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An Overview of Pulmonary Surfactant in the Neonate: Genetics, Metabolism, and the Role of Surfactant in Health and Disease

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Abstract

Pulmonary surfactant is a complex mixture of phospholipids (PL) and proteins (SP) that reduce surface tension at the air-liquid interface of the alveolus. It is made up of about 70% to 80% PL, mainly dipalmitoylphosphatidylcholine (DPPC), 10% SP-A, B, C and D, and 10% neutral lipids, mainly cholesterol. Surfactant is synthesized, assembled, transported and secreted into the alveolus where it is degraded and then recycled. Metabolism of surfactant is slower in newborns, especially preterm, than in adults. Defective pulmonary surfactant metabolism results in respiratory distress with attendant morbidity and mortality. This occurs due to accelerated breakdown by oxidation, proteolytic degradation, inhibition or inherited defects of surfactant metabolism. Prenatal corticosteroids, surfactant replacement, whole lung lavage and lung transplantation have yielded results in managing some of these defects. Gene therapy could prove valuable in treating inherited defects of surfactant metabolism.

Keywords

Dipalmitoylphosphatidylcholine; Phospholipids; Prematurity; Respiratory Distress Syndrome; Surface tension; Surfactant; Surfactant proteins; Thyroid transcription factor-1; Type II alveolar cells

Introduction

I. OVERVIEW OF SURFACTANT

Pulmonary surfactant is a complex mixture of phospholipids (PL) and proteins (SP) that reduce surface tension at the air-liquid interface of the alveolus, thus preventing its collapse during end-exhalation [1,2]. It also participates in innate host defense against inhaled pathogens [2].

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Surfactant is synthesized and secreted by Type II alveolar epithelial cells, also called pneumocytes, which differentiate between 24 and 34 weeks of gestation in the human. It is made up of 70% to 80% phospholipids, approximately 10% protein and 10% neutral lipids, mainly cholesterol [3]. The primary surface-active material found in surfactant is the phospholipid, dipalmitoylphosphatidylcholine (DPPC), while the surfactant proteins are SP-A, SP-B, SP-C and SP-D. Surfactant increases surface pressure while lowering surface tension. High surface pressure resists a decrease in alveolar surface area, while low surface tension stabilizes the lung by decreasing the pressure gradient across the alveolar lining layer [4].

(A) Function—The theory of surfactant protein B (SP-B) induced lateral stability has been proposed as the mechanism responsible for the functional ability of surfactant to lower and vary surface tension with changing surface area in the stable alveolus [5]. This theory evolved from studies of peptides synthesized according to sequences of SP-B amino acids or mimicking these sequences which showed that SP-B provided cohesiveness to molecules of phospholipids [5,6]. The peptides and SP-B are hydrophobic and are positioned in the acyl side chains of the phospholipid monolayer, with strong electrostatic interactions between the positively charged amino acids and the negatively charged phospholipids. This bonding of SP-B, peptide and phospholipid molecules confers lateral stability to the phospholipid molecules in the monolayer of the alveolus and by virtue of this; the cohesive monolayer is able to prevent collapse of the alveolus [5,7].

(1) Lung Anatomy: The lung first appears as a ventral bud off the esophagus just caudal to the laryngotracheal sulcus. The bud later elongates within the surrounding mesenchyme and divides to form the future main stem bronchus while further branching gives rise to the conducting airways [3]. The canalicular stage of lung development is between 16 to 26 weeks. During this period, the primordial gas exchange region of the peripheral lung is formed along with the differentiation of the respiratory epithelium. Components of the surfactant system begin to appear as well [8]. Alveolarization occurs from 36 weeks preterm to 36 months postnatal in humans, and involves a progressive decrease in the size of the alveolar air spaces, together with a concomitant increase in the total number of alveoli [9].

Several factors, including hormonal, biochemical, and physical factors play a role in the regulation of alveolarization. Thyroxine and retinoic acid stimulate alveolarization in experimental models [3]. The differentiation of type II alveolar epithelial cells is partially regulated by a balance between glucocorticoid and TGF-β signaling [10] while transdifferentiation of type II alveolar epithelial cells into type I alveolar epithelial cells is influenced by TGF-β/Smad2, 3, 4 signaling [11]. The degree of TGF-β signaling must, however, be precisely controlled, because both up- and down-regulation of TGF-β signaling impairs the alveolarization process [12]. BMP signaling may play a role in homeostasis during the saccular and early alveolarization stages of lung development [13]. In transgenic mice, overexpression of TGF-α, IL-6 or 11, and TNF-α in the pulmonary epithelium interferes with alveolar development [3], while targeted deletion of FGF-9 and 10 results in lung hypoplasia and agenesis respectively [8]. The human fetal lung is not mature clinically until after approximately 35 weeks gestation.

(2) Alveolus: The alveolus is the primary site of gas exchange with the blood in mammalian lungs. In humans, it consists of an epithelial layer and extracellular matrix surrounded by capillaries and has a radius of about 0.1mm, and a wall thickness of about 0.2μm. Two types of epithelial cells are found in the alveoli: Type I cells and Type II cells. Type I cells, make up 95% of the alveolus, while the Type II cells account for the remaining 5% [14]. Type I cells form the alveolar wall while the Type II cells synthesize and secrete surfactant. The alveoli have an innate tendency to collapse because of their spherical shape, small size and the

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contribution of water vapor to surface tension. Surfactant helps to lower surface tension thus preventing collapse.

(3) Surface Tension: Surface tension arises from the difference between the attractive forces on molecules at an air-liquid interface. As a result of this, there is a force or tension in the surface film that resists expansion of the bubble and consequently acts to contract surface area. This force is surface tension and has a value of 70 mN/m or 70 dynes/cm of water at 37 C [4]. The primary surface-active material found in surfactant is the phospholipid, DPPC. A pure film of DPPC on a surface balance is capable of lowering the surface tension to near 0 mN/m under dynamic compression [4]. DPPC is insoluble in water but can form lipid bilayers. The close packed nature of the saturated palmitic acids allows the DPPC molecules to organize into a two dimensional surface gel at 37 C, giving it stability [4]. This ultimately helps in lowering surface tension and preventing collapse of the alveoli. Natural surfactant generally lowers surface tension to <6 dynes/cm [15]. At concentrations that mimic that of surfactant in the epithelial lining layer (4mg of PL/ml), clinical surfactants lower surface tension to 5–10 dynes/cm [16].

The total surfactant content can be divided into an intra-alveolar and an intracellular pool [17]. However, the total surfactant pool size is not equivalent to the amount of active surfactant. Maintaining adequate surfactant pools within the air space is essential for lung function and is dependent on the dynamic cycle of surfactant metabolism [18]. It is reduced to less than 10 mg/kg surfactant in preterm infants who have Respiratory Distress Syndrome (RDS) compared with term infants who have an estimated pool size of 100 mg/kg surfactant [19]. Exogenous surfactants are given at doses between 10–20 times the normal pool sizes during surfactant replacement therapy which approximates the pool size in term infants [19].

(B) Protein Components—Four surfactant proteins, called SP-A, SP-B, SP-C, and SP-D, have been identified. SP-B and SP-C have been characterized as hydrophobic polypeptides (PP) that enhance the adsorption of lipid to the surface of the alveoli, whereas SP-A and SP-D are hydrophilic and participate in the innate host defense immune system [20].

(1) SP-A: SP-A is a 26–35 kDa glycoprotein synthesized by respiratory cells in the developing fetal lung. It binds and aggregates phospholipids in a calcium-dependent manner. It activates alveolar macrophages *in vitro* and enhances the opsonization of bacterial, fungal and viral pathogens and may therefore play an important defense role in neonatal and mature lung. The human SPA gene locus consists of two functional genes, SP-A1 and SP-A2 which have four coding exons represented by approximately 4.6 kb of DNA located on the long arm of human chromosome 10 [21]. SP-A transcription is regulated by thyroid transcription factor-1 (TTF-1). The expression of SP-A in amniotic fluid increases in late gestation and is a useful marker for determining fetal lung maturity [22]; however, measurement of SP concentrations in amniotic fluid does not have a higher specificity or sensitivity for predicting lack of respiratory distress syndrome than more routinely measured phospholipid indices, such as the lecithin/sphingomyelin ratio [23].

(2) SP-B: SP-B is a 17.4 kDa homodimeric protein comprising two subunits with 79 amino acids each. Each 79 residue polypeptide chain contains three disulfide bridges and the dimer is held together by a disulfide bond linking the CYS48 of the two subunits [24]. The dimeric structure of SP-B may account for its ability to cross-link different lipid membranes. SP-B is tightly associated with surfactant PLs and forms tubular myelin in the presence of SP-A, phospholipids and calcium [3]. SP-B is critical for lamellar body formation [3] and its deficiency results in abnormal processing of SP-C due to abnormal lamellar body formation. SP-B interaction with polar head groups resists surface tension by increasing the lateral stability

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of the phospholipid monolayer [6] and interacts as a helical peptide conformation with the lipid head groups based on NMR spectroscopy data [25].

SP-B is an important component of surfactant replacement mixtures made by organic solvent extraction of pulmonary surfactant or lung minces. It alters PL membrane organization, enhancing the surfactant-like properties and the uptake of PL vesicles by Type II cells *in vitro* while resisting surface tension by increasing the lateral stability of the phospholipids monolayer [6]. The sequence region 64–79 of SP-B contains peptides with two or more basic residues that include arginine or lysine. This sequence has been shown to be most effective in lowering surface tension [7]. These hydrophobic, positively charged, basic residues are positioned at periodic intervals throughout the amino acid sequence [7].

The human SP-B gene consists of 10 exons in 9.5 kb of DNA located on the short arm of human chromosome 2 and its transcription is regulated by TTF-1. The concentration of SP-B increases with advancing gestation, as does SP-A. SP-B is absolutely essential for breathing and SP-B (−/−) mice, and infants with mutations in SP-B gene die of respiratory distress after birth [8]; and conventional therapies for SP-B deficiency in infants are ineffective unless lung transplantation is provided [26].

(3) SP-C: SP-C is a transmembrane protein with a short extra-membranous domain expressed exclusively alveolar by Type II cells postnatally [27]. The SP-C proprotein is proteolytically cleaved in multivesicular bodies removing the N- and C terminal peptides generating the 35 amino acid peptide which is stored in lamellar bodies with surfactant phospholipids until secretion into the alveolar space where it enhances the stability and spreading of phospholipids [28]. The SP-C peptide is highly hydrophobic and contains two cysteine residues in an NH2-terminal domain that are palmitolyated. This hydrophobic region forms an alpha-helical structure that spans the lipid bilayer [29]. Both alpha-helical domain and the cysteine-linked palmitoyl groups are associated with phospholipids by recruiting them to monolayers and multilayers at the air-liquid interface [30]. The human SP-C gene consists of 6 exons in 3.5 kb of DNA located on the short arm of human Chromosome 8, and its transcription is regulated by TTF-1 [31]. SP-C (−/−) Swiss black mice do not demonstrate abnormalities in lung structure at birth [32]. In a congenic 129/Sv strain, SP-C deficient mice developed severe, progressive pulmonary disease associated with emphysema, alpha-smooth muscle actin staining, monocytic infiltrates, and epithelial cell dysplasia in conducting and peripheral airways [33]. Mice strain dependent influences on the SP-C (−/−) phenotype and the variety of lung pathology that vary in severity and time are similar to finding in patients with a familial idiopathic fibrosis caused by mutations in the SP-C gene [34]. An SP-C allele polymorphism (138Asn-186Asn) was found associated with very premature females' birth that may affect SP-C secretion at birth and the occurrence of bronchopulmonary dysplasia among a Finnish cohort of preterm and term infants [35].

(4) SP-D: SP-D is a hydrophilic 43kDa collectin belonging to the superfamily of collagen containing C-type lectins, and is structurally similar to SP-A. Produced in Type II alveolar cells, it is also found in epithelial cells and secretory glands of the gastrointestinal tract. SP-D plays an important role in the innate immune system by binding to specific carbohydrate and lipid structures on the surface of bacteria, viral particles, fungi and protozoa through a calcium-dependent interaction [20]. It has also been thought to have a role in the control of lung inflammation [31]. The SP-D gene consists of 8 exons spanning >11 kb of DNA located on the long arm of human chromosome 10 [36]. Levels of SP-D and SP-A are potential markers for lung maturation because studies of amniotic fluid and lung tissue have demonstrated increasing levels of SP-D with increasing gestational age [31]. Transcription of SP-D is regulated by direct interaction of nuclear factor of activated T cells (NFAT) with TTF-1 [37].

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II. METABOLISM OF SURFACTANT

A. Overview of Surfactant Metabolism—Pulmonary surfactant is synthesized, assembled, transported and secreted into the alveolus where it is degraded. It is then recycled in a highly complex and regulated mechanism. This process is slower in newborns (especially those born prematurely) than in adults or those with lung injury.

The rate of synthesis and the half-life of surfactant are influenced by many factors. Surfactant synthesis and turnover in preterm infants using stable isotopes of glucose, acetate and palmitic acid demonstrates that synthesis from glucose to surfactant phosphatidylcholine (PC) takes approximately 19 hours and reaches a peak at 70 hours after labeling. The absolute production rate of PC is 4.2 mg/kg/day while the half-life is 113 (± 25) hours [38]. The fractional synthesis rate of surfactant PC from plasma palmitate was significantly higher than that from palmitate synthesized *de novo* from acetate or glucose, but only accounted for half of the total surfactant production in preterm infants [39].

Surfactant secretion can be stimulated by a number of mechanisms. Type II cells have beta-adrenergic receptors and respond to beta-agonists with increased surfactant secretion [40]. Purines, such as adenosine triphosphate are potent stimulators of surfactant secretion and may be important for its secretion at birth. Mechanical stretch such as lung distension and hyperventilation, have also been found to be involved in stimulating surfactant secretion. Stretch-mediated enhancement of surfactant secretion during exercise prevents a loss of alveolar surfactant [41]. Hormones also play a role in surfactant secretion. Thyroxine accelerates Type II cell differentiation while acting synergistically with glucocorticoids to enhance the distensibility of the lung and DPPC synthesis. However, glucocorticoids alone are used in clinical practice to induce lung maturity because studies have not shown that the synergistic effect with thyroxine is greater than the effect achieved by glucocorticoids alone [3].

Type II cells, macrophages and the alveolar lining play a major role in surfactant turnover. Cyclical changes in the alveolar surface appear to promote conversion of newly secreted, apoprotein-rich, active surfactant aggregates into protein-poor, inactive forms that are ready for clearance [3]. Surfactant components are removed from air spaces through uptake by Type II cells and alveolar macrophages, with the bulk done by the Type II cells. The phospholipids are taken up by endocytosis into the Type II cells where they are recycled and re-secreted, whereas the SPs are recycled back into the lamellar bodies for re-secretion with surfactant. Surfactant is also transformed during the cyclic compression and expansion of alveoli from large, highly surface active aggregates into smaller, less active subtypes [42].

(B) Defects in Surfactant Metabolism—Defective surfactant metabolism leads to both morbidity and mortality in preterm and term neonates. In general, defects in surfactant metabolism occur due to accelerated breakdown of the surfactant complex by oxidation, proteolytic degradation, and inhibition [43,44]. Some inherited surfactant gene defects have also been implicated.

(1) Respiratory Distress Syndrome: Respiratory Distress Syndrome (RDS) is one of the most common causes of morbidity in preterm neonates. It occurs worldwide with a slight male predominance [31]. Patients present shortly after birth with apnea, cyanosis, grunting, inspiratory stridor, nasal flaring, poor feeding, and tachypnea. There may also be intercostal or subcostal retractions. Radiological findings include a diffuse reticulogranular "ground glass" appearance (resulting from alveolar atelectasis) with superimposed air bronchograms [31]. The preterm infant who has RDS has low amounts of surfactant that contains a lower percent of disaturated phosphatidylcholine species, less phosphatidylglycerol, and less of all the surfactant proteins than surfactant from a mature lung. Minimal surface tensions are also higher

for surfactant from preterm than term infants [19]. The diagnosis can be confirmed by biochemical evidence of surfactant deficiency or pathologically. Lungs of infants who have died from RDS show alveolar atelectasis, alveolar and interstitial edema and diffuse hyaline membranes in distorted small airways [45]. Prenatal corticosteroids and postnatal surfactant replacement therapy significantly reduce the incidence, severity and mortality associated with RDS, and surfactant therapy has become the standard of care in management of preterm infants with RDS [46].

(2) Meconium Aspiration Syndrome: Meconium Aspiration Syndrome (MAS) is an important cause of morbidity and mortality from respiratory distress in the perinatal period and affects an estimated 25,000 neonates in the United States each year [47]. Meconium staining of the amniotic fluid or fetus is an indication of fetal distress. Fetal respiration is associated with movement of fluid from the airways out into the amniotic fluid. However, in the presence of fetal distress, gasping may be initiated *in utero* leading to aspiration of amniotic fluid and its contents, which includes meconium, into the large airways [48]. Acute lung injury is characterized by airway obstruction, pneumonitis, pulmonary hypertension, ventilation/perfusion mismatch, acidosis and hypoxemia [49].

The mechanisms underlying surfactant inactivation by meconium are not fully understood, but it has been shown that meconium destroys the fibrillary structure of surfactant and decreases its surface adsorption rate [50]. MAS is associated with an inflammatory response characterized by the presence of elevated cell count and pro-inflammatory cytokines IL-1β, IL-6, and IL-8 as early as in the first 6 hours and significantly decreased by 96 hours of life [49]. Phospholipase-A2, (PLA_2) present in meconium, has been found to inhibit the activity of surfactant *in vitro* in a dose-dependent manner, through the competitive displacement of surfactant from the alveolar film [51]. PLA_2 is also known to induce hydrolysis of DPPC, releasing free fatty acids and lyso-PC which damage the alveolar capillary membrane and induce intrapulmonary sequestration of neutrophils [52]. Exogenous surfactant replacement either as bolus therapy or with a diluted surfactant lung lavage have been shown to reverse the hypoxemia and reduce pneumothoraces caused by meconium aspiration, decrease requirement for extracorporeal membrane oxygenation (ECMO), decrease duration of oxygen therapy and mechanical ventilation, and reduce the duration of hospital stays [47,53]. A comparison of various surfactant treatment regimens in MAS did not find the superiority of one form of therapy over another, and may be related to the heterogeneous nature of this form of lung injury [54]. In an underpowered randomized trial comparing bolus (N=6) versus surfactant lavage (N=7) followed by inhaled nitric oxide, infants receiving surfactant lavage has significant improvements in oxygenation, decreases in mean airway pressure, and arterial-alveolar oxygen tension gradients; however there were no significant differences in duration of assisted ventilation, nitric oxide therapy, or hospitalization[55].

(3) Pulmonary Hemorrhage: Pulmonary hemorrhage may also be associated with Respiratory Distress Syndrome (RDS) and can be difficult to differentiate from it by radiography [46]. It occurs subsequent to a rise in lung capillary pressure due to the effects of hypoxia, volume overload, congestive heart failure, or it may be induced by trauma from mechanical suctioning of the newborn airway. There is a strong association between significant left to right ductal shunting and pulmonary hemorrhage in preterm babies [45,56]. There is a build up of the capillary filtrate in the interstitial space which can then burst through into the airspaces through the pulmonary epithelium. Neutrophils are released following endothelial damage and they, in turn, express proteases, oxygen free-radicals and cytokines. These free oxygen molecules damage the Type II cells that produce SPs, thus inhibiting production of the proteins. Elastase, one of these proteases, damages and degrades SP-A, thereby inhibiting SP-A mediated surfactant lipid aggregation and adsorption *in vitro* [2]. Pulmonary hemorrhage is also considered a rare adverse event associated with surfactant replacement therapy [45,46].

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(4) Acute Respiratory Distress Syndrome: Acute Respiratory Distress Syndrome (ARDS) is a significant cause of morbidity and mortality in all age groups following sepsis, hemorrhage, or other forms of lung injury. It is defined as a severe form of acute lung injury (ALI) and a syndrome of acute pulmonary inflammation. ALI/ARDS is characterized by sudden onset, impaired gas exchange, decreased static compliance, and by a non-hydrostatic pulmonary edema [57].

Infection is the most common cause of development of ARDS in children [58]. The lungs appear particularly vulnerable in the first year of life. Premature neonates with chronic lung disease who develop viral pneumonia, older children with immune deficiency syndromes, and those with childhood malignancies are especially at risk [58].

The hallmark in the pathophysiology of the acute event is an increase in the permeability of the alveolar-capillary barrier as a result of injury to the endothelium and/or alveolar lining cells. Damage to the alveolar Type I cells leads to an influx of protein-rich edema-fluid into the alveoli, as well as decreased fluid clearance from the alveolar space. Neutrophils are attracted into the airways by host bacterial and chemotactic factors and express enzymes and cytokines which further damage the alveolar epithelial cells [2]. Type II epithelial cell injury leads to a decrease in surfactant production, with resultant alveolar collapse.

Four clinical criteria must be met to establish a clinical diagnosis of ARDS: (i) acute disease onset, (ii) bilateral pulmonary infiltrates on chest radiograph, (iii) pulmonary capillary wedge pressure < 18 mmHg or absence of clinical evidence of left atrial hypertension, and (iv), ratio between arterial oxygen partial pressure (PaO2) and the fraction of inspired oxygen (FiO2) $<$ 200 [57]. In contrast, patients that meet the first three criteria, but exhibit a PaO2/FiO2 ratio between 200 and 300, are defined as having ALI.

Despite the introduction of novel treatments, the mortality from ARDS in the pediatric age group still remains high. Attempts to treat ARDS with an SP-C surfactant, Venticute® (Altana Pharma, Germany), were ineffective [59]. However, the use of calfactant (Infasurf®) in younger children with ALI was effective in reducing ventilator days and increasing survival [60].

(5) Pulmonary Alveolar Proteinosis: Pulmonary Alveolar Proteinosis (PAP) is a rare lung disease in which the alveoli fill with PL-rich proteinaceous material. This substance stains for periodic acid-Schiff and is nearly identical to surfactant [61]. PAP occurs in three clinically distinct forms; congenital, secondary and acquired. Congenital PAP is an uncommon cause of respiratory failure in full-term newborns known to be caused by inborn errors of surfactant protein metabolism [62]. Lysinuric protein intolerance has also been implicated as a secondary cause of congenital/infantile PAP [63]. Although the specific cellular pathogenesis is unknown, recent observations in genetically altered mice have led to the speculation that either absolute deficiency of alveolar cells or hypo-responsiveness of the alveolar cells to Granulocyte-Macrophage Colony Stimulating Factor (GM-CS F) is etiologic to PAP [61]. However, the role of GM-CSF in congenital PAP is not clear as antibodies against GM-CSF have not been identified in infants with this condition. The standard of care is the use of whole lung lavage to relieve the symptoms [61]. The prognosis for infants with congenital PAP has been uniformly poor and they die within the first year of life, despite maximal medical therapy [62,64]. However, a recent report also showed successful treatment of congenital PAP with monthly doses of intravenous Immunoglobulin with the patient remaining free of respiratory symptoms for more than 3 years [65].

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(B) Inherited Defects of Surfactant Metabolism

(1) Hereditary SP-B Deficiency: Human SP-B gene (SFTPB) mutations lead to surfactant dysfunction and lethal respiratory distress and was first recognized in term infants with severe respiratory distress after birth [8]. Hereditary SP-B deficiency is inherited as an autosomal recessive condition due to mutation in the SFTPB gene located on chromosome 2. The carrier rate for SFTPB mutation is estimated to be ~1 in 1000 [66]. SFTPB mutations include nonsense, missense, frameshift and splicing defects with the most common being a frameshift mutation (121ins2) occurring in exon 4 has accounted for approximately two thirds of the mutant alleles identified to date [66,67]. Mutations in SFTPB typically result in SP-B mRNA deficiency or the formation of abnormal SP-B proteins, and ultimately respiratory failure in the newborn period [8].

The normal packaging and routing of SP-C is also disturbed by SP-B deficiency, resulting in immature SP-C in the airspaces [68]. Clinical features suggestive of RDS are observed within a few hours of birth. Radiographic findings include alveolar infiltrates and collapse, reticular-granular infiltrates and air bronchograms in term infants with no other underlying cause of respiratory failure [8]. A definitive diagnosis is made by the identification of both mutations in alleles of the SFTPB gene. Most infants die within the first month, despite maximal medical therapy. Surfactant replacement is not effective. Lung transplant has provided relief in some of these patients and it has been found that long-term outcomes after lung transplantation for SP-B–deficient infants are similar to those of infants transplanted for other indications [26].

(2) Hereditary SP-C Associated Disorder: Mutation of the human SP-C gene (SFTPC) resulting in the lack of SP-C are associated with acute and chronic lung disease (CLD) in infants and adults [8]. SP-C deficiency is inherited as an autosomal dominant disorder due to mutation in the SFTPC gene located on the short arm of chromosome 8. The mutation could be familial or *de novo* [69] and is associated with interstitial lung disease and susceptibility to ARDS following lung injury and infection. The pathophysiology of the lung disease caused by SFTPC mutations may involve multiple mechanisms, including both direct toxicity of abnormal proSP-C, and deficiency of mature SP-C [68]. SP-C is derived from a precursor protein, proSP-C. A misfolding leads to formation and accumulation of abnormal proSP-C in the lung tissue and alveolar spaces which interferes with routing and processing of the proSP-C produced from the normal SFTPC allele [69]. Definitive diagnosis can only be made by the identification of a mutation in the SFTPC gene [8]. Variability in disease severity has been observed with SFTPC mutations, including severe cases resulting in death in early infancy and lung transplantation. The mechanisms for this extreme variability are poorly understood. However, considerable allelic heterogeneity exists but there is no obvious correlation between genotype and disease severity [70].

(3) ABCA3 Transporter Gene Mutation: Mutations in the ABCA3 transporter gene have also been identified as a cause of ARDS in infants, and CLD in older individuals [71]. ABCA3 is a member of the ATP-Binding Cassette (ABC) transporter family and is highly expressed in the Type II epithelial cells of the lung, predominantly at the limiting membrane of the lamellar bodies [72]. The human ABCA3 gene spans 80 kb of DNA located on the short arm of chromosome 16 and encodes a 1704 amino acid protein [73]. ABCA3 is critical for the proper formation of lamellar bodies and intracellular lipid homeostasis and over 70 ABCA3 mutations including missense, nonsense, splicesite and frameshift mutations have been identified in association with lethal RDS in newborns and chronic respiratory insufficiency [71]. Lung disease from ABCA3 gene mutation is inherited in an autosomal recessive manner with phenotypic heterogeneity ranging from fatal to milder forms [72]. A history of consanguinity and a family history of fatal neonatal RDS support the likelihood of this form of inheritance [8]. For instance, patients with fatal surfactant deficiency carrying a type I

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homozygous ABCA3 mutation (W1142X/W1142X, L101P/L101P, or L1553P/L1553P) or a type I/type II compound heterozygous mutation (L982P/G1221S or Ins1518/L1580P) die within the neonatal period while patients carrying a type II/type II ABCA3 mutation (E292V/T1114M or E292V/E690K) exhibit pediatric forms of interstitial lung disease suggesting that the type II/type II ABCA3 mutation produces a milder phenotype [71,72]. Mutations in full-term newborns are associated with a defective assembly of lamellar bodies, an abnormal staining pattern of type II pneumocytes for SP-B, and fatal surfactant deficiency [71]. Newborn infants present with grunting, chest retractions and cyanosis followed by rapidly progressive respiratory failure refractory to ventilation and ECMO, and have also presented as persistent pulmonary hypertension of the newborn [74]. Pulmonary opacification, reticular-granular infiltrates and air bronchograms are seen on chest radiographs. ABCA3 (−/−) mice have grossly reduced surfactant phosphatidyl glycerol levels and die of respiratory failure soon after birth [75]. There are no known treatments for lung disease resulting from this mutation and the infants die within the first month of life despite maximal medical therapy.

SUMMARY

An understanding of the complex metabolic process involving phospholipids and surfactant proteins is the key in the management of respiratory failure secondary to defects in surfactant metabolism. The combined use of prenatal corticosteroids and postnatal surfactant replacement therapy can be credited with a dramatic improvement in the outcome of patients with RDS [45]. Lung transplantation has been successful in treating infants with inherited SP-B deficiency and has also afforded the opportunity to investigate surfactant composition and function. Additional experience with infants with inherited mutations in the SP-C gene will help predict the natural history and provide more informed decision-making about lung transplant in these patients [36]. Whole lung lavage is currently the mainstay of treatment in Pulmonary Alveolar Proteinosis [59] and further studies to ascertain the role of SP-B and GM-CSF will help in advancing further ground breaking therapy for this disease. Gene therapy could overcome the limitations of surfactant replacement therapy in inherited defects of surfactant metabolism.

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