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Review

The Pulmonary Surfactant System: Biochemical and Clinical Aspects

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Abstract. This article starts with a brief account of the history of research on pulmonary surfactant. We will then discuss the morphological aspects and composition of the pulmonary surfactant system. We describe the hydrophilic surfactant proteins A and D and the hydrophobic surfactant proteins B and C, with focus on the crucial roles of these proteins in the dynamics, metabolism, and functions of pulmonary surfactant. Next we discuss the major disorders of the surfactant system. The final part of the review will be focused on the potentials and complications of surfactant therapy in the treatment of some of these disorders. It is our belief that increased knowledge of the surfactant system and its functions will lead to a more optimal composition of the exogenous surfactants and, perhaps, widen their applicability to treatment of surfactant disorders other than neonatal respiratory distress syndrome.

Key words: Surfactant protein—Pulmonary surfactant—Respiratory distress syndrome.

History

Research on surfactant goes back to 1929 when von Neergaard published the first paper about the difference in pressure needed to inflate lungs with air or with liquid [333]. He found that the pressure necessary for filling the lungs with air was higher than when the lungs were filled with liquid. To explain this result he stated that the alveoli were stabilized by lowering the naturally high surface tension of the air/water interface. In 1946 Thannhauser and co-workers reported that lung tissue has a remarkably high content of the lipid dipalmityl lecithin (current name, dipalmitoylphosphatidylcholine)

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Abbreviations: DPPC, dipalmitoylphosphatidylcholine; PG, phosphatidylglycerol; RDS, respiratory distress syndrome; SP-A, SP-B, SP-C, and SP-D, surfactant protein A, B, C, and D; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; CRD, carbohydrate recognition domain; LPS, lipopolysaccharide; HIV, human immunodeficiency virus; ALTE, apparent life-threatening events; SIDS, sudden infant death syndrome; PEEP, positive end-expiratory pressure.

[307]. At that time no connection was made between the high content of this lipid and stabilization of the alveoli. Nine years later, in 1955, Pattle proposed that bubbles, made of lung fluid material, obtained their stability through the quantity and quality of the surface-active material [246]. Subsequently, Clements showed, with the help of a modified surface balance, that surface tension dropped to low values upon compression of surface films from lung extracts [41]. This was followed by a theoretical attempt to clarify the role of surfactant in the structural stability of the lung [42].

It was also the group of Clements which investigated for the first time the surface tension-lowering properties of several lipid fractions. These investigators found that it was the phospholipid fraction that reduced the surface tension and that this reduction was inhibited by other lipid fractions (cholesterol, triacylglycerols, and fatty acids). In the same paper it was reported that the activity of synthetic dipalmitoylphosphatidylcholine (DPPC) was similar to that of phospholipids isolated from fresh beef lung [172]. In the meantime, Avery and Mead showed that the surface tension of lung extracts of infants under 1,100–1,200 g and of those dying with hyaline membrane disease was higher than expected [10]. They associated this with surface-active material deficiency.

In 1967, it was shown that DPPC was produced during the development of the lung and secreted into the alveolar space [93]. A few years later a diagnostic test, using the lecithin/sphingomyelin ratio of amniotic fluid, was developed to determine the maturity of the fetal lung [92]. In 1975 Hallman and co-workers discovered the importance of phosphatidylglycerol (PG) in contributing to surfactant spreading and the decreased levels of this phospholipid in children suffering from respiratory distress syndrome (RDS) [112]. The demonstration of a protein in surfactant was important for the recognition that proteins could be important constituents of surfactant [171]. A landmark was the first successful treatment of neonatal RDS with surfactant replacement therapy [87]. Next, it was recognized that lipid extracts alone were not sufficiently efficient, and attention was focused on the presence and role of proteins in surfactant. This resulted in a rapid extension of research to get insight into the molecular biology, structure, and properties of pulmonary surfactant proteins. In 1988 a new nomenclature for surfactant proteins was proposed: the proteins were termed surfactant protein A, B, and C [257]. Consequently, a newly discovered protein, which is, at least partly, associated with surfactant phospholipids, was named SP-D [250]. Apart from the biophysical role of surfactant, it became clear that surfactant had also a role in lung defense [320].

Although our knowledge of the composition of surfactant and the structure of the surfactant proteins has advanced greatly, the various functions of the surfactant proteins remain incompletely understood. Nonetheless surfactant has been introduced in clinical treatment with much success [278].

Anatomic Aspects of the Lung

The lung is a large organ (6% of the body volume, irrespective of the body weight) with a large inner surface, continuously in contact with the environment. Mammalian lungs are membranous sacs, divided into alveoli, small sacs that vastly increase the surface

area available for gas exchange. Measurement of the surface of the human lung indicates that 1 cm^3 of lung tissue has a total gas exchange surface of 300 cm^2. Because warm blooded animals require a high rate of oxygen uptake, the large surface is essential [275].

Gas exchange in the lung takes place in the alveoli; the bronchi and their branches are only connective tubes. The alveoli are bubble-shaped, and have a high curvature (Fig. 1). Oxygen diffuses from the alveoli to the capillaries, and carbon dioxide leaves the capillaries and diffuses into the alveoli. The surface tension of the moist inner surface originates from the attraction between the molecules in a fluid and is responsible for the tendency to make the bubbles contract and eventually disappear. Without prevention this would result in lung collapse. This tendency is minimized by the presence of a substance that reduces the surface tension on the inner surface of the alveoli to very low values. Although it is sometimes stated that this value is near zero, it is theoretically impossible to eliminate surface tension completely [15, 16]. The surface tension lowering substance, which is found in the lungs of all mammals, is called lung *surfactant.* Clements demonstrated that the tension of a surface film varies with the surface area [41]. Exhalation results in a decreased surface area and a decreased surface tension, whereas a relatively high surface tension is found when the surface area of the lung is large (after inhalation). This mechanism prevents the alveoli from collapsing during expiration.

Composition of Surfactant

Surfactant is produced by the alveolar type II cells in the lung. Two major surfactant pools can be distinguished: an intracellular surfactant compartment and an extracellular surfactant compartment. The intracellular compartment consists of the lamellar bodies in the alveolar type II cells. Their function is storage of surfactant before it is released into the alveolar space [105, 314]. The extracellular surfactant compartment is surfactant that is secreted into the alveolar space. Collection of this surfactant is done easily by bronchoalveolar lavage.

When (extracellular) surfactant from several species is compared, a highly consistent chemical composition is seen [46, 258]. Pulmonary surfactant is composed of two main fractions: lipids and surfactant-specific proteins. Lipids account for approximately 90%, and phospholipids from the bulk of the lipids. Other lipids that are found are: cholesterol, triacylglycerol, and free fatty acids. Phosphatidylcholine (PC) is identified as the most abundant component of surfactant and is always found in a quantity of 70–80% of the total amount of lipid. Approximately 50–70% of PC is saturated, especially in the dipalmitoylated form (DPPC). The anionic PG accounts for approximately 8%. Other lipids are phosphatidylethanolamine (PE, ±5%), phosphatidylinositol (PI, ±3%); and phosphatidylserine (PS), lysophosphatidylcholine, and sphingomyelin in small quantities (less than 2%) [46, 94]. The plasmalogen analog of PC has been identified as an important component in pulmonary surfactant [262]. Cholesterol accounts for 2.4 weight% of the total composition of surfactant [258]. The phospholipid composition of the lamellar bodies is very similar to the composition of the extracellular compartment [3, 146, 239].

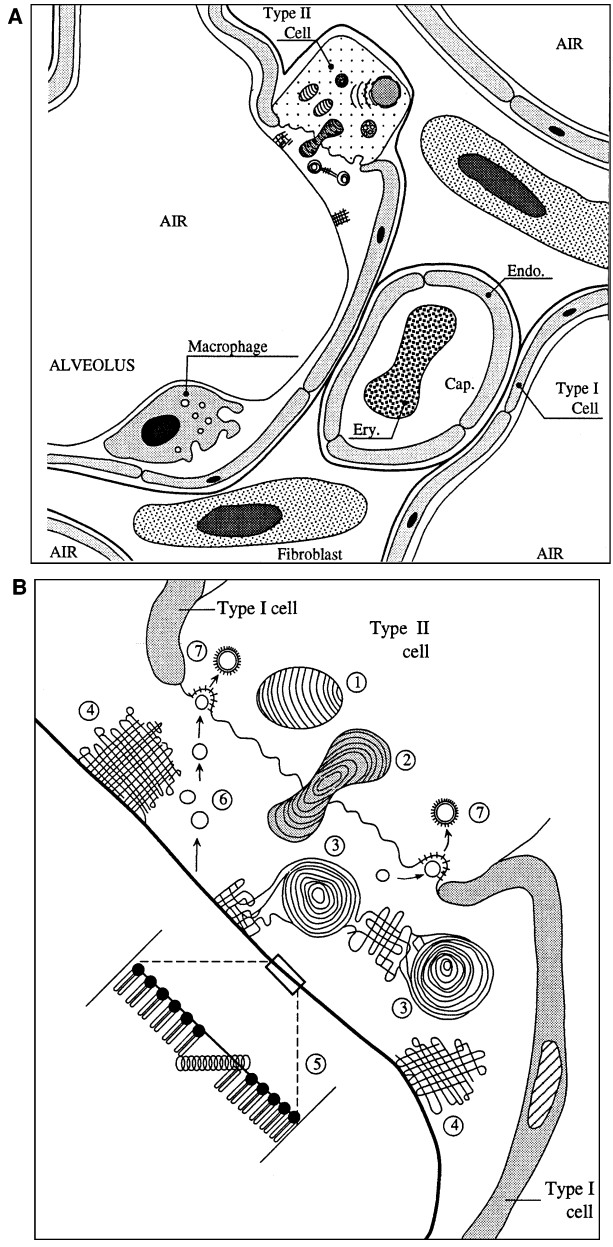

Fig. 1. *A*, schematic drawing of lung tissue. *B*, type II cells produce surfactant, which is stored in lamellar bodies (*1*) and secreted into the alveolar space (*2*). The surfactant is transformed (*3*) into tubular myelin (*4*), from which the monolayer (5) is formed. After the surfactant is used, it is taken up again (*6*) by the type II cells and reused (*7*).

Although most of surfactant consists of lipids, it comprises approximately 10% protein. Four surfactant-associated proteins have been described (for a review; see Ref. 150). These proteins can be divided into two groups: the hydrophilic surfactant proteins SP-A and SP-D, and the hydrophobic surfactant proteins SP-B and SP-C. The surfactant proteins are either exclusively lung associated or predominantly found in the lung. SP-A and SP-D may play important roles in the first line defense against inhaled pathogens, and SP-A may have a regulatory function in the formation of the monolayer that lowers the surface tension. In 1972 King and Clements reported that canine surfactant lipids were able to form stable surface films with low surface tension but that this process was much faster when complete canine surfactant with the proteins included was used [170]. This important observation indicated that the presence of the surfactant-associated proteins was required for an optimal functioning of the lung.

Regulation of Phospholipid Synthesis and Secretion

The lamellar bodies contain all lipid and protein components of surfactant [17, 235] and are secreted into the fluid layer lining the alveoli (Fig. 1). Several factors influence surfactant phospholipid synthesis and secretion (for reviews, see Refs. 19 and 201. Some investigations to determine physiologic and pharmacologic regulation of surfactant secretion have been carried out with the intact lung (whole animal and perfused lung), allowing the involvement of nerve influence, paracrine factors, and physical forces. However, most experiments designed to study regulation of surfactant secretion have been performed with isolated type II cells that had been cultured overnight in the presence of labeled choline. Subsequently, secretion is quantified by measurement of the amount of radioactivity accumulated in lipid extracts of cell media and expressed as the percentage of label secreted. Secretion is stimulated by mechanical stretch and various agents, including agonists for β-adrenergic, purino-, and vasopressin- receptors, and is associated with increased cytosolic Ca^{2+}, cellular cAMP, and activation of protein kinases. The reader is referred to reviews for further information on the regulation of surfactant secretion [37, 201, 349]. Interestingly, the composition of surfactant phospholipids can be influenced by factors such as diet [21, 241], age [232], and physical effort [66, 237].

Extracellular Surfactant Metabolism

After secretion, surfactant is transformed into specific structures, called *tubular myelin*, from which insertion of phospholipids into an air-liquid interface is thought to take place (Fig. 1). The thickness of the alveolar lining liquid layer in the rat lung is 0.24 μm, with a variation of 25 nm to some micrometers [18]. The phospholipid molecules are found with their hydrophobic fatty acid chains up in the air and their (polar) headgroups in the subphase. The fatty acid chains are tilted at an angle of 21.5 to 29°, depending on the relative humidity [162]. Surfactant phospholipids form stable surface films with low surface tension upon compression; adsorption of phospholipids from the subphase into the surface film is highly accelerated when hydrophobic surfactant proteins are present [121]. Phospholipid adsorption is required to ensure molecular

occupation of the air-water interface during inflation of the lung. Not only is the formation of the monolayer stimulated by the hydrophobic proteins, but it has been reported that SP-B alone may also reduce the surface tension by increasing the lateral stability of the phospholipid layer [43]. The composition of the monolayer is also an important factor in the adsorption of the surface-active material into the monolayer [234, 355].

During expiration the surface tension at the air-water interface of the lung is reduced. To reach a low surface tension, the monolayer becomes enriched in DPPC. This process may occur either by selective insertion of DPPC during adsorption or by selective exclusion of other components of the surface film during reduction of the surface area. Evidence of the latter possibility, which results in the formation of different types of remnants, has been provided [245, 276]. During the next inhalation, and expansion of the surface area of the alveoli, the hydrophobic surfactant proteins improve the respreading of lipids [234, 303]. During this process surfactant components are lost from the interface and taken back into the type II cell for recycling.

Hydrophilic Surfactant Proteins

Two hydrophilic surfactant proteins have been isolated, SP-A and SP-D. These two proteins are related and belong to a subgroup of mammalian lectins called *collectins* (or C-type lectins, group III). This is a group of soluble proteins which consists of oligomers with COOH-terminal carbohydrate recognition domains in association with NH_2-terminal collagen-like domains. The collectins can be divided into a group wtih a bouquet form (mannan-binding protein, SP-A) and a group with a cruciform shape (conglutinin, SP-D) [69, 130]. SP-A and SP-D may be involved in the first-line defense system of the lung [313].

Structure of SP-A

The predominant surfactant-associated protein is the large and complex glycoprotein SP-A (Fig. 2). Almost all of the protein in bronchoalveolar lavage is found associated with surfactant lipids. SP-A was the first of the surfactant proteins that was purified [171] and analyzed for its primary structure [343]. Butanol extraction is a widely used method to purify SP-A, but recent work from our laboratory suggests that some of the functional characteristics of SP-A are lost during this extraction procedure [317]. The molecular mass of the monomeric form is 28–36 kDa, and human SP-A comprises 248 amino acid residues [341]. When comparing the primary structure of SP-A from several species (human, dog, rabbit, rat, mouse), the homology is striking [26, 29, 175, 273, 343]. The primary structure of SP-A comprises four domains: an amino-terminal domain, a collagenous domain, a neck domain, and a carbohydrate recognition domain (CRD). The amino-terminal segment of secreted SP-A is a short peptide of 7 amino acids, with a cysteine residue at position 6 which forms an interchain disulfide bond. This cysteine may help to align SP-A subunits during assembly of the mature oligomers. In some species (e.g. rat, dog)

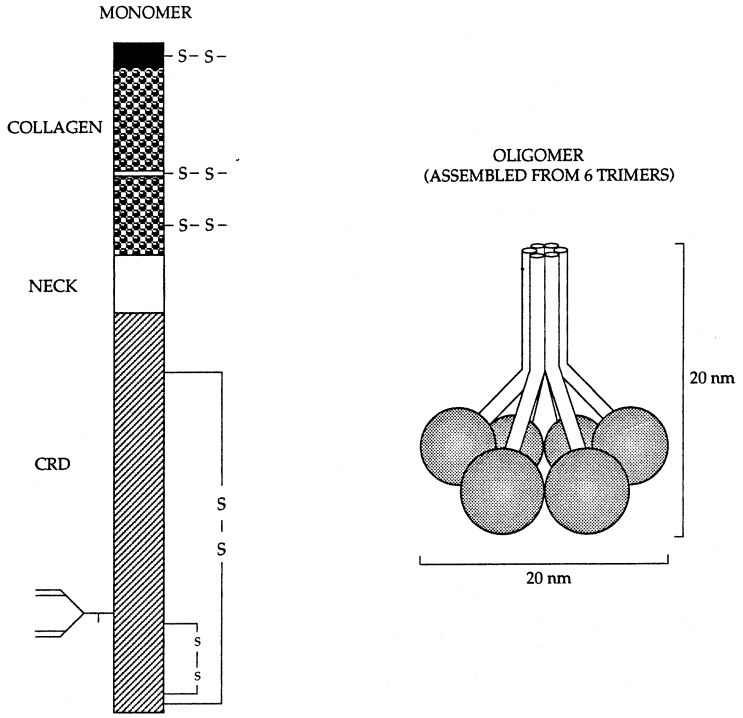

Fig. 2. Structure of SP-A.

a potential glycosylation site has been detected in this area, whereas in other species (human, rabbit) no glycosylation site is present.

The next part of SP-A is a 73-amino acid collagen-like segment, consisting of 23 repeating tripeptides with the sequence glycine-Xaa-Yaa (in 13 of the 24 triplets, Yaa is hydroxyproline), only interrupted between the 13th and the 14th Gly-Xaa-Yaa triplet as a result of a proline residue, and the substitution of a cysteine for a glycine in the triplet sequence [119]. This region is folded as a triple helix involving three highly homologous subunits. Six of these triple helices are assembled into a bundle of 18 monomers of SP-A. Electron microscopic images of SP-A obtained after rotary shadowing indicate that this region of SP-A is organized into a rod-like structure of approximately 20 nm. The interruption in the collagen-like repeating sequence after the 13th triplet introduces a flexible kink in the collagen rod. After this interruption, the trimers are no longer bundled, but they bend outward from the central axis into six directions [338]. The carboxyl-terminal region (divided into a neck region and the CRD) is composed of 148-residues, forming a C-type lectin domain [67, 68].

The neck region may be involved in phospholipid binding [269], although this domain cannot account for all the lipid binding activity of SP-A [230]. Epitope mapping indicated that the CRD is also involved in lipid binding of SP-A [183], and especially the region Glu^{202} to Met^{207} is important for expression of the biologic activities [127]. The CRD contains a Ca^{2+}-dependent specific carbohydrate binding site

Table 1. Putative functions of SP-A

• Formation of tubular myelin
• Regulation of phospholipid insertion into the monolayer
• Modulation of uptake and secretion of phospholipids by type II cells
• Activation of alveolar macrophages
• Binding and clearance of bacteria
• Binding and clearance of viruses
• Chemotactic stimulation of alveolar macrophages

[103, 118]. The positions of the four cysteine residues in this region are conserved in all members of the class of calcium-dependent lectins. Disulfide bonds have been described between residue 135 and 226, and residue 204 and 218 [106], and their function is probably stabilization of the structure. The CRD is glycosylated at position 187. The carbohydrate moiety may be involved in lipid aggregation [102] and virus recognition [25, 319].

Properties of SP-A

SP-A was the first surfactant-associated protein discovered, and the properties and putative functions of SP-A (Table 1) were studied more extensively than those of the other surfactant proteins. Obviously, SP-A is not directly responsible for the surface tension lowering properties of pulmonary surfactant, although SP-A has possibly a regulating role [121, 276]. The fact that excess SP-A could be detected in tracheal and bronchial glands and in the epithelium of conducting airways [169] also suggests the importance of non-surfactant-associated functions of SP-A and contributes to the proposed role of SP-A in the host defense. SP-A (and SP-D) may even have a function in the amniotic fluid in the antibody-independent recognition and clearance of pathogens [212].

Formation of Tubular Myelin

In bronchoalveolar lavage, surfactant exists as various morphologically different complexes. Pulmonary surfactant is transformed into tubular myelin after the secretion of lamellar bodies into the fluid layer, which lines the alveolar space (Fig. 1). In tubular myelin, SP-A is localized at the corners of the tubular myelin lattice [334]. The phospholipids and proteins are thought to be stored extracellularly in this structure before they are used to incorporate phospholipids into the monolayer that lines the alveoli [94]. By in vitro reconstitution, it became clear that SP-A is essential for the formation of this lattice [259, 301, 347]. SP-A aggregates lipid vesicles in a calcium-dependent manner [104, 120]. SP-A-induced aggregation is dependent on an intact collagenous domain [269]. At physiologic extracellular Ca^{2+} concentrations SP-A shows self-aggregation [104]. These interactions and radial SP-A–SP-A interactions via the CRD and oligosaccharide moiety may be important for the SP-A-induced formation of tubular myelin [102]. The formation of large membrane structures could

be important to protect surfactant from inactivation by serum proteins. In line with this notion is the observation by Cockshutt and co-workers that SP-A reverses inhibition of the surface activity of lipid extract surfactant by serum proteins in vitro [47]. In lungs of patients with RDS a lack in tubular myelin is found together with a shortage of SP-A, supporting the importance of SP-A in tubular myelin formation [58].

Regulation of Phospholipid Insertion into the Monolayer

SP-A is also considered to play a biophysical role as a regulator of phospholipid insertion into the monolayer. This function is probably related to tubular myelin formation. The addition of SP-A to hydrophobic surfactant components leads to an enhanced phospholipid adsorption in vitro [39, 121, 276]. SP-A is able to bind to phospholipids in a calcium-independent way. Lipid binding requires the lipids to be in the gel phase [35]. In addition, SP-A has a high affinity for DPPC as was determined by binding studies on thin layer chromatography plates [181] and by fluorescence studies [35]. These properties may be important for enriching the surface film with DPPC during hydrophobic surfactant protein-induced insertion of phospholipids into the monolayer.

Modulation of Phospholipid Uptake and Secretion

Another physiologic function of SP-A may be the regulation of surfactant homeostasis [341, 349]. SP-A binds specifically to type II cells [182, 348] and inhibits secretion of labeled PC from these cells [65, 264]. It has been shown that the carboxyl-terminal domain of SP-A is responsible for the binding to type II cells, thereby regulating phospholipid secretion [230]. Results, mainly from studies with isolated type II cells, suggest that the removal of phospholipids from the alveoli by alveolar pneumocytes may be enhanced by SP-A [20, 308, 350, 354]. Several type II cell molecules have been described which bind SP-A, but so far none of these molecules was shown to be a functional receptor. Local concentration-dependent uptake rather than SP-A receptor-mediated endocytosis could be the explantation for the effects of SP-A on lipid uptake [101]. Part of the clearance of lipids is done by alveolar macrophages, and this process is also enhanced by SP-A [352].

Activation of Alveolar Macrophages

Human SP-A, purified from the lavage of alveolar proteinosis patients, enhances the lucigenin-dependent chemiluminescence response by rat alveolar macrophages [320]. In addition, the chemiluminescence response induced by rat surfactant can be abolished by antibodies against SP-A. These observations indicate that SP-A may also induce killing of microorganisms. The SP-A-induced stimulation of superoxide radical production is not observed with peritoneal macrophages, polymorphonuclear leukocytes, or monocytes [320]. SP-A surface interactions are required to release oxygen radicals from alveolar macrophages in vitro [342].

Clearance of Bacteria

Drickamer and co-workers described a sequence similarity between SP-A and mannose-binding proteins and suggested that SP-A could also have carbohydrate binding properties [68]. Shortly afterward, calcium-dependent binding of SP-A to monosaccharides was described [103]. Ba^{2+}, Sr^{2+}, and Mn^{2+}, but not Mg^{2+}, could also substitute for Ca^{2+}. As each human SP-A monomer binds two to three calcium ions, an assembled SP-A molecule binds 36–54 calcium ions [104]. It was proposed that SP-A may play a role in the lung defense [103]. Two reasons supported this notion: SP-A is able to bind carbohydrates, and SP-A is structurally similar to C1q. Evidence was found that SP-A potentiates the antibacterial functions of the alveolar macrophages but not of peritoneal macrophages, polymorphonuclear leukocytes, or monocytes [306, 320]. This is possible because SP-A can bind both bacterial components and alveolar macrophages. SP-A recognizes and binds endotoxin (also known as lipopolysaccharides or LPS) on the membrane of Gram-negative bacteria [157, 315]. The lipid A region of LPS has been implicated in the calcium-dependent binding of SP-A to Gram-negative bacteria [315]. The opsonization of bacteria is selective; e.g. *Staphylococcus aureus* is opsonized, but *Streptococcus pneumoniae* is not [206]. Recently, it has been shown that only rough LPS-containing bacteria are opsonized [254]. The killing of *S. aureus* is mediated by the binding of SP-A to the C1q receptor of monocytes [90]. Apart from opsonization, SP-A is also able to aggregate type A, but not type B, *Hemophilus influenzae* [206].

The growth of group B streptococs, intratracheally inoculated, was mitigated by treatment with surfactant devoid of SP-A [124, 285], indicating that other surfactant components also have bactericidal activity. The presence of SP-A potentiates the antibacterial functions of alveolar macrophages [177] by modulating the immune cell function in the lung by regulating the cytokine production and immunoglobulin secretion [176].

Clearance of Viruses

SP-A has also been reported to act as an opsonin in the phagocytosis of herpes simplex virus type 1 by rat alveolar macrophages [318]. Compared with the opsonic capacity of serum, SP-A was found to be twice as potent. SP-A binds herpes simplex virus, as was shown indirectly by the increased binding to virus-infected cells expressing viral proteins at the cell surface [319]. Binding of SP-A to infected cells is inhibited by heparin, but not by yeast mannan. Interestingly, deglycosylated SP-A, obtained by digestion with *N*-glycosidase F, did not bind to infected cells. These observations suggest that the carbohydrate moiety of SP-A is involved in recognition of viruses [319]. The carbohydrate moiety is not required for macrophage stimulation. Benne and co-workers found recently that the carbohydrate moiety of SP-A is also involved in virus neutralization. Infection of LL-C MK2- cells with influenza A (H3N2) virus was prevented by preincubation of the virus with SP-A. Viral infectivity was measured by the appearance of viral proteins on the cell surface. After removal of the carbohydrate moiety of SP-A by enzymatic digestion with *N*-glucosidase F, SP-A no longer prevented viral infection

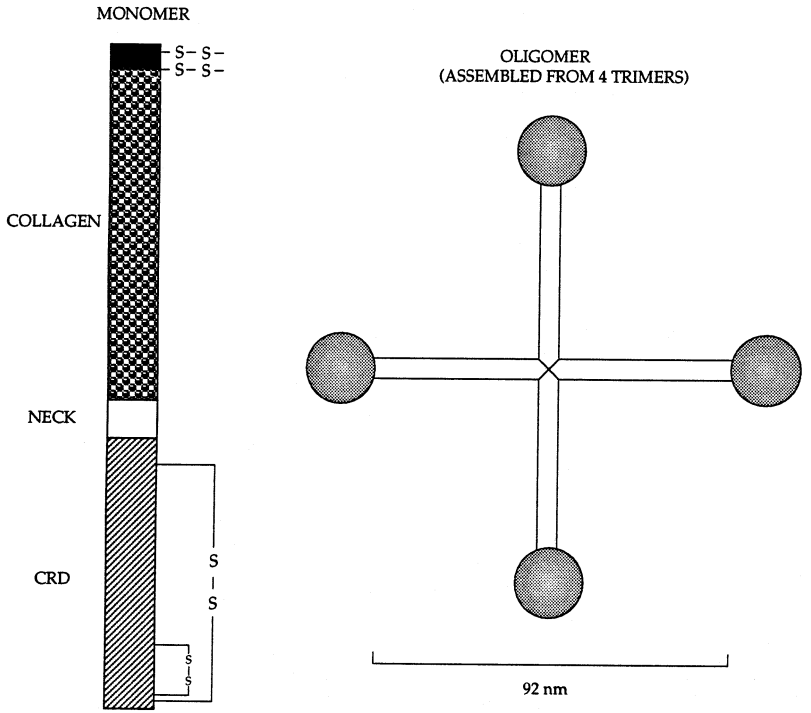

Fig. 3. Structure of SP-D.

of the cells. It was shown that SP-A binds to influenza A virus via its sialic acid residues and thereby neutralizes the virus [25]. SP-A may bind influenza virus partly via interaction with neuraminidase [200].

Stimulation of Alveolar Macrophage Chemotaxis

Wright and Youmans reported that SP-A stimulated alveolar macrophage migration. As the migration is directed into one specific direction, it is called chemotaxis [351]. This mechanism may contribute to the direct attack of the invaded microorganisms.

Structure of SP-D

The other hydrophilic collagenous glycoprotein found in bronchoalveolar lavage is SP-D (Fig. 3) [251]. It may be argued that SP-D is not a true surfactant protein. Only a small part of SP-D (less than 10%) is associated with surfactant phospholipids [250], and the production of SP-D is not exclusively in the lung; SP-D mRNA is also found in gastric tissue [83]. The mature human SP-D polypeptide chain contains 355 amino acid residues, and the molecular mass of this protein is 43 kDa under reducing conditions [198]. SP-D has many structural characteristics in common with other C-type

Table 2. Putative functions of SP-D

- Activation of alveolar macrophages
- Agglutination of bacteria
- Protection against nonbacterial microorganisms and viruses
- Role in phosphatidylinositol metabolism

lectins such as SP-A and conglutinin. The nucleotide sequence of SP-D contains 87% nucleotides in positions similar to those of bovine conglutinin [192]. Like SP-A, the monomeric subunit of SP-D consists of four regions: a short amino-terminal sequence, a collagen domain that comprises 59 Gly-Xaa-Yaa repeats, a short neck region, and the carboxyl-terminal CRD. The collagen domain of SP-D is larger than that of SP-A (59 Gly-Xaa-Yaa repeats vs 24 Gly-Xaa-Yaa repeats, respectively) [199]. A second difference is that the collagen domain of SP-D is very regular, without the interruption caused by an extra proline residue, as is found in SP-A. This results in a stretched structure without a bend [99]. Collagen triple helices can cluster in a tail-to-tail conformation, forming dimers/trimers/tetramers of collagenous chains. A tetramer consists of 12 polypeptide chains and has a molecular mass of 630 kDa under nondissociating conditions. Electromicroscopy reveals a highly homogenous quaternary structure of SP-D in the form of a cross, which is very similar to conglutinin. From the central point (hub), four identical rod arms of 46 nm emanate and end in a globular terminal expansion, consisting of the CRD of three SP-D molecules [53, 198].

Properties of SP-D

SP-D does not seem to have a role in the classical function of surfactant. Most putative functions described so far are related to lung defense (Table 2).

Binding of Bacteria and Activation of Alveolar Macrophages

SP-D is a calcium-dependent lectin-like protein that associates with carbohydrates; it binds especially to α-glucosyl residues [249]. It has been demonstrated that SP-D binds to LPS of several bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella paratyphi*, and *Pseudomonas aeruginosa*), but not to Gram-positive *S. aureus* [179, 190]. SP-D can also bind with a high affinity to alveolar macrophages, and it induces production of oxygen radicals by alveolar macrophages [212, 316]. The binding of SP-D to both bacteria and alveolar macrophages and the subsequent induction of oxygen radicals could be very important in lung defense. SP-D may also scavenge free LPS (endotoxin). This would prevent the binding of LPS to granulocytes and would consequently protect against septic shock.

Bacterial Agglutination

SP-D has an ideal shape for agglutination reactions. The four clusters of CRDs at the end of the arms span a long distance, a feature that may be important for the agglu-

tination of microorganisms [179]. Agglutinated bacteria may be cleared more rapidly from the lung via mucociliary transport.

Protection against Nonbacterial Microorganisms and Viruses

Recently, evidence was provided that SP-D may have a role in the protection against nonbacterial microorganisms and viruses. In a patient suffering from human immunodeficiency virus (HIV), abnormalities were found in the pulmonary surfactant. The development of *Pneumocystis carinii* enhances these abnormalities [78]. During *P. carinii* pneumonia, SP-D accumulates in the lung [191], interacts with gpA (the major surface antigen of *P. carinii*), and augments the binding of *P. carinii* to alveolar macrophages [227]. SP-D interacts with the mannose-rich antigen gp120, which modulates an interaction with the alveolar macrophages. In this way SP-D acts as an opsonin. SP-D may also protect against viruses such as influenza A by binding to the virus, and SP-D is even ten times more effective in inhibiting hemagglutination [115].

Phosphatidylinositol Binding

A striking finding is that SP-D can bind PI in a calcium-dependent way [228, 252]. In fact, this is the only known interaction of this surfactant protein and phospholipids. The importance of the interaction between this acidic phospholipid and SP-D regarding homeostasis and metabolism is not clear. Only about 3% of the phospholipids in pulmonary surfactant is PI. Studies done with chimeras of SP-A and SP-D identify the CRDs as essential for interaction with phospholipids [230]. The physiologic significance of binding to PI remains a mystery, but it is conceivable that SP-D may play a role in intracellular lipid sorting or signal transduction.

Hydrophobic Surfactant Proteins

Phizackerley and co-workers were the first to describe the presence of hydrophobic surfactant proteins [253]. Two hydrophobic surfactant proteins are known: SP-B and SP-C. These proteins are soluble in organic solvents such as chloroform/methanol or acetonitrile/water mixtures [247]. Both proteins are secreted by the alveolar type II cells and require specialized intracellular processing events to produce their mature forms [22, 23, 335, 336] because of the extremely hydrophobic nature of these proteins.

Structure of SP-B

SP-B is a small hydrophobic protein of 79 amino acid residues (Fig. 4), known for its high cysteine content [56]. In the species for which the sequence has been described, the primary structure (and especially the positions of the cysteine residues) is conserved (±80% of the mature protein). The cysteine residues form a unique disulfide pattern of three intramolecular bonds and one intermolecular disulfide bond, which stabilize the

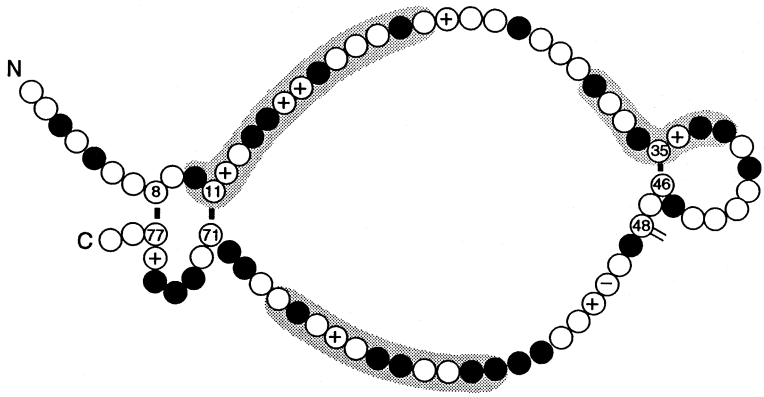

Fig. 4. Structure of SP-B.

protein and produce a dimeric form of SP-B [149, 152]. Mature SP-B contains a small disulfide loop within a larger loop. The secondary structure of SP-B is mainly α-helical [32, 215, 322]. The helices have an amphipathic character.

Properties of SP-B

Promotion of Phospholipid Insertion into the Air-Liquid Interface

Definitely the most important property of SP-B is to enhance the biophysical properties of surfactant lipids (Table 3). A rapid insertion of phospholipids into the air-liquid interface is obligatory for the maintenance of alveoli integrity. SP-B greatly enhances the formation of a stable surface film by inducing the insertion of phospholipids into the monolayer [121, 174, 233, 234]. The positive charges of the protein are essential for the activity of the protein [43], and the interaction with (negatively charged) PG enhances phospholipid adsorption [34, 355, 356]. During expiration, the surface area is decreased, and hence the surface pressure in the monolayer is increased. Experiments with positively charged peptides resembling fragments of SP-B showed an increase of collapse pressure of palmitic acid up to 70 mN/m [195]. At a surface pressure higher than 40–45 mN/m, SP-B is squeezed out of the monolayer, together with two or three phospholipid molecules per SP-B dimer [304]. Later, during expansion, a new cycle is started by SP-B-catalyzed insertion of phospholipids into the monolayer [165]. During this process part of SP-B may be degraded as was shown in vitro by continuous alteration of the surface area [325].

In vivo experiments in preterm rabbits [265] and selective blocking of SP-B [267] did confirm the significance of this protein. Recently, Nogee and co-workers described a frame shift mutation in the SP-B cDNA [223], which resulted in children unable to produce SP-B. They were suffering from severe respiratory failure, eventually leading to death [222].

Table 3. Putative functions of SP-B

• Promotion of rapid phospholipid insertion into air-liquid interface
• Formation of tubular myelin
• Influence on molecular ordering of phospholipid layer

Formation of Tubular Myelin

SP-B is, together with SP-A, necessary for the formation of tubular myelin structures [259, 301, 347]. SP-B is able to induce the calcium-dependent fusion of membranes [236, 259]. In SP-B-deficiency an abundance of alveolar concentric multilamellar structures is found, but no tubular myelin [60]. It is hypothesized that SP-B induces the formation of contact sites between bilayers in tubular myelin which enable flow of lipids from the outer leaflet of a bilayer to the adjacent bilayer (or monolayer).

The activity of surfactant in lowering the surface tension is reduced by serum proteins. Surfactant inactivation by serum is reduced by synthetically produced SP-B [5]. This may be explained by the fact that lipids in large membrane structures, like tubular myelin, are more protected from exogenous factors that could impair surface activity.

Molecular Ordering of the Phospholipid Layer

The addition of SP-B increases the inter- and intramolecular ordering of bilayer membranes [43, 330], especially under the gel to fluid phase transition temperature. This ordering is possibly the result of a specific interaction of the positively charged SP-B with the PG headgroup [12, 330, 356]. One monomeric SP-B molecule influences 50–70 molecules of phospholipid [286]. It has been suggested that SP-B reduces the surface tension by an increase of the lateral stability of the phospholipid layer [43]. In contrast, Vincent and co-workers found that synthetic peptide fragments, which resemble SP-B, increase the lipid disorder. Dynamic bilayer microheterogeneities caused by the interactions of SP-B and PG may be essential for pulmonary mechanics [331, 356]. For more information regarding protein-lipid interactions and biophysical properties of surfactant the reader is referred to other review articles [100, 165].

Structure of SP-C

The second member of the group of hydrophobic surfactant proteins is SP-C (Fig. 5). The unique properties and metabolism of this protein have recently been reviewed [24]. This small protein of only 35 amino acid residues is only soluble in organic compounds such as chloroform or 80% acetonitrile in water [247]. The protein is extremely hydrophobic, and is characterized by a high content of valine residues. Two thirds of the protein consists of a continuous hydrophobic stretch, and the secondary structure of this part of the protein is a regular α-helix [154, 244, 286] which is able to span a DPPC bilayer [216]. It has been shown that the long axis of the α-helix is oriented parallel to

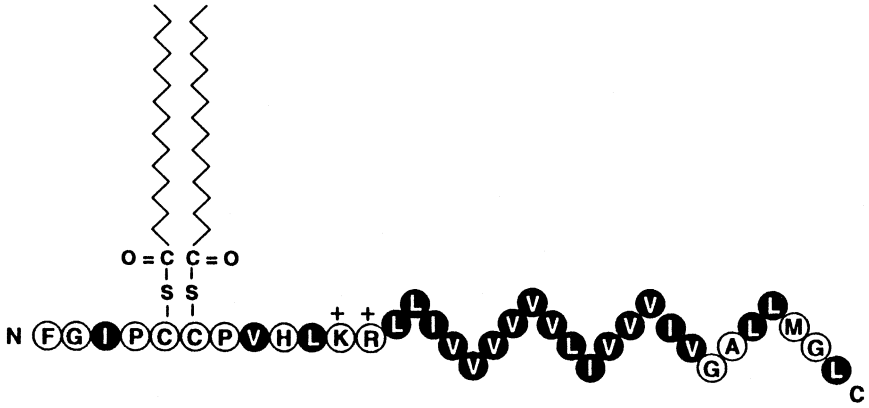

Fig. 5. Structure of human SP-C.

the lipid acyl chains [323]. Palmitoylation of the two cysteine residues adds to the hydrophobic character of the protein [57]. Canine SP-C contains only one (palmitoylated) cysteine residue [153]. The palmitoyl chains are linked to the cysteines with a thioester [296]. The function of the acylation is not clear, but it is speculated that palmitoylation leads to a better binding of a protein to a membrane [50, 197], influences the conformation and orientation of peptides [161], or plays a role in membrane fusion [220]. Positively charged lysine and arginine residues are found at positions 11 and 12, respectively. These positive charges are important for the binding of the protein to negatively charged phospholipids [49]. Both monomeric and dimeric forms of SP-C are found, but the function of the two forms remains to be clarified. Dimeric proteins form structures that may have dynamic properties that are different from single chain surfactants [158]. In bovine SP-C, the dimeric form appears to have a secondary structure that is almost exclusively β-sheet [13]. However, the dimeric form of canine SP-C is mainly α-helical [51]. Recently, it was shown that the secondary structure of SP-C depends on the solvent in which the protein is dissolved. When SP-C is allowed to form protein-protein interactions, mainly β-sheet is formed [54]. SP-C gradually self-associates when present in a mixture resembling pulmonary surfactant, even at a temperature below 38°C [135]. In the species analyzed (rat [82], human, porcine [57, 151] a marked conservation in primary structure of SP-C exists, which implies a strong evolutionary pressure [117].

Properties of SP-C

Promotion of Phospholipid Insertion into the Air-Liquid Monolayer

SP-C is able to stimulate insertion of phospholipids out of a subphase into the air-liquid interface in a calcium-dependent way (Table 4) [233, 302]. This process is preceded by the SP-C-dependent binding of phospholipid vesicles to the monolayer [234]. It is likely that SP-C is present in the monolayer but at high pressures (higher than 55

Table 4. Putative functions of SP-C

• Promotion of rapid phospholipid insertion into air-liquid interface
• Regulation of phospholipid ordering

mN/m) SP-C is squeezed out [166]. When SP-C is squeezed out, eight to ten PC molecules/molecule of SP-C accompany the protein. This raises the possibility for SP-C to modify the composition of the monolayer [305].

Ordering of Phospholipids

In mixtures of SP-C and phospholipids, the protein alters the arrangement of the lipid bilayers [347] and the packing of phospholipids in monolayers [248]. One SP-C molecule binds 20–35 lipid molecules [286, 289]. Incorporation of SP-C into a phospholipid bilayer increases the phospholipid ordering parameter, and thereby it may increase the lateral pressure within the bilayer [136]. SP-C causes an increase in the limiting anisotropy in both the gel and liquid crystal phases [75]. In contrast, SP-C appears to disrupt the lipid structure in its immediate vicinity, whereas SP-B lacks this quality [137]. SP-C (and cholesterol) can increase the miscibility of PC and PG mixtures [290]. SP-C is not able to induce lipid mixing of vesicles, unless (part of) the vesicles lacks anionic lipids [236]. Interestingly, SP-C, which lacks most of its positively charged residues, is able to induce lipid mixing of vesicles even in the presence of negatively charged lipids [49].

The presence of serum proteins reduces surfactant activity. Surfactant proteins, especially SP-C, may be a target of serum proteins [281]. It was discussed previously that SP-A and SP-B could protect surfactant inactivation to a certain extent. Excess SP-C may also prevent surfactant inactivation. The negative effect of serum constituents on surfactant activity could be reduced by (synthetically) produced SP-C in the presence of calcium [5, 6]. In a preliminary study, it has been reported that SP-C is capable—in the presence of calcium ions—of enhancing the lipid aggregation caused by SP-A [36]. Protein-protein interactions in surfactant have not been studied extensively, although these interactions may turn out to be essential for proper surfactant function.

Disorders of Surfactant

Surfactant consists of a complex mixture that is impaired in several diseases. This was noticed for the first time by Avery and Mead, who described that a shortage of surface active material leads to a higher surface tension at the air-liquid interface in the lungs in neonatal RDS [10]. Measurement of pulmonary compliance and the gestational age generates the highest accuracy in predicting the appearance of RDS [28, 288]. The main cause of RDS is a shortage of surfactant, and leakage of serum proteins to the alveolar space probably contributes to the disease. Lungs of infants dying from RDS contain all normal components except tubular myelin [58]. As SP-A and SP-B are

essential for the formation of tubular myelin, this could indicate that one or both of these proteins are nonfunctional or missing. This was confirmed by a study showing that neonates seem to have an immature SP-A metabolism [217].

In 1993, a pulmonary SP-B deficiency was described (named *congenital alveolar proteinosis*), originating from a deficiency of SP-B mRNA [222]. By determination of the sequence of the SP-B transcript in affected children, it was discovered that a frame shift mutation is responsible for this disease [59, 223]. In mice it has been demonstrated that only the animals homozygous for this allele were affected [40]. Interestingly, to date, six different mutations in this gene were identified [345]. The SP-B deficiency is associated with SP-A and SP-C abnormalities. Ultrastructural abnormalities, such as a reduced number of lamellar bodies or the absence of tubular myelin, suggest a significant derangement of surfactant metabolism [59]. The results of the treatment of infants suffering from this disease are still very poor. Up to now, total cardiopulmonary support, involving extracorporeal membrane oxygenation, repeated surfactant instillations, and corticosteroid therapy has not led to successful treatment [114, 345].

A disease probably caused by a complex of factors is adult (or acute) respiratory distress syndrome (ARDS). The potential of endogenous surfactant is diminished by the presence of serum proteins, but a shortage of surfactant may also play a role in this disease. It is shown that the chemical composition and the functional activity of surfactant are changed as a result of ARDS [96].

Alveolar proteinosis is a disease in which the quantity of the alveolar material is increased but in which the composition is changed. Most notably, the content of SP-A is elevated [131, 184], but the ratio of SP-A to protein is approximately the same as in healthy patients. An accumulation of SP-D in the lungs of alveolar proteinosis patients was also reported [52]. In serum of alveolar proteinosis patients SP-A is present as a complex with immunoglobulins [132, 184].

A case report describes two children with recurrent cyanotic periods who had a lower content of surfactant [126]. In children with recurrent ALTE (apparent life-threatening events), definable abnormalities in the physical properties of surfactant have also been described [202]. These findings may provide a sensitive means of identifying those at risk of recurrent ALTE or sudden infant death syndrome (SIDS).

Multiple mechanisms, such as pH change [107] or the presence of LysoPC, can inactivate performed pulmonary surfactant surface films, an effect that is opposed by the hydrophobic surfactant proteins or the addition of calcium ions [5, 6]. The serum proteins are an important cause of the deterioration of the function of surfactant [280, 281]. The presence of fibrinogen is fatal for surfactant activity [279]. Polymerizing fibrin incorporates surfactant; but after lysis of the fibrin clot, the activity of the surfactant is restored [97]. Anesthetics such as halothane [213], toxic agents such as polyurethane smoke [240], or drugs [98] can negatively influence the biosynthesis and function of pulmonary surfactant in vivo and in vitro. An optimal function of surfactant is dependent on a delicate balance of its constituents and is only seen when all constituents are present, and no inhibition is found from exogenous factors.

Several other factors are known which impair surfactant synthesis and function. Among them are oxidant gases (e.g. nitrogen dioxide exposition [208, 218, 219] or ozone exposure [98, 260]), shortage of copper during the gestation period (associated with a lower birth weight and neonatal lung abnormality [2]), or iron-transferrin ac-

cumulation in epithelial lining fluid (promoting the formation of free radicals, which inactivates the surfactant system [113]).

Therapeutic Effects of Hormones

The administration of hormones can be used to influence the biosynthesis and function of surfactant. Several factors improve surfactant biosynthesis and function. A deficiency of surfactant can be prevented by maternal administration of glucocorticoids (for a review, see Ref. 221). This therapy results in an increased ventilatory and cardiovascular response [294]. The effect is a decrease of morbidity [159], in spite of a brief period of suppression of the basal corticoid concentration. Postnatal glucocorticoid therapy shows no clear evidence of long term benefits [160]. However, the combined use of corticosteroids with surfactant improved the outcome of therapy compared with the use of surfactant alone [147]. In experimental animals, all four surfactant proteins are increased as a result of the treatment with corticosteroids. In rabbits, it has been shown that corticosteroids (glucocorticoids) cause an increase in SP-B mRNA and a large increase in SP-A mRNA [48, 71, 84]. However, there is a difference between the two proteins in the magnitude of the response, indicating that the expression of SP-A and SP-B may be regulated independently [63, 287]. It has been reported that the regulation of SP-A may be dependent on the dose and the time of exposure [140]. There is also a dexamethasone-induced pre- and postnatal increase of the production of SP-D, an effect that is absent when dexamethasone is administered to adult rats [229]. The extent of regulation of SP-C mRNA is still under debate, varying from no increase [48] to a 35-fold increase of SP-C mRNA compared with a control group [326]. Differential glucocorticoid regulation of both hydrophobic proteins has been reported [77, 326]. There is still discussion regarding by which mechanisms corticosteroids accelerate surfactant lipid synthesis [9, 268, 282].

The administration of corticosteroids is a cause of growth retardation and is a potential risk for the mother. To overcome these problems, alternatives have been investigated. A single dose of betamethasone instead of multiple doses showed a negative response, as there was no lung maturity observed, but still growth retardation of the newborn animals [299]. A second approach is ultrasound-guided single fetal corticosteroid treatment. An intramuscular injection of corticosteroids was the most promising technique to obtain improved postnatal lung function in lambs [148]. The ultrasound-guided, intramuscular injection of thyroxine did not augment the corticosteroid effects [38].

A second factor contributing to the beneficial effects of glucocorticoids is thyrotropin. There are indications that the SP-B gene promoter is a target for thyroid transcription factor 1, thereby regulating the transcription of the SP-B gene [30, 353]. Combined maternal treatment with thyrotropin-releasing hormone and glucocorticoids in preterm lambs increases lung compliance, the total amount of phospholipids, and the saturated PC content in alveolar lavage in preterm lambs [214]. In premature rabbits that had received combined therapy 2 days before birth, no increased surfactant metabolism or mobilization of saturated PC was seen [282]. A randomized, controlled trial of antepartum thyrotropin-releasing hormone and betamethasone indicated a reduction in the incidence of RDS and improved survival of preterm infants [173].

Other potential factors such as retinol [86] or endothelin-1 [283] have been studied in animals, but their therapeutic effect remains to be resolved.

Therapeutic Use of Surfactant

Surfactant has had therapeutic use since Fujiwara and co-workers demonstrated its clinical potential in 1980 [87]. A review of pulmonary surfactant therapy was published recently [145]. Convincing evidence has been collected showing that the severity of neonatal RDS can be reduced by replacement of surfactant. Surfactant can be used prophylactically or given to infants who have developed the disease. Treatment of infants with RDS with isolated surfactant or with synthetic surfactant has a beneficial effect on the alveolar ventilation [272] and results in a rapid increase of the arterial oxygen tension [87, 144, 226, 266]. It also increases lung volume and respiratory mechanics as a result of the opening of new distal airways [329]. Surfactant therapy leads to improved aeration, suggesting an end-expiratory increased volume of air [211]. The overall effect is a significantly improved survival rate [194, 204, 209].

There has been discussion as to whether surfactant should be given prophylactically or as rescue therapy. It has been reported that prophylactic administration of surfactant is more effective than early treatment of RDS (rescue therapy), especially in infants under 28 weeks gestation and in infants weighing less than 1,000 g [72, 164]. Less ventilation is required, and lower mortality is seen in this group [163, 238]. Despite these reports in favor of the prophylactic use of surfactant, rescue therapy is normally used. Its advantages are less oxygen dependence and less use of surfactant (and hence, lower cost) [145, 204]. It is not yet established whether neurologic differences are induced in infants who received surfactant therapy vs infants who did not. In a follow-up investigation, no difference in neurologic outcome was found between the surfactant-treated and the control groups [80]. In contrast, it was reported that infants who received surfactant had lower mean mental and motor scores. This would favor giving replacement therapy only to children with postnatal evidence for RDS [324]. Treatment with surfactant consists of one or two doses. There are no indications that a third or fourth dose would be useful [238]. When comparing the different surfactant preparations, there are only small differences in the rate of mortality or bronchopulmonary dysplasia [55, 133]. The best regime for the treatment of RDS is still under investigation [27, 62, 70, 110, 256, 291, 292, 358]. However, despite all efforts, a group of infants (up to 30%) remains which does not respond to surfactant treatment [79].

After prolonged ventilation of the immature lung, destruction of lung parenchyma can be seen. This is mainly a result of barotrauma [28]. Surfactant is given to improve lung function, but surfactant therapy has also proven to have a positive effect on the structure of the lung. Surfactant replacement results in maintenance of more normal parenchyma with less atelectasis during prolonged ventilation of the immature lung. No effect is seen on the alveolar type II cells after surfactant treatment [255, 256], and the therapy is beneficial for long term resistive air flow properties [1]. Children who received surfactant showed less wheezing when they were 24 months old compared with children who did not receive surfactant [300]. Nowadays, the effects of surfactant treatment of premature infants are considerable. Mortality and morbidity of the preterm

neonates are significantly reduced as an effect of surfactant therapy [204, 242]. Eighty percent of the decline of United States infant mortality rate between 1989 and 1990 could be attributed solely to the use of surfactant. Morbidity also was reduced; leading to lower costs in the American health care [278]. In some populations, the limit of viability is now decreased to 23–25 weeks gestation [4, 80], and the mortality of children weighing 600–1,300 g at birth is decreased by approximately 20% [134].

Surfactant treatment could still be optimized. Several techniques are under investigation: antepartum addition of a combination of thyrotropin-releasing hormone and betamethasone [173], addition of antithrombin III to surfactant (to form a complex with thrombin, thereby neutralizing its effect) [274], dilution of surfactant with a saline solution to obtain a better distribution [312], supplementation with inositol in premature infants to increase survival and decrease retinopathy [111], or a combined treatment of a single dose of surfactant and nasal continuous positive airway pressure [328].

A second disease for which administration of synthetic surfactant could be useful is ARDS [8, 95]. (For a review, see Ref. 188.) To study ARDS in an animal model, several procedures have been developed. One example is aspiration of hydrochloric acid in the lungs, which results in reduced gas exchange. The acid causes damage to the alveolar septa, resulting in alveolar edema, a damaged surfactant system, and an inhibition of the surfactant because of the proteins in the edema fluid. Administration of surfactant as soon as possible after the aspiration prevents reduced gas exchange [73]. Bronchoalveolar lavage prior to the surfactant instillation is even more effective [74]. In animal modes of ARDS, lung surfactant improves gas exchange [108]. In small studies the effectiveness of surfactant in ARDS treatment has been studied [116, 122, 143, 293]. The application of surfactant alone will probably not be enough to treat patients suffering from ARDS, and additional therapy will be necessary. The potential therapeutic benefit of the addition of pentoxifylline has been studied to see whether this addition prevents intraalveolar fibrosis in ARDS [180]. As most studies are not complete, and ARDS has multiple causes, it is difficult to predict the place of surfactant in the treatment of this disease.

A third disease in which surfactant therapy promises to be a potential tool is meconium aspiration [298]. In a rat model, meconium aspiration induced diffuse and prominent atelectasis, intraalveolar edema, and hyaline membranes. These morphologic abnormalities were reversed by a high dose regimen of exogenous surfactant [299]. However, in a piglet model, no improvement in oxygenation, surface tension, or lung histology was observed after surfactant therapy, combined with high frequency jet ventilation [332]. Surfactant therapy in full term infants with respiratory failure due to meconium aspiration was often effective in improving gas exchange. A randomized controlled trial of surfactant therapy for this indication has to be performed [167].

There are several other diseases or situations in which the composition or the quality of surfactant is affected. The rationale for surfactant treatment has to be investigated for each of these diseases (Table 5).

The use of surfactant is relatively simple and successful. So far no specific immunologic response to the proteins present in surfactant has been discovered [14, 295, 344]. Administration can be done by instillation or by nebulization. Nebulization of surfactant gives a better distribution [186, 187, 189, 309], but the alveolar recovery of exogenous surfactant was better when instillation was performed [188]. The response

Table 5. Possible indications for use of surfactant

Use	References
• Congenital diaphragmatic hernia	31, 297, 346
• Bacterial, viral, and *Pneumocystis* pneumonia	78, 128, 185, 210, 311
• Clinically significant pulmonary hemorrhage	243
• Improvement of pulmonary outcome after ECMO	33, 196
• Delivery of material to the lung	
—Technetium sulfur colloid, pentamidine	168
—Antioxidants in surfactant liposomes	339
—DNA	11
—Amoxicillin, ceftazidime, tobramycin	321
• Prevention of pulmonary complications after cardiac surgery	64
• Contribution to preservation of lung grafts	76, 178, 224, 225, 325
• Enhancement of mucus clearance	61
• Near drowning	205
• Asthma attack (prophylactically)	193

to a surfactant is determined by both the surfactant composition and the ventilation strategy [231]. The activity of surfactant is improved when hydrophobic surfactant proteins are constituents of the surfactant [109], whereas positive end-expiratory pressure (PEEP) improved the response to supplied surfactant [265, 277, 284]. The application of high frequency oscillatory ventilation may be useful for the prevention of lung injury (especially air leak syndrome) [125, 142, 203] and does not alter the turnover of surfactant [340]. In animal experiments it has been demonstrated that intraamniotic surfactant is taken up from the amniotic fluid [89]. A single treatment with surfactant in utero significantly improved the clinical course but did not completely prevent hyaline membrane disease [88].

The serum proteins present in the affected lung can inactivate the administered surfactant, especially in the presence of lysophosphatidylcholine [45]. Surfactant inactivation can be reduced by SP-B and SP-C [7, 327], by SP-A [47, 357], or by palmitic acid [44]. In a study in which surfactant is instilled into the lungs of preterm lambs and recovered after 5 h of ventilation, it has been shown that the newly recovered surfactant is more active than the original exogenous surfactant preparation [141]. Exogenous surfactant probably associates with components of the endogenous surfactant. This indicates that the clinical efficacy of the surfactant preparation is not optimal, and that the biologic system adds properties to the surfactant which may be important [129].

Complications in Surfactant Therapy

With the increased use of surfactant, negative effects have been observed. In infants, cerebral perfusion was affected during and at 10 min after surfactant instillation [310]. Despite increased pulmonary function, a short decrease in cerebral activity is observed after surfactant treatment [123]. A comparison of the hemodynamics of preterm neonates with RDS suggests that rapid instillation of surfactant leads to a uniform distribution in the lungs. This may be the reason for an increase in cerebral blood flow [271,

309]. It has been shown that prenatal dexamethasone treatment combined with exogenous surfactant therapy has some benefits over the standard therapy; it decreases cerebral complications [159].

A serious problem is that pulmonary complications such as pulmonary hemorrhage are associated with the use of exogenous surfactant [261]. Prenatal dexamethasone treatment combined with exogenous surfactant therapy decreases pulmonary morbidity [159]. Also, the use of high frequency oscillatory ventilation in infants with severe RDS improves oxygenation and reduces the occurrence of an air leak syndrome [125].

A third problem is represented by left-to-right shunting, which appears to be a common event following surfactant treatment [310]. Synthetic surfactant replacement in infants with RDS reduces pulmonary vascular resistance, resulting in a significant but transitory reduction in pulmonary arterial pressure and an increase in ductal flow velocity [155, 156]. The mean arterial blood pressure is decreased by 9.3 mmHg after surfactant adjustment [123]. Recently, it has been described that therapeutic pulmonary surfactant may be associated with in vitro lysis of red blood cells. This cytotoxicity differs for different surfactants and different dosages [81]. In vitro, it has been demonstrated that synthetic surfactants can act as an antioxidant; in vivo, surfactants have been shown to scavenge oxidants to protect against hyperoxic lung injury [85, 91]. The antioxidant function of alveolar surfactant is caused by the presence of lipophilic antioxidantia, such as vitamin E [270]. In a preliminary study in primates, however, porcine surfactant did not protect the lung against oxygen injury [138]. Neither incidence nor intensity of retinopathy was affected by use of prophylactic surfactant therapy [263].

In conclusion, increased knowledge of surfactant will lead to a more optimal composition of the surfactants and a better treatment regime. This will eventually result in a reduced incidence of serious pulmonary and nonpulmonary complications.

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