

Pulmonary Surfactant-Update on Function, Molecular Biology and Clinical Implications

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Abstract: This review is based on the contents of an international congress entitled, "Surfactant 2004", organized by Drs. B. Lachmann and L.M.G. van Golde and held in Berlin, Germany.

This is the fourth meeting of its kind; the first one was held in 1989. The purpose was to bring together investigators, interested in surfactant research from different disciplines to review progress in basic and clinical sciences, evaluate findings from clinical trials, and build upon the current knowledge to design better clinical trials for the prematurely born infant and other groups of patients, who are identified with surfactant dysfunction, as well as formulate new hypotheses for surfactant investigation both at the basic science and clinical science levels. Although the importance of surfactant in normal lung function was initially appreciated in the case of the prematurely born infant the importance of surfactant throughout life and the roles, especially of the surfactant proteins, not only in surfactant-related activities but also in the innate host defense of the lung has led to a tremendous increase in research activity in the field. The work presented in this meeting is summarized under four general topics: biophysical, innate host defense, surfactant proteins and pulmonary disease, and clinical studies of surfactant.

Keywords: Surfactant proteins, host defense, lung disease, surfactant replacement therapy, polymorphisms.

1. BIOPHYSICAL STUDIES

The classical function of pulmonary surfactant components on the surface of the alveolar lining layer is to lower the surface tension at the air-liquid interface during breathing allowing stable lung volume even at low transpulmonary pressures. This function creates during the first breath and maintains throughout life the tremendous gas exchange area in lungs [1, 2]. Whether or not the surface tension has to reach 0 mN/m for this function remains unclear. Since any interface vanishes at a surface tension of 0 mN/m, it is more likely that surface tensions in lungs oscillate around the equilibrium tension value of 23 mN/m. This view is supported by surface rheology data of surfactant layers [3].

There is no doubt that SP-B is an essential component of pulmonary surfactant. However, the controversial role of SP-B in monolayer refining and the formation of a DPPC enriched layer was discussed. The theory that SP-B facilitates enrichment of DPPC in the surfactant monolayer either by "squeeze-out" of non-DPPC materials from the monolayer or by insertion of DPPC from the subphase into the monolayer is only one way to address SP-B function. An alternative theory has been advanced that SP-B functions by bringing lateral stability to the DPPC-rich monolayer of phospholipids by both electrostatic and hydrophobic

interactions between the SP-B protein, or SP-B-derived peptides, and the phospholipid molecules. Through this stabilization of the phospholipid film, the surfactant acts to maintain alveolar expansion [4].

Several groups reported on structure and morphology of interfacial layers containing pulmonary surfactant components. Time-of-flight secondary ion mass spectrometry is an experimental tool for structure analyses of surfactant films [5, 6]. Using this tool it was demonstrated that deuterated phospholipids do not differ in their interfacial behaviour from non-deuterated species, and SP-B becomes enriched in the interfacial lipid layer at surface pressures above 50 mN/m (which roughly compares with the equilibrium tension). Lipids and SP-B form a three-dimensional network at the interface which, however, might be altered by several subphase properties [7].

Another tool for structure analyses of surface films is scanning force microscopy [8]. Scanning force microscopy shows a non-homogeneous distribution of lipids in the liquid-expanded (LE) phase, in the form of condensed nanodomains, that are not detected by other techniques such as epifluorescence microscopy. SP-B also perturbs the morphology of condensed nanodomains in the LE phase by reducing their size and their interconnectivity. The effect of SP-B was associated with a substantially higher resistance to mechanical deformation and breakage of the films. These results suggest that hydrophobic surfactant proteins alter the structure of surfactant films to optimize film rheological behaviour for the dynamic conditions imposed by the lungs [9, 10].

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Observation by fluorescent confocal microscopy of giant unilamellar vesicles prepared from native surfactant, in the absence of any detergent or organic solvent but in the presence of traces of particular fluorescent lipid probes, detected coexistence of two segregated liquid phases at a range of temperatures including 37°C. The two phases were postulated to be a DPPC- and cholesterol- enriched liquid-ordered phase and a cholesterol-depleted liquid-disordered phase. Coexistence of the two phases was not dependent on the presence of surfactant proteins but was critically dependent on the presence of cholesterol. SP-B, as well as SP-C, distributed exclusively into the liquid-disordered phase. This feature suggests that protein action in native membranes could be dependent on a proper environment regarding lipid composition and protein densities, that has to be considered in order to design new effective surfactant substitutes [11].

Film balance experiments using DPPC/DPPG (80:20) as a lipid matrix with truncated SP-C in various concentrations demonstrated that there was no plateau region in the pressure-area isotherms, which is in contrast to the ternary system containing native SP-C. Native SP-C may feature a different phase behaviour compared to truncated proteins. By means of film balance experiments and using fluorescence light and scanning force microscopy truncated N-terminal forms of SP-C are not able to build up protrusions and to stabilize bilayer formation. Therefore, the C-terminal part of SP-C plays a major role in multilayer formation during the breathing cycle in pulmonary surfactant [12, 13].

SP-C can transform from a monomeric alpha-helix into beta-sheet aggregates, reminiscent of structural changes that are supposed to occur in amyloid fibril formation [14]. The native helical conformation of SP-C is transformed into β sheets depending on the condition of surfactant protein preparation and occurs preferably at high lipid/protein concentration [15]. Surface pressure/area isotherms and surface rheological behavior of pulmonary films differ remarkably depending on the secondary structure of SP-C. The squeeze-out mechanism is altered especially in the presence of β -SP-C. Thus, β -SP-C does not meet the functional demands of the pulmonary surfactant layer in lungs [16].

Functional pulmonary surfactant also plays a role in particle displacement in pulmonary conducting airways [17]. For all sampling sites in the central and peripheral airways and regardless of the particle nature, all particles with 1-6 μm in diameter were found submersed in the aqueous layer or coated with layer material. The particles were in close association with the epithelial cells, which were often indented. Quantitative light microscopic analysis of particle deposition showed that the particles had been immersed into the lining layer after their deposition in the airways. The *in vitro* studies on the wetting and immersion of Teflon particles and puffball spores demonstrated that the surfactant film surface tension had to be lowered to at least 15 mN/m to submerge the particles. These findings give support to the existence of a surface tension gradient from the alveoli to the ciliated airways and finally to the trachea. This gradient is likely important for particle clearance by Marangoni flow from the alveolar region to the ciliated airways [18]. The size of the particles determines uptake rate and cellular distribution [19].

2. INNATE HOST-DEFENSE

A number of papers were presented dealing with the effects of surfactant, particularly SP-A and SP-D, on processes related to host defense function. Most of these reports fall into two broad categories: 1) those demonstrating direct interactions of surfactant components with potentially injurious substances; and 2) those in which surfactant components alter the behavior of immune effector cells of various types.

The binding of the pulmonary collectins (SP-A and SP-D) to various microorganisms has been examined in a number of studies [20, 21]. The list of bound organisms continues to expand and increased attention is being paid to identifying the specific molecules on the pathogen surface that serves as the ligand. SP-D, for example, binds to lipoteichoic acid and peptidoglycan on gram-positive bacteria [22]. SP-D also binds, *via* its carbohydrate recognition domain (CRD), to capsular components of the fungal pathogen *Cryptococcus neoformans* and to the parasite *Schistosoma mansoni*. There are also some species-specific differences in the binding of SP-D to influenza A virus. The pig was proposed as a good model for study of the role of collectins, SP-A and SP-D, in host defense for the following reasons: its similarity to humans, and its importance as a production animal and as a reservoir for zoonotic diseases [23]. In pig SP-D, but not SP-A, was expressed in several extrapulmonary tissues-duodenum, jejunum, colon, trachea, thyroid, liver and skin. SP-D binds to glycosylated proteins (hemagglutinin and neuraminidase) present on the envelope of influenza A virus (IAV) *via* its CRD, and SP-A binds to the sialic acid receptor of IAV *via* its conserved sialylated N-linked oligosaccharide. The porcine SP-D (but not SP-D from other species) has a sialylated, highly heterogeneous N-linked oligosaccharide moiety on its CRD. Porcine SP-A and SP-D exhibit greater inhibitory activity against human, swine, and horse IAV, when compared to collectins from other species. The greater activity of pig SP-D is attributed to its special feature (i.e., the sialylated N-linked oligosaccharide present in its CRD). It is hypothesized that the presence of a sialylated N-linked oligosaccharide in the CRD provides an additional mechanism for IAV attachment. It is believed that pigs exchange IAV strains with humans and birds and may be critical in the emergence of new IAV strains that lead to human IAV pandemics. Therefore, study of pig collectins could be directly applicable to human disease.

SP-A and SP-D bind to various lipids on the surface of *Mycoplasma pneumoniae* [24]. Disaturated phosphatidylglycerols, particularly dipalmitoylphosphatidylglycerol, are bound with high affinity, whereas unsaturated phosphatidylglycerol species appear to be only weak ligands. In some cases where organisms are bound by both collectins it has not been clear which is more important for normal host defense. Studies of influenza A infection in mice [25] with SP-D^{-/-}, SP-A^{-/-}, and SP-AD^{-/-} double knockout mice have demonstrated the key role of SP-D in the clearance of influenza A virus. Although the collectins bind to various pathogen molecules it has also been shown that [26] peptidoglycan and zymosan, both of which are agonists of Toll-like receptor-2 (TLR-2), are not directly bound by SP-

A, but that binding of SP-A to TLR-2 inhibits the cellular response to peptidoglycan and zymosan.

However, collectin binding is not restricted to pathogen-associated molecules. Grass pollen starch granules are bound by collectins [27]. SP-D (but not SP-A) binds the granules avidly, apparently through its CRD. Interestingly, despite the fact that the binding of SP-D to these granules enhanced their uptake by phagocytosis, the increased uptake had little or no effect on the production of TNF- α or nitrite by the macrophages that phagocytosed them.

In addition to binding to pathogens and other foreign matter, SP-D has been shown to bind *via* its CRD to apoptotic cells and DNA originating from either apoptotic or necrotic cells or bacteria [28]. The consequences of this binding are evident in SP-D-/- mice where an excess of apoptotic cells is seen and rescue with exogenous SP-D reduces the number of apoptotic or necrotic alveolar macrophages and a reduction in inflammation lessens the likelihood for anti-DNA antibodies to be formed.

Various surfactant components have modulatory effects on the activity of various immune cells. Although the macrophage and processes related to innate immunity had been the focus of many studies [21, 29, 30], recently surfactant, and collectins in particular, have been shown to influence the behavior of other immune cells, such as mast cells, dendritic cells, and lymphocytes [31]. Both SP-A and SP-D bind to dendritic cells, but while SP-A inhibits their maturation, SP-D has no effect on maturation and enhances the ability of the cells to take up and present antigen, thereby enhancing adaptive immunity.

The presence or absence of collectin can have diverse effects in different animal models [32]. SP-D-/- mice develop emphysema-like pathology in their lungs. A pathologic picture is seen in GM-CSF-/- mice, although there are differences in the proliferation and surfactant metabolism of type II cells between the two types of knockouts. Double knockouts lacking SP-D and GM-CSF had more severe lesions than either of the single gene knockouts. Intratracheal bleomycin, a model of non-infectious inflammatory injury [33] exerts different effects in SP-A and SP-D knockout mice versus wild-type animals. SP-D-/- mice have a more pronounced response and greater mortality following bleomycin treatment suggesting an anti-inflammatory action for SP-D and increased inflammation in its absence. SP-A-/- mice, on the other hand, have a diminished response in the early stages of bleomycin treatment, but a more pronounced response at later stages. These actions suggest that SP-A can either have pro- or anti-inflammatory activity depending on the degree of activation of the tissue. An interesting example of these diverse responses [34] is seen during lung maturation, where accelerated expression of the surfactant proteins is observed in fetal lungs exposed in intraamniotic endotoxin or interleukin-1 and several other agonists of TLR-2 and TLR-3, but not to TNF- α or interferon- γ . The mechanism of these changes is not known but may be due to either direct effects of these agonists on the type II cell or to the modulation of immune cell function that in turn has a regulatory influence on the type II cells.

A number of studies indicate that SP-B may protect the lung from injury [35] and that a member of the signal

transducers and activators of transcription (STATs) family, namely STAT-3 regulates SP-B expression *in vitro* [36] and *in vivo*. Mice with a STAT-3 deletion when exposed to 95% oxygen exhibit rapid and progressive lung injury that includes alveolar capillary leak, increased cytokine production (IL-1, IL-6), and deranged surfactant lipid and protein levels in BAL. SP-B was absent in BAL from STAT-3-/- mice, but exogenous addition of SP-B during hyperoxia improved both survival and lung histology in these mice [37], suggesting a role of SP-B in lung function during hyperoxia.

Although the available literature indicates that surfactant treatment of patients with asthma should have beneficial effects by restoring surface activity and modulating allergic inflammation, recent findings indicate that the allergen-induced pro-inflammatory response was increased following surfactant treatment [38]. Currently, it is unknown whether this finding is specific to a given surfactant preparation. However, these findings point to underlying complexities and to the need for further experimentation, where multiple parameters are considered and whereby the sum of their effects have a beneficial effect on all the deranged disease-specific processes.

3. SURFACTANT PROTEINS AND PULMONARY DISEASE (POLYMORPHISMS/MUTATIONS, AND OTHER DETERMINANTS)

a) Surfactant Protein Variants, RDS, and ARDS

Association of SP-A and SP-B variants with susceptibility to diseases of infants that include respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD) and respiratory syncytial virus (RSV) have been made [39, 40]. Although the SP-A locus is linked to RDS, the tremendous complexity of human SP-A observed at the protein, mRNA, and DNA levels [41] has precluded a full understanding of the mechanisms involved in RDS pathogenesis and pulmonary disease in general.

Humans and primates have two functional SP-A genes whereas all other species studied have a single SP-A gene. The two *in vitro* expressed SP-A gene products differ in the level of their functional abilities in proinflammatory cytokine production [42, 43], phagocytosis [44] and inhibition of surfactant secretion [45]. These also differ in their biochemical [45, 46], biophysical [47], and carbohydrate binding properties [48], as well as in the regulation of their expression [49]. In addition, subtle differences among SP-A1 and SP-A2 variants have been detected. A role of the oligomerization status in the function of SP-A has also been suggested [50, 51]. Further *in vitro* investigation of the SP-A variants, a better understanding of the role of the various cysteines in the degree of oligomerization, the study of human variants in animal models, as well as investigations on the role of epigenetics on SP-A function and expression [52], are key to our better understanding of the significance of the associations made between SP-A variants and disease susceptibility.

SP-B variants, at the single nucleotide polymorphism (SNP) 1580 that either maintains or loses an N-linked glycosylation site [53] and at intron 4 (deletion variants)

have been shown to associate with susceptibility to pulmonary disease. The SNP1580 has been shown to associate with RDS (when present in combination with certain SP-A variants) [54, 55] and ARDS [56], and the SP-B intron 4 with RDS [57], BPD and COPD [58, 59]. Moreover, pro-SP-B (but not pro-SP-C) forms of 25-26 and 19-21 kDa were frequently observed in BAL samples under normal conditions [60]. Currently, it is unknown whether the amounts of the various SP-B forms change in disease states and/or whether these can be used as markers of the SP-B producing epithelial cells in the lung.

The question whether RDS is an inflammatory disease was raised [61]. Following initiation of mechanical ventilation, an increase in neutrophils in lung tissue and a decrease in circulating neutrophils were correlated with the degree of lung edema formation in animal models [62]. Alveolar macrophages (within 4 days) reach maximum levels in airways of patients with RDS, and a 15- and 10-fold increase is observed in the number of CD68 positive macrophages and neutrophils, respectively, in infants who died after birth compared to stillborns of comparable age.

Moreover, the migration of circulating cells into airways requires expression of adhesion molecules and complex interactions between endothelial and inflammatory cells are likely to occur. Infants with RDS with poor response to surfactant therapy were identified with high levels of circulating adhesion molecules. Furthermore, an imbalance in the production of proinflammatory and anti-inflammatory cytokines, and/or between elastase and proteinase inhibitors [63, 64], an increased rate of apoptosis in distal airway cells [65], changes in factors that affect the alveolar-capillary membrane permeability, and several other molecules/processes may contribute to the pathogenesis of RDS. Therefore, given the multifactorial etiology of RDS, different mechanisms may be responsible for RDS pathogenesis in different patient subgroups. Each RDS subgroup may, in turn, be best identified by a different set of markers and may require different therapies. An individualized course of therapy would be the most likely approach in the future.

b) SP-C and Interstitial Lung Disease (ILD)

SP-C mutations have been observed in cases of familial and sporadic interstitial lung disease [66-69] and recently SP-C missense, splice, or frameshift mutations have been identified in several infants with chronic lung disease [70]. SP-C is an integral membrane proprotein of approximately 191 or 197 residues, which is processed within the secretory pathway and specifically within the multivesicular body of the epithelial T2 cell into a 35 residue (residues 24-58 of the proprotein) mature peptide. During the processing step about two thirds of the mature peptide sequence resides within the endoplasmic reticulum (ER) membrane and the remaining in the cytosol side [71]. Although the ER luminal domain (or the C-terminal peptide) of the SP-C proprotein is dispensable for trafficking [72, 73], virtually all known mutations (except the SP-C^{P30L}) are located within the ER luminal domain of the proprotein. This has led to speculation that mutations in the C-terminal peptide lead to protein misfolding, which in turn is toxic to the cell. The available data support this speculation. The mutated allele

appears to exhibit a dominant negative phenotype whereby the levels of the wild type proprotein are significantly reduced [67], suggesting trapping of the wild-type proprotein in the ER *via* oligomerization of the mutant and wild-type followed by degradation [69]. Therefore, SP-C mutations appear to contribute to disease pathogenesis *via* protein misfolding, causing chronic ER stress through the accumulation of SP-C aggregates [74] and inability of the cell to clear these, leading to cell toxicity.

Disease due to protein misfolding is a growing area of research. The mechanisms involved in SP-C-misfolding-mediated disease are not known and thus prevention strategies are currently not possible. However, evidence indicates that the N-terminal 12-residue peptide of the proSP-C intermediate stabilizes the α -helix (residues 9-34) by locking the metastable SP-C polyvaline portion of 16 residues (containing 10-12 valines) in the α -helical conformation [75, 76]. Usually, α -branched amino acids (Val, Ile) are more frequently found in α -strands and less so in α -helices [77]. SP-C exhibits dual conformational preference as it can convert from helical conformation to β -strand aggregates. It is predicted that SP-C can be found in α -helix and β -sheet conformation depending whether there is a lipid or a polar environment [75, 78]. Moreover, SP-C can form amyloid fibrils under certain conditions [14, 79] and proSP-C aggregation has been observed with various SP-C mutations [74]. However, the role of conformational stability or protein misfolding of SP-C in disease pathogenesis remains to be determined. Studies from SP-C knockout mice showed that lung morphological changes started at two weeks of age and progressed to pulmonary disease with characteristics similar to those observed in humans with interstitial lung disease (ILD) [80]. The morphological changes included increased inflammatory and remodeling activity, some airspace loss, and impaired clearance of *P. aeruginosa*, suggesting that inflammatory processes contribute to ILD especially in the presence of SP-C mutations and environmental challenges, such as infection.

4. CLINICAL STUDIES

Clinical studies have extended knowledge of surfactant biology and advanced understanding of the role that exogenous surfactant may play in the treatment of diseases of neonates, children, and adults.

Respiratory Disease of the Newborn

Pulmonary inflammation is a prominent feature of respiratory distress syndrome (RDS) in the preterm infant. The number of neutrophils and macrophages in distal airways increases dramatically in response to a variety of chemotactic and chemokinetic factors. Endothelial cell expression of adhesion molecules is reflected in high plasma concentration of soluble adhesion molecules [63, 64]. Proinflammatory cytokines including tumor necrosis factor- α , IL-1 and IL-6 are found in airway secretions [81], and IL-8 expression in bronchoalveolar epithelium is marked [82]. In contrast, expression of the anti-inflammatory cytokine IL-10 is reduced in the lungs of preterm infants with RDS. Reactive oxygen species and proteinases derived from

neutrophils and macrophages contribute to lung injury as well.

While premature infants are at risk of developing RDS, both premature and term infants are subject to respiratory syncytial virus (RSV) infection. Population studies of nearly 1000 high-risk infants or normal infants of Finnish origin show that allelic variants of surfactant proteins influence the risk of severe respiratory failure in the presence of various environmental risk factors [83, 84]. These variants may influence processing, sorting, or transport of the surfactant protein, such that adequate surfactant pools are not available at birth. Variants of SP-A and SP-D may result in expression of collectins that fail to interact optimally with RSV [39, 85]. Understanding structure-function relationships of these allelic variants may help in prevention or treatment of these neonatal diseases.

To study surfactant synthesis, pool size, and half-life in neonates with severe respiratory insufficiency who require extracorporeal membrane oxygenation (ECMO), patients have been infused with [U - ^{13}C]glucose (to study label incorporation into palmitate groups) or administered intratracheal deuterium-labeled DPPC; PC was subsequently isolated from sequential samples of tracheal aspirate. The fraction of the PC pool synthesized per day averaged 2.4 to 3.3 %/day for infants on ECMO, whereas it averaged 8.0 %/day for control neonates. PC pool size and half-life were not different, suggesting increased catabolism and recycling in patients with severe lung disease [86].

Treatment of RDS with exogenous surfactant is of proven benefit. To explore the efficacy of a synthetic peptide-containing surfactant (Surfaxin) in the treatment of RDS in preterm infants, two international multicenter trials have compared Surfaxin to Exosurf, Curosurf, or Survanta. These trials have shown that Surfaxin is as efficacious as Curosurf in preventing death or development of bronchopulmonary dysplasia by day 28, and that Surfaxin is more effective than Exosurf in preventing development of RDS or RDS-related death [87].

Respiratory Disease of the Child

Analysis of surfactant in lavage fluid recovered from normal children or children with chronic bronchitis, congenital alveolar proteinosis (CAP), or juvenile alveolar proteinosis (PAP) may provide clues to the etiology of these diseases. These analyses show presence of SP-B in all patients except those with CAP, where SP-B is rarely detected. Pro-SP-B forms are frequently detected, and truncated processing products are occasionally identified in disease states. SP-C is readily identified in almost all patients; pro-SP-C forms are not found in normal children, and are seen in patients with CAP with the 121ins2-mutation [60].

The value of surfactant in treating the acute respiratory distress syndrome (ARDS) in children and adults remains uncertain. A randomized, controlled blinded study of calfactant (a natural surfactant preparation) in the treatment of children with ARDS has been performed in which 77 children received calfactant and 75 received placebo treatment. Calfactant treatment was significantly associated

with improvement in oxygenation and decreased mortality (19% vs. 36%) [88].

Treatment with exogenous surfactant is most often accomplished by instilling the surfactant through a catheter to the trachea. An alternative approach is to administer the surfactant as a bronchoalveolar lavage. Six children (3 months to 7 years of age) have recently been treated using Curosurf diluted to a concentration of 8 mg/ml and given in 4 aliquots for a total dose of 25 mg/kg (3.1 ml/kg). Although some required retreatment, all were successfully extubated with good pulmonary outcome [89]. Older children and adults with ARDS secondary to aspiration of gastric contents have been treated with selective lavage of segmental bronchi using a fiberoptic bronchoscope. In one uncontrolled study, 14 patients received five aliquots of 5 to 20 ml (depending on age). Blood gas improvement was observed within one hour of treatment [90].

Acute Respiratory Distress Syndrome of the Child and Adult

Lung surfactant abnormalities are well documented in patients with ALI/ARDS, and include alterations in the phospholipid and surfactant protein composition, a decrease in the fraction of surfactant present in the large aggregate form, and decrease in surface tension lowering ability [91]. The neutral lipid/phospholipid ratio is significantly elevated in patients with ARDS, remains elevated despite administration of exogenous surfactant, and might contribute to impairment of surfactant function [92]. Recognition of these abnormalities has prompted efforts to restore surfactant activity to the lung through administration of exogenous surfactant. Trials of surfactant treatment of ARDS with Surfaxin, a peptide-containing surfactant, and Venticute, a surfactant containing a modified recombinant human SP-C, are currently in progress. Two prior phase III trials of Venticute demonstrated a greater improvement in blood oxygenation with surfactant treatment, but failed to show improvement in survival [93]. A phase III trial of HL10, a natural surfactant containing SP-B and SP-C was recently terminated when no difference in mortality was observed between treated and control patients. All phase III trials completed to date have enrolled patients with ARDS associated with a wide variety of precipitating causes. It may be that only an ARDS patient subset, perhaps just those patients with a specific predisposing cause, will benefit from surfactant treatment.

A role for surfactant in the treatment of neonates at risk for or with established RDS is well accepted. Treatment of ARDS in children appears to also be of significant benefit, although confirmatory studies will be of value. Benefit from surfactant treatment of adults with ARDS is not yet demonstrated, perhaps because of the confounding influence on survival of the many comorbidities present in adults with that syndrome.

Asthma

Patients with disease other than ARDS have also been considered to potentially benefit from surfactant administration. Bronchoalveolar lavage fluid from patients

with asthma has decreased surface tension lowering activity due to inhibition by plasma and secreted proteins, hydrolysis of surfactant phospholipids, and altered synthesis of hydrophobic surfactant proteins [94]. Treatment with exogenous surfactant might restore biophysical activity and might reduce inflammation, as both SP-A and SP-D inhibit eosinophilic inflammation in animal models of asthma and promote a Th-1 response. Clinical investigations of surfactant treatment of patients with asthma are few and provide conflicting results. Investigations of the effect of porcine surfactant on inflammation induced by segmental bronchial lavage with allergen show an actual enhanced response after surfactant pre-treatment [38]. The investigators point out that understanding the sum of effects on surface tension, allergen presentation, and immunomodulation will be critical in reducing the inflammatory and bronchoconstrictive response.

Pulmonary Tuberculosis

In several case-controlled studies, patients with multi-drug resistant pulmonary tuberculosis have been administered 4 or 5 anti-tuberculous drugs and also Surfactant-BL (Biosurf, St. Petersburg, Russia) by inhalation. Investigators report improved conversion of sputum to negative, resolution of pulmonary infiltrates, and reduction or closure of cavities [95, 96].

Interstitial Pulmonary Disease

Mutations in the SP-C gene have recently been described in patients with familial or sporadic interstitial pulmonary disease. Deletion, missense, splice or frameshift mutations have now all been identified in infants with chronic lung disease of unknown etiology [66, 67]. Almost all of these map to the luminal C-terminal portion of the pro-protein and may lead to misfolding and interrupted processing of the pro-protein. The affected allele appears to exert a dominant negative effect, such that mature SP-C may be reduced or absent in individuals with a single affected allele. The misfolded protein may have a cytotoxic effect, leading to cell injury or death, the consequences of which are postulated to contribute to the development of interstitial lung disease.

5. COMMENT

The beginning of surfactant research is dated back to 1929, when Kurt von Neergaard identified surface tension as playing an important role in lung expansion, and investigation of the substance (that later came to be known as surfactant) that modulates surface tension began in the 1950's. The importance of surfactant in normal lung function was readily appreciated, especially in the case of the prematurely born infant. Basic and clinical research led to surfactant replacement therapy in the prematurely born infant and to steroid therapy of mothers threatening to deliver prematurely to accelerate surfactant production in the prematurely born infant. However, although a normally functioning surfactant is essential throughout life, surfactant replacement therapies in patient groups exhibiting surfactant dysfunction show varying results. This may reflect the

phenotypic complexity of the surfactant dysfunction patient compared to the surfactant deficient prematurely born infant and/or the relatively early stages of surfactant replacement studies in patients with surfactant dysfunction. Furthermore, the recognition of the multiple roles the surfactant proteins (SPs) in the last couple of decades, not only in surfactant-related activities but also in the innate host defense of the lung, has increased tremendously the research activity in the field.

The purpose here was to provide the reader with an overview of the Surfactant 2004 conference and surfactant research today. In the interval since a meeting of this type was last summarized [97] many new roles for surfactant have emerged. And while some aspects of surfactant biology are now well understood, many questions remain about its function, its complexity, and its role in lung health and disease.

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