate surfactant. These findings suggest that the trafficking and/or processing of normal proSP-C was disrupted by the presence of mutated proSP-C, altering surfactant composition and function.

Inherited mutations in the SP-C gene have been identified in individuals with familial ILD and *de novo* mutations have been identified in individuals with sporadic ILD, suggesting that the mutations are causally linked with disease (11–13). This infant's novel deletion in the presence of ILD and the absence of the mutation and disease in the parents further strengthens this association. In addition, similar to all previously reported cases, the mutation was present on only one allele, suggesting a dominant negative effect.

The exact mechanisms of lung disease resulting from mutations in the SP-C gene remain to be fully elucidated. However, the information derived from our current studies supports previous *in vitro* studies and provides insights into other mechanisms that may promote respiratory dysfunction in these patients. First, we demonstrated aggregation of proSP-C within Type II cells in explanted lung tissue, similar to findings with transfected lung epithelial cells. A549 cells transfected with constructs containing mutated proSP-C sequences have demonstrated that alterations in the COOH terminus of rat SP-C (deletion of Leu 185-Ile194) or human SP-C (deletion of Exon 4: Leu 110-Gln 144) result in accumulation of the mutant proSP-C in aggresomes (6, 14). In addition, transfection of SP-C cDNA containing the point mutation seen in a family with ILD (Exon 5 + 128 T>A) into mouse lung epithelial cells resulted in cytotoxicity (12). In the subject of this article, the absence of mature SP-C in the large aggregate

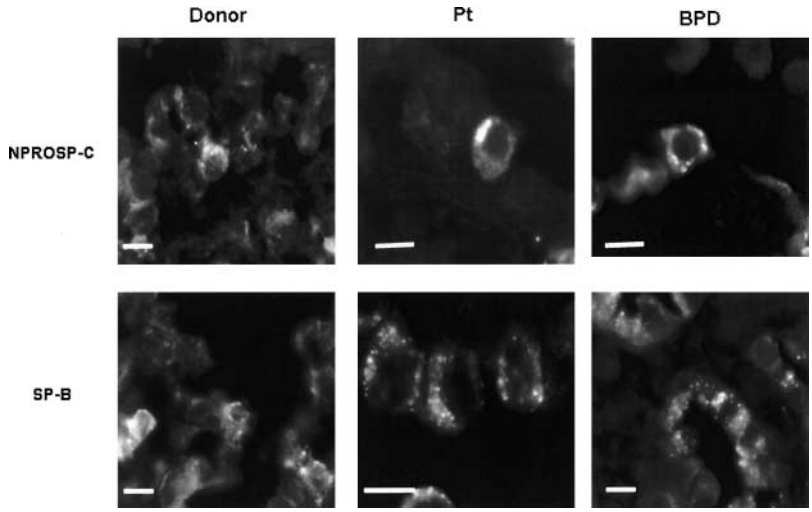

Figure 5. ProSP-C and SP-B expression in lung of a transplant donor, the case patient, and an infant with BPD. Sections were stained with primary polyclonal anti–NPROSP-C (*top row*) and anti-human mature SP-B (*bottom row*) and secondary Texas Red anti-rabbit IgG. Fluorescence microscopic images demonstrate intracellular proSP-C aggregates in alveolar type II cells in the case patient, in contrast to the control subjects, in which, despite increased numbers of type II cells, fluorescence shows proSP-C to be distributed in cytosolic vesicles. Staining for SP-B demonstrated distribution in cytosolic vesicles in all samples. *Scale bars* equal 10 μm.

surfactant and the juxtanuclear accumulation of proSP-C are consistent with a dominant negative effect of a presumably misfolded peptide that prevents normal proSP-C trafficking into the multivesicular and lamellar bodies (6). It has been speculated that the aggregation of misfolded proSP-C may interfere with the "unfolded protein response" and result in toxic gain of function similar to that seen in mice that overexpress SP-C, or in patients with Alzheimer's disease, α1-antitrypsin deficiency, and cystic fibrosis (35, 36). In addition, amino acids 94–191 of the COOH terminus of SP-C share sequence similarity with propeptide domains of BRI2, chondromodulin-I, and CA11, the "BRICHOS" family (37). Mutations in these domains result in dementia, chondrosarcoma, and stomach cancer, respectively, likely due to disrupted peptide processing, targeting, and/or aggregation, mechanisms which appear to be operative with SP-C mutations, as well.

Surfactant dysfunction, not previously reported in patients with SP-C mutations, is another factor that probably contributed to this infant's decline in respiratory function. Whether the decreased surfactant function was specific to disrupted SP-C metabolism or was a more generalized response to cell injury and inflammation is unknown. The lack of mature SP-B and SP-C in the large aggregate surfactant is a prominent finding that would explain lack of surface activity and respiratory dysfunction. Murine lineages conditionally expressing SP-B develop respiratory failure, altered surfactant composition and decreased surfactant function when SP-B levels reached 25% of normal values (38). *In vitro,* surfactant phospholipids in the absence of surfactant proteins have a slow adsorption rate to the surface film and do not achieve low surface tensions; normal surfactant properties are restored in a dose-dependent fashion by reconstitution of lipids with SP-B (39). Thus, the absence of detectable mature SP-B and SP-C in our patient's surfactant would disrupt the surfactant phospholipid layer and decrease surfactant function. Another possibility is that the misfolded proSP-C itself may inhibit surfactant function; however, there is no evidence that this proSP-C is secreted into the alveolar space. The presence of proSP-B immunostaining in the alveolar space, the presence of disorganized lamellar bodies on electron microscopy, and apparent intracellular aggregation of proSP-C suggest that the metabolism and assembly of the surfactant complex was disrupted, similar to that seen with SP-B–deficient infants (7, 16, 34, 40).

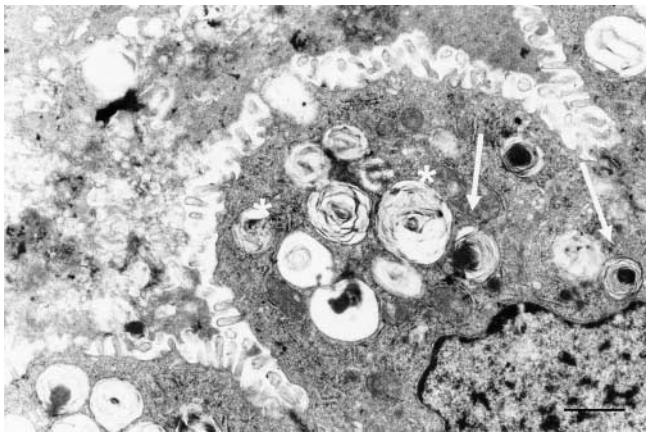

Figure 6. EM of the patient's explanted lung tissue shows hyperplastic type II pneumocytes with numerous lamellar bodies in apical cytoplasm, some of which appear normal (*arrow*) and others which appear to be disorganized (*asterisk*). *Scale bar* equals 1 μm.

Although these alterations in surfactant composition and function may be specific for mutations in the SP-C gene, another possibility is that the alterations are a nonspecific response to cell injury. Decreased or absent SP-B and SP-C are features of other lung diseases associated with inflammation, such as ILD without mutations in the SP-C gene, in adults with acute respiratory distress syndrome, in premature infants with chronic lung disease, and in animals with bleomycin-induced lung injury (32, 40, 41). Because this infant presumably had normal pulmonary function for several months after birth, we speculate that, like the Swiss black SP-C null mice, surfactant function was normal under normal conditions, but was easily disrupted once challenged with an inflammatory response either from accumulation of proSP-C or from a secondary insult such as aspiration or infection.

This is the first infant known to have a mutation in the SP-C gene to be considered for lung transplantation. In contrast to the known and rapidly lethal outcome for infants with SP-B deficiency for whom discussions with the family about lung transplantation can be initiated at the time of diagnosis, infants and adults with ILD due to mutations in the SP-C gene have more variable, and hence, unpredictable short- and long-term outcomes (11, 12, 15). Thus the consideration for lung transplantation is not as straightforward as that for infants with inherited SP-B deficiency. In this case, the criteria regarding commitment to lung transplantation that are currently applied for other forms of progressive lung

disease were applied. Only when it became apparent that the patient's condition was deteriorating and refractory to medical therapy was she listed for transplantation.

In summary, lung transplantation for an infant with ILD due to a mutation in the SP-C gene afforded the opportunity to investigate surfactant composition and function. These studies provide further indication that altered SP-C processing leads to a chronic inflammatory state that disrupts surfactant metabolism and function. Additional experience with infants with mutations in the SP-C gene will permit genotype–phenotype correlations that will help predict the natural history and provide more informed decision-making about the need for lung transplantation.

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