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Review

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The surfactant system of the adult lung: physiology and clinical perspectives

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Summary. Pulmonary surfactant is synthesized and secreted by alveolar type II cells and constitutes an important component of the alveolar lining fluid. It comprises a unique mixture of phospholipids and surfactant-specific proteins. More than 30 years after its first biochemical characterization, knowledge of the composition and functions of the surfactant complex has grown considerably. Its classically known role is to decrease surface tension in alveolar air spaces to a degree that facilitates adequate ventilation of the peripheral lung. More recently, other important surfactant functions have come into view. Probably most notable among these, surfactant has been demonstrated to enhance local pulmonary defense mechanisms and to modulate immune responses in the alveolar milieu. These findings have prompted interest in the role and the possible alterations of the surfactant system in a variety of lung diseases and in environmental impacts on the lung. However, only a limited number of studies investigating surfactant changes in human lung disease have hitherto been published. Preliminary results suggest that surfactant analyses, e.g., from bronchoalveolar lavage fluids, may reveal quantitative and qualitative abnormalities of the surfactant system in human lung disorders. It is hypothesized that in the future, surfactant studies may become one of our clinical tools to evaluate the activity and severity of peripheral lung diseases. In certain disorders they may also gain diagnostic significance. Further clinical studies will be necessary to investigate the potential therapeutic benefits of surfactant substitution and the usefulness of pharmacologic manipulation of the secretory activity of alveolar type II cells in pulmonary medicine.

Key words: Pulmonary surfactant – Phospholipids – Surfactant proteins – Alveolar stability – Air pollution – Pulmonary defense – Adult respiratory distress syndrome – Surfactant therapy – Bronchoalveolar lavage

Surfactant (=*sur*face *act*ive *agent*) is a material capable of lowering surface tension. The existence of a pulmonary surface active substance was first postulated by Van Neergard in 1929 [181]. He found the calculated surface tension of the alveolar air-liquid interface to be too high to prevent end-expiratory alveolar collapse and atelectasis. Therefore, he predicted the presence of an agent able to exert and maintain a low alveolar surface tension as a prerequisite for the adequate ventilation of the peripheral airways and for normal lung function.

It was almost another 30 years until Pattle [135] and Clements [20] found a substance in lung edema fluid and in lung extracts that indeed lowered the surface tension dramatically. The material was found to be composed of a phospholipid and a protein fraction.

In 1959, Avery and Mead [4] drew attention to the role of a surfactant deficit in hyaline membrane disease (IRDS) of premature infants. Thus, clinical relevance and a first therapeutic perspective became apparent in surfactant research.

More than another 30 years later, the understanding of the pulmonary surfactant system has grown tremendously. The precise composition of the surfactant is known down to the genetic codes of surfactent-specific proteins, making the industrial production of different surfactants a realistic prospect. Much has been learnt about surfactant synthesis in the alveolar type II cell and its regulation and metabolism. Intratracheal surfactant re-

Abbreviations: PC=phospatidylcholine; DPPC=dipalmitoylphosphatidylcholine; PG=phosphatidylglycerol; PI=phosphatidylinositol; SP-A, SP-B SP-C, SP-D=surfactant-specific proteins A, B, C, and D; ARDS=adult respiratory distress syndrome; IRDS=infant respiratory distress syndrome; BAL=bronchoalveolar lavage; IPF=idiopathic pulmonary fibrosis; HP=hypersensitivity pneumonitis; DIPD=drug-induced pulmonary disease

placement is on the verge of becoming a routine life-saving therapy in IRDS. Accumulating evidence suggests that in adult respiratory distress syndrome (ARDS) a similar disturbance of the surfactant system is involved which may possibly be ameliorated by substitution therapy.

In recent years, surfactant functions other than the maintenance of normal lung function have come into view. Perhaps most important among these findings is that surfactant plays a major role in pulmonary defense mechanisms and local immunomodulation. Therefore, the role of surfactant in different lung diseases and in the defense against various environmental impacts on the respiratory tract is attracting growing attention.

The purpose of this article is to present a review of the current knowledge on the pulmonary surfactant system with emphasis on possible clinical implications and future perspectives for adult pulmonary medicine.

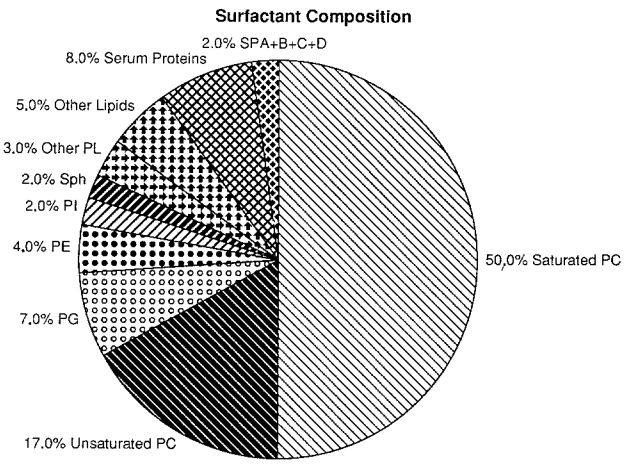

Fig. 1. Components of isolated whole surfactant material from bronchoalveolar lavage (approximate percentage of total weight): PC = phosphtidylcholine; PG = phosphatidylglycerol; PE = phosphatidylethanolamine; PI = phosphatidylinositol; Sph = sphingomyelin; PL = phospholipids; SP = surfactant protein

Biochemical aspects and composition

Surfactant is a complex mixture of lipids and proteins (Fig. 1). Additionally, carbohydrate components are found in both the lipid [165] and the protein fractions [189], but their precise functions remain to be established. Most of the present data on surfactant composition is based on analyses of lung lavages [65, 174], which are thought to reflect adequately the situation in the alveolar lining fluid. However, it has to be kept in mind that lavage specimens may to some degree be contaminated with lipids of nonsurfactant origin, e.g., lipids stemming from cell membranes or lipids secreted by airway epithelial cells [93]. Fewer data exist on the intracellular surfactant composition, e.g., in the lamellar bodies of alveolar type II cells. However, the surfactant composition of the intra- and extracellular compartments seems to be similar [55, 65].

The pool size of extracellular surfactant has been investigated in animals and ranges from about 10–15 mg/kg body weight in adults. Mature newborns have 5- to 10-fold higher values [83]. Assuming similar values in man, a 70 kg person would thus have an estimated alveolar surfactant pool of approximately 0.7–1.0 g. However, there are no available data on the normal surfactant pool size in man, and there may possibly be considerable interindividual variations.

Surfactant lipids

Lipids are the major surfactant component by weight (Fig. 1). They make up about 85%–90% of whole isolated surfactant [65]. Approximately 90% of this lipid fraction consists of a mixture of phospholipids. The remaining 10% are composed of other lipids, mainly cholesterol, which seems to be blood-derived and is of uncertain functional significance [66].

Phospholipids combine hydrophobic and hydrophilic properties and are therefore involved in the coating of boundary areas and surfaces. They possess the ability to achieve low surface tensions at air-liquid interfaces and support, for example, the formation of micelles and lamellae. However, phospholipids not only have structural functions but may in many ways be involved in different dynamic biological processes [64].

The phospholipid composition of human lung surfactant is shown in Fig. 1. In other mammals, this distribution is very much the same. None of these phospholipids is unique to surfactant, but in contrast to the phospholipid profile in other organs, the relative concentrations of phospatidylcholine and phosphatidylglycerol are higher. Surfactants of amphibians and birds lack phosphatidylglycerol, suggesting that this phospholipid was introduced late in evolution [65]. In human fetal lung development, phosphatidylglycerol becomes detectable only late in pregnancy and may serve as an indicator of fetal pulmonary maturity [59], although it does not seem to contribute to the reduction of alveolar surface tension [15].

Phosphatidylcholine accounts for approximately 80% of total surfactant phospholipids and for about two-thirds of whole surfactant (Fig. 1).

Table 1. Some characteristics of the surfactant-specific proteins (SP)

	Molecular weight (kDa, reduced)	Hydrophobicity	Main known functions
SP-A	~29	+	Support of alveolar macrophage activities, regulation of surfactant secretion
SP-B	~ 8	++	Optimizing surface activity
SP-C	~ 5	+++	Optimizing surface activity
SP-D	~43	+	Probable role in host defence, opsonin-like activity?

Approximately 70% of its fatty acids are saturated under normal conditions [65], the most common saturated acid being palmitic acid. Dipalmitoylphosphatidylcholine (DPPC) is the surfactant component which is predominantly responsible for the reduction of alveolar surface tension [9]. Its hydrophilic (choline) residue associates with the alveolar liquid phase while the hydrophobic (palmitic acid) residue reaches into the air phase [197].

Surfactant proteins

Pattle [135] first noted that a protein component in surfactant material seemed necessary for proper surfactant function. In 1973, King et al. [94] could, for the first time, demonstrate the existence of specific surfactant proteins.

By weight, protein accounts for approximately 10% of whole isolated surfactant. About 80% of this protein portion consists of contaminating serum proteins while only 20% are made up by the surfactant-specific proteins (Fig. 1).

Four surfactant specific proteins (SP) have so far been identified (Table 1). A simplified nomenclature of these proteins has recently been proposed [145] and is increasingly being accepted, despite certain difficulties and disadvantages [33]. The first three proteins are simply termed surfactant protein A, B, and C in descending rank of their molecular masses. More recently, a fourth protein called SP-D has been described. The primary structures of surfactant proteins A, B, and C have been identified, and their commercial production by modern techniques of molecular biology is possible [67, 158].

SP-A

SP-A is the major surfactant protein in regard to relative abundance as well as size. In vivo, SP-A is found as a group of isoforms with a molecular weight ranging from approximately 28 to 36 kDa, depending on the extent of posttranslational modifications [67]. It has structural homologies with C1q, a protein of the classical complement pathway [171, 182], and contains a collagen-like domain [28] which is the probable association site of SP-A monomeres. After alveolar secretion, SP-A is predominantly found as a multimeric molecule resembling a flower bouquet [182]. Recent evidence suggests that in man, there are at least two different SP-A subtypes encoded on two separate genes [44, 188]. This may have structural implications for the arrangement of the naturally occurring SP-A multimeres [183], but the functional significance of these findings awaits further clarification.

SP-A seems to play an important role in the formation of a preliminary alveolar surfactant layer called tubular myelin which is found immediately after alveolar secretion [145, 181, 191]. In concert with SP-B and SP-C, SP-A probably enhances the surface activity of the surfactant monolayer [43]. However, the importance of the presence of SP-A regarding this aspect of surfactant function is still debated [146].

SP-A seems to be unique among the surfactant specific proteins as it apparently has additional functions in the surfactant complex apart from influencing surface activity. The structural homologies to the complement protein C1q stimulated investigations of possible common biological functions of these two proteins. Indeed, it was found that the presence of SP-A enhances the phagocytosis of opsonized sheep erythrocytes by macrophages and monocytes in a concentration-dependent manner [171]. Furthermore, SP-A is able to increase the phagocytosis of *Staphyloccocus aureus* [178], herpes simplex virus type 1 [178], and colloidal gold particles [43]. Thus, SP-A seems to play an important role in the local host defense mechanisms of the lung.

Another probable function of SP-A is its ability to regulate the alveolar surfactant concentration. In vitro, SP-A inhibits the secretion of phosphatidylcholine from cultured alveolar type II cells [34] and enhances the uptake of surfactant lipids [198]. Possibly, these SP-A effects are mediated by an alveolar type II cell receptor [157].

SP-B

SP-B is a small protein of a molecular weight of approximately 8 kDa under reducing conditions [186]. Although it is very hydrophobic, it remains soluble in aqueous solutions to some extent. SP-B forms thiol-dependent oligomers of different sizes with the dimer probably being the most common form in vivo [84, 185]. It has no known immunomodulatory or regulatory function but seems to be a key protein in the formation of a functionally optimal and stable surfactant monolayer on the alveolar surface [5, 23, 169]. Also, SP-B seems to play a role in the formation of tubular myelin in cooperation with SP-A [191]. Its amino acid sequence contains high amounts of cysteine, suggesting that disulfide bridges may be important to the role of this protein in the surfactant complex [67]. Indeed, intramolecular disulfide bridges seem to contribute to the structural properties of the SP-B polypeptide chain, and an intermolecular disulfide link may explain the frequent natural occurrence of SP-B dimers [84]. Furthermore, SP-B has a strong positive net charge (at physiological pH) which seems to be important to the interaction between SP-B and the anionic phospholipids [23, 84]. However, the structural interaction between SP-B and other surfactant components still has to be more clearly defined.

SP-C

SP-C is a very small protein with a molecular weight of approximately 5 kDa. It is extremely hydrophobic, which is in part due to a high content of the hydrophobic amino acid valine [145]. It is therefore only soluble in organic solvents. Small size, hydrophobicity, and low immunogenicity make the investigation of this protein a difficult task. As far as its functional role in the surfactant complex is presently understood, it contributes to the formation and stabilization of the alveolar surfactant monolayer in cooperation with SP-B [169]. Probably, SP-C has no role in tubular myelin formation [191]. The molecular structure and most of the properties of SP-C are substantially different from SP-B, suggesting that both proteins have separate roles in the surfactant complex. Indeed, in vitro studies indicate that SP-C may be more important to the adsorption of phospholipids, while SP-B supports the reduction of surface tension more effectively [199]. There seem to be no similarities of SP-C to other proteins of known functions that would suggest an additional role of SP-C [185].

SP-D

SP-D is a collagenous glycoprotein synthesized by alveolar type II cells which has only recently been described [137, 138]. The question still remains whether this protein is a true surfactant protein or a protein that is synthesized independently of the surfactent pathway and is only functionally associated with the surfactant complex. In rat bronchoalveolar lavage (BAL) fluids, the total SP-D content was found to be approximately 12% of that of SP-A [99]. It has a molecular size of approximately 43 kDa (reduced) and appears to build polymeric complexes comprised of the 43-kDa subunits. SP-D has certain structural similarities with SP-A and probably is readily soluble in the alveolar milieu. Like SP-A, SP-D does not contribute to the surface activity of the surfactant complex [137]. Its function is still hypothetical. Structural analogies with proteins like mannose-binding protein, conglutinin, and SP-A suggest that it may have a role in local host defense [184], perhaps by functioning like an opsonin. A recent study indicates that SP-D may also have regulatory functions by counteracting the inhibitory effects of SP-A on phospholipid secretion by alveolar type II cells [98].

Morphological and ultrastructural aspects

The site of alveolar surfactant synthesis and secretion is the cuboidal alveolar type II cell which covers less than 5% of the alveolar surface. There is evidence to suggest that surfactant synthesis and secretion in the lung are not exclusively restricted to the alveolar type II cell but that they may also take place in higher parts of the airways, for instance in Clara cells [3, 170] and possibly even in the tracheal epithelium [8]. This may contribute to normal mucociliary function [190]. However, the significance of these findings awaits further investigation. The alveolar surfactant components are synthesized and assembled in the endoplasmic reticulum of alveolar type II cells and then transferred to the Golgi apparatus prior to forming so-called lamellar bodies in the cytoplasm (Fig. 2). This process has been followed by autoradiography and by immunocytochemistry for phospholipids and SP-A [33]. As shown by transmission electron microscopy [122], lamellar bodies undergo a process of maturation while travelling through the cytoplasm and are eventually transported into the alveolar space by merocrine secretion after fusion with the cell membrane. Here, the lamellar bodies

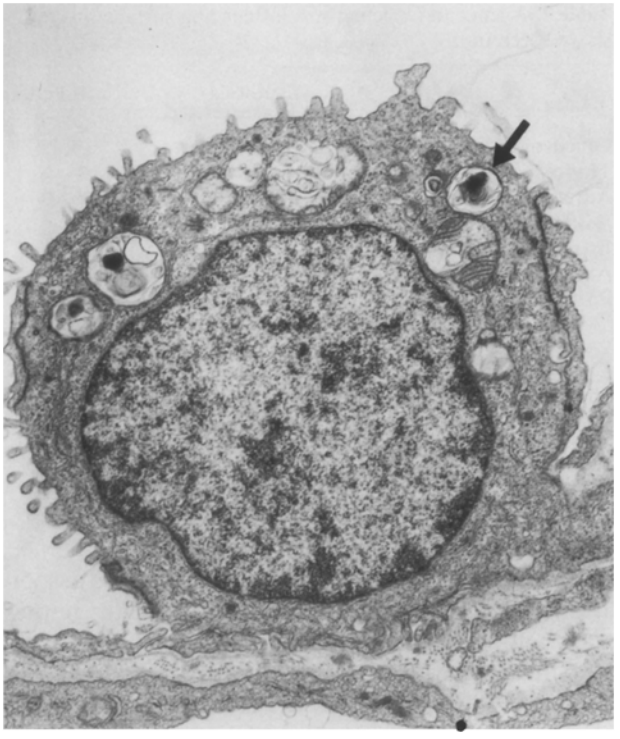

Fig. 2. Transmission electron microscopy view of a single alveolar type II cell. Note the cuboidal cell shape, its protrusion into the alveolar air space and lamellar bodies (*arrow*) in the cytoplasm (×16000)

rapidly transform into tubular myelin, an intermediate surfactant material that is composed of a lattice of highly ordered tubules. SP-A is thought to play a role in the formation of tubular myelin and has recently been located at the corners of the tubular framework by immune electron microscopic techniques [181]. Another recent in vitro study [191] suggests that in tubular myelin formation the presence of SP-B but not of SP-C is necessary in addition to SP-A. Finally, this material is spread to reach its definitive form, the surfactant monolayer (Figs. 3, 4).

Synthesis, regulation, and metabolism

Pulmonary surfactant is not a static accessory of the alveolus but undergoes a constant dynamic process of turnover and metabolism. This review will present only a short summary of the present knowledge on these processes. For more detailed information, the interested reader is referred to a number of recently published reviews which emphasize these aspects [17, 18, 56, 65, 174–176, 185, 196, 197].

Basically, all phospholipid components of surfactant seem to be synthesized and incorporated into the lamellar bodies within the alveolar type

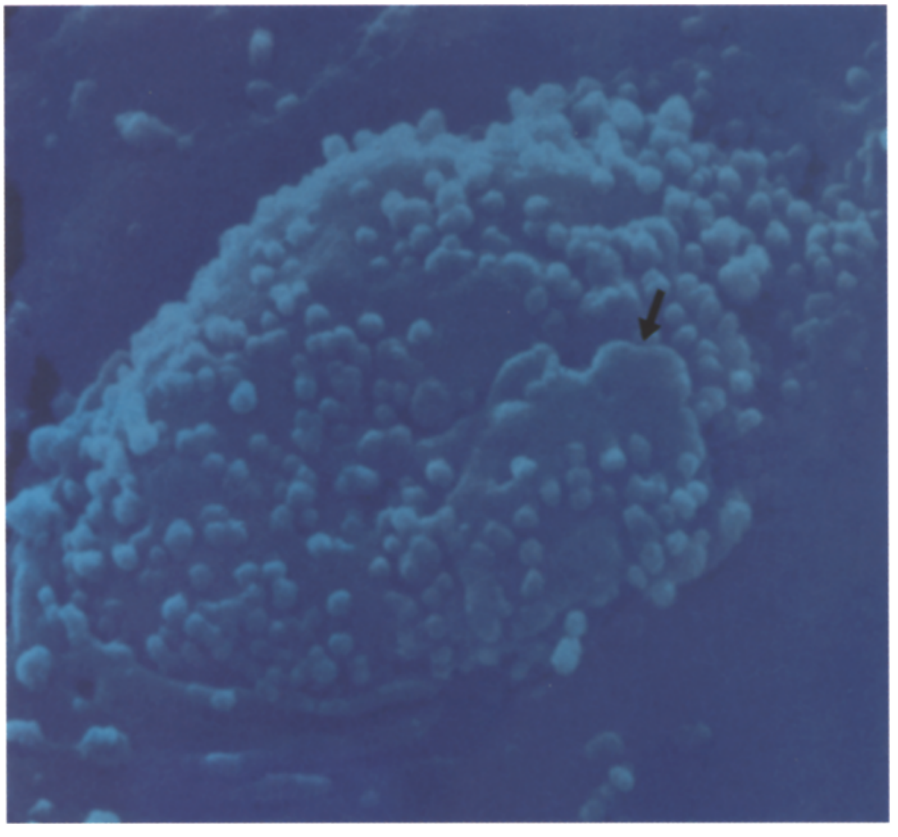

Fig. 3. Scanning electron microscopy view of alveolar type II cell protruding into alveolar air space. Note densely arranged microvilli and spreading of probable surfactant material (*arrow*) on cell surface

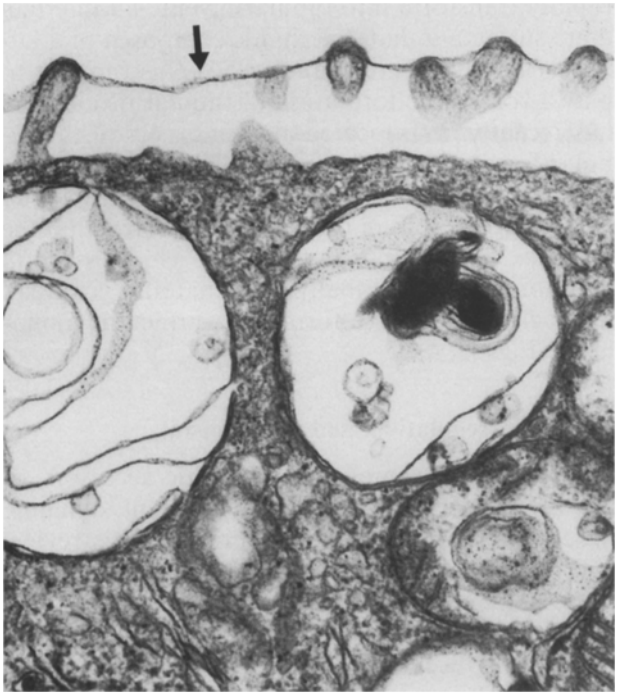

Fig. 4. Transmission electron microscopy view of alveolar type II cell surface (detail). Note lamellar bodies in cytoplasm and secreted surfactant layer (*arrow*) on microvilli

II cell. This is supported by findings that the phospholipid composition of isolated lamellar bodies is virtually identical to that of BAL [197]. DPPC is the best studied phospholipid regarding intracellular synthesis pathways. It is de novo synthesized from blood-derived phospholipid precursors and can probably also be remodelled from unsaturated or recycled phosphatidylcholine.

Less evidence exists on the synthesis and precise pathways of secretion of the surfactant-specific proteins. Alveolar SP-A gene expression is restricted to the alveolar type II cell as shown by in situ hybridization [141]. It is synthesized as a preprotein of approximately 29 kDa and a second variant of approximately 31 kDa. Different posttranslational modifications of this protein like sialylation, acetylation, and sulfation have been described [145]. Single SP-A monomeres are oligomerized to hexameric bundles resembling flower bouquets [182]. Probably, surfactant proteins and the phospholipids are all assembled and introduced into the lamellar bodies within the type II cell before secretion [185]. However, many details of this process remain to be investigated. For instance, it is not yet quite certain whether all of the individual proteins are introduced into the lamellar bodies or if some of them join the surfactant complex after secretion into the alveolar space [56]. Furthermore, no evidence exists on the possible association of SP-D with intracellular lamellar bodies and their secretion.

Table 2. Agents and mechanisms influencing surfactant synthesis and secretion

	Synthesis	Secretion
Glucocorticoids	+	∅
Thyroxine	+	+
Adrenergic stimuli	+	+
β-Blockade	∅	−
Estrogens	+	+
Androgens	−	∅
Prostaglandins	∅	+
cAMP	+	∅
Ventilation	∅	+
Ambroxol	+	+
SP-A (intraalveolar)	∅	−

+ = Stimulation; − = inhibition; ∅ = no known influence or controversial data

Surfactant synthesis has been found to be influenced by a number of different stimuli [7] (Table 2). Glucocorticoids, cAMP, oestrogens, and thyroid hormones, among others, have been described as enhancing surfactant synthesis. However, the in vivo role and importance of these factors is not clearly determined. Some of these stimuli, e.g., glucocorticoids, may vary in their effects depending on dose and time [105], and there may be different pathways for the regulation of surfactant phospholipid and protein synthesis [56]. A recent in vivo study [42] has investigated the influence of exogenously administered glucocorticoids and of adrenalectomy on the regulation of surfactant proteins. Glucocorticoid administration resulted in the accumulation of mRNAs of surfactant proteins SP-A, B, and C, with the highest response being SP-B mRNA. However, adrenalectomy did not change the mRNA levels but decreased the total pulmonary SP-A levels. This study demonstrates that exogenous glucocorticoids enhance surfactant protein synthesis and suggests that adrenal hormones may have a role in the pulmonary response to stress. On the other hand, endogenous steroids under normal conditions do not seem to be important to baseline surfactant protein synthesis at the mRNA level but may to a minor degree contribute to translational or posttranslational processing. The inhibition of surfactant production is possibly controlled by a feedback mechanism involving a surfactant protein [172].

Surfactant secretion into the alveolar space is accomplished by exocytosis of the lamellar bodies. Experimental data suggest that various stimuli like

high volume lung inflation and increased ventilation rate, adrenergic agents, estrogens, and thyroid hormones may enhance surfactant secretion, while beta-blockade and an SP-A-dependent feedback mechanism have inhibitory effects [18, 33, 65] (Table 2). SP-D seems to counteract the inhibitory effect of SP-A [98]. Again, the in vivo significance of these experimental data remains under discussion.

Turnover studies with different labeled surfactant phospholipids after secretion have demonstrated half-lives of between 15 and 30 h [60, 155]. The fate of secreted surfactant material seems to be determined by five mechanisms:
- Intraalveolar catabolism
- Phagocytosis and degradation by alveolar macrophages [110, 118]
- Removal by the mucociliary escalator
- Recycling into the alveolar type II cell
- Redistribution into other surrounding tissue

Clearance studies in rabbits [140] have shown that approximately 7% of radiolabeled phosphatidylcholine is removed via the upper airways in 24 h, suggesting that this pathway is only of minor importance. Further work by the same group [139] supports evidence that most surfactant material is probably redistributed into the surrounding tissue or is recycled into alveolar type II cells.

Many aspects of the regulation of these processes remain to be clarified. SP-A has been shown to enhance the uptake of liposomes into the alveolar type II cell [198]. This process is probably mediated by an SP-A receptor on the epithelial surface of type II cells, which also controls the reuptake of SP-A [157].

Surfactant and lung function

This review will only give a short introduction into the role of surfactant in alveolar stability and in the work of breathing. The interested reader is referred to a number of articles [22, 69, 70, 136, 194] which discuss these aspects in detail.

The lowering of surface tension is the best known function of surfactant material and led to its discovery. However, this classical surfactant function probably was not the initial reason for the development of this material in evolution since animals with less complex lung architectures and thus without a need for surface-tension lowering agents already possess a pulmonary surfactant system [52].

Surfactant material has been shown to reduce the surface tension at the alveolar air-liquid interface down to levels that are required for normal ventilation of the peripheral lung. It reduces the respiratory work load throughout the respiratory cycle and improves lung compliance. The most important surfactant component in this regard is saturated phosphatidylcholine. Other surfactant components like SP-A and more importantly, SP-B and SP-C, have been described to enhance the surface activity of this phospholipid. The hydrophobic saturated fatty acids of saturated phosphatidylcholine are aligned in parallel and rise out of the liquid phase into the alveolar air. The hydrophilic choline residues are packed in the aqueous phase of the alveolus. This arrangement remains stable through ventilatory compression and extension of the alveolus and reduces the strong alveolar cohesive forces close to zero. Thus, alveolar surfactant material successfully prevents alveolar collapse and atelectasis as observed in surfactant-deficient lungs, e.g., in IRDS.

Surfactant and pulmonary defense

Surfactant material may contribute to pulmonary defense mechanisms and local immunomodulation in four different ways:
- Support of nonspecific defense mechanisms
- Direct bactericidal properties of surfactant components
- Immunomodulatory action on lymphocytes
- Augmentation of macrophage activities in the alveolar milieu

Nonspecific defense mechanisms

Surfactant is part of the alveolar and bronchial epithelial lining fluid which is thought to act as a nonspecific barrier against adhesion and invasion of microorganisms. Also, surfactant has antioxidant activities [115] which may contribute to the protection of the alveolar epithelium by scavenging toxic (reduced) oxygen species.

Bactericidal properties

Several reports have addressed the possible antibacterial properties of surfactant material. Studies of rat alveolar lining material identified long-chain free fatty acids as bactericidal surfactant components and demonstrated their antibiotic action against pneumococci in vitro [24]. However, studies of human alveolar lining material obtained by BAL could not demonstrate antibacterial effects against pneumococci or *Haemophilus influenzae* [86]. The in vivo significance of these findings

is still uncertain, and the antibiotic effect of surfactant remains controversial.

Lung surfactant has been shown to influence the activities of lymphocytes and macrophages. These influences are probably of significant in vivo importance for the maintenance of a balance between excessive immune responses and favorable cellular defense mechanisms.

Surfactant and lymphocyte activity

Surfactant suppresses the activation and the proliferative response of lymphocytes to various stimuli in a dose-dependent manner [17, 164, 192]. This suppressor activity is contained in the lipid fraction of surfactant [17]. The major surfactant phospholipids phosphatidylcholine, phosphatidylglycerol, and phosphatidylinositol were shown to be responsible for this immunoregulatory effect. The mechanism of this effect has not yet been clarified but may be related to changes in cell membrane dynamics [193]. Surfactant exerts its effects only on the resting lymphocyte or on the early stage of lymphocyte activation. Activated lymphocytes are not affected. The suppression seems to be largely irreversible, even after the removal of surfactant material from the medium. The inhibitory effects of surfactant have been shown for a variety of lymphocyte activities such as proliferation, differentiation, immunoglobulin production, and natural killer cell activity [10, 17, 164, 192, 193].

Surfactant and alveolar macrophage activity

Nearly all studies on the influence of surfactant on alveolar macrophage activity report an enhancement of macrophage functions. In detail, it has been shown that surfactant material supports phagocytosis [131, 177] and intracellular killing [88, 131] of *Staphylococcus aureus* and the phagocytosis of herpes simplex virus type 1 [178]. It may also enhance the migration of alveolar macrophages [159] and their cytotoxicity against tumor cells [13]. It has to be stressed that these studies report the results of in vitro investigations mostly with animal material. Thus, the significance of these findings for normal human lung defense mechanisms is not yet definitely established. In general, alveolar macrophages are thought to be less active than blood monocytes or macrophages residing in other tissues [75, 150].

Recent studies [171, 177, 178] have shown that SP-A is probably responsible for the enhancement of alveolar macrophage functions as isolated SP-A had the same stimulant effect on macrophages as whole surfactant, while surfactant lipids had no effect [177].

Probably, this macrophage stimulation is mediated by a macrophage receptor which binds SP-A. The specific binding and uptake of SP-A by macrophages has been demonstrated by electron microscopy [110]. A recent report [109] suggests that the SP-A receptor may be identical with the leukocyte C1q receptor, which is a tempting hypothesis since SP-A has structural homologies with the complement protein C1q.

Surfactant, air pollutants, and other pulmonary toxicants

Surfactant material, as part of the alveolar epithelial lining fluid, is thought to represent a first defense line against inhaled particles and gases reaching the alveolar space. Apart from building a "mechanical" barrier, it probably plays an active role in the elimination of foreign particles, e.g., by enhancing macrophage activities and by exerting antioxidant effects [115] against a variety of oxidant gases. On the other hand, the surfactant system itself may be damaged by inhaled particles and gases. A number of studies have been published addressing the impact of air pollutants and other toxicants on the pulmonary surfactant system. Varying study designs such as the use of different animal or in vitro models and different doses and exposure times have led to divergent and sometimes conflicting results. Furthermore, some of the studies focussing only on phospholipid alterations leave some doubt as to whether these changes are truly related to surfactant abnormalities or rather reflect other mechanisms like unspecific cell membrane damage. It certainly has to be kept in mind that the surfactant system is only one of the potential targets of pollutants and toxicants reaching the lung periphery, and hazardous effects on the surfactant system may be of a direct or indirect or as yet unknown nature. This review will only give a short overview of the known or proposed effects of some pollutants and toxicants on the surfactant system. For further information, the reader is referred to a number of reviews and articles focussing on this subject [40, 55, 119, 120, 155].

Ozone

Ozone is a major component of photochemical smog. It acts as a highly aggressive oxidant and leads to the transudation of blood proteins and to edema in the alveolar space even at comparatively low concentrations. Furthermore, chronic

low-dose ozone exposure is known to increase the susceptibility to pulmonary infections. It is believed that the pulmonary toxicity of ozone is at least in part due to impairment of the surfactant system [55]. Several reports support this hypothesis. In rats exposed to 0.3 ppm of ozone for 16 days, giant lamellar bodies were observed in the alveolar type II cells after day 11. This could suggest that ozone may impair surfactant secretion [163]. Short-term exposure (2.5 h) of isolated rat alveolar type II cells to variable amounts of ozone resulted in impaired intracellular synthesis of phospholipids [57]. Exposure of bonnet monkeys to variable low-dose concentrations of ozone for 21–90 days led to changes of fatty acid compositions and a marked increase in phosphatidylcholine levels in lung lavage fluids [149]. Short-term (1–8 h), high dose (3 ppm) ozone exposure of rats resulted in ultrastructural alterations of intracellular lamellar bodies and inhibited proper unfolding of secreted lamellar body membranes in the alveolar space [6]. In vitro ozone exposure of SP-A led to impairments of important physiologic SP-A functions like self-association and SP-A-mediated lipid aggregation [132].

These studies sugest that ozone even at low levels leads to changes in surfactant metabolism and secretion and to alterations of composition and properties of the secreted surfactant material. Thus, it seems likely that the pulmonary toxicity of ozone is in part due to impairment of the surfactant system. One of the many remaining questions is whether ozone-induced surfactant abnormalities are also involved in the increased susceptibility to pulmonary infections of chronically exposed individuals.

Nitrogen dioxide

The majority of atmospheric nitrogen oxides is derived from natural sources. However, in urban areas, nitrogen oxides from energy utilization largely determine air pollution levels with these gases [123]. The pulmonary toxicity of nitrogen dioxide is similar to that of ozone, inducing free radical reactions and lipid autoxidation [55]. Probably, both these air pollutants have synergistic toxic effects on the lung. Short-term exposure (5 h) of rats to high levels (40 ppm) of nitrogen dioxide resulted in phosphatidylcholine and phosphatidylglycerol accumulation in lung tissue with a peak at 48 h postexposure. Incorporation studies suggested that this increase was due to enhanced phospholipid synthesis [14]. Long-term exposure (9 months) to low levels (2.9 ppm), by contrast, led to a significant decrease in the lung lipid content and changes in the phospholipid fatty acid composition [2].

These studies may indicate that the acute effect of nitrogen dioxide on alveolar type II cells is enhanced surfactant lipid synthesis, while chronic low-dose exposure leads to a decrease in surfactant synthesis capacity. However, the evidence is still scarce and not all observed phospholipid changes are necessarily related to the surfactant system. Further studies are necessary to define more precisely the possible impact of nitrogen dioxide on alveolar type II cells and surfactant material. Also, in view of a more realistic approach to urban air pollution, it seems important to learn more about the co-toxicity of ozone and nitrogen dioxide.

Oxygen

The toxicity of hyperbaric oxygen or oxygen at high concentrations is well-known and represents one of the problems of mechanical high oxygen ventilation, e.g., in intensive care units. The toxic effect is due to aggressive oxygen-derived free radicals which attack various cell constituents and probably also the surfactant system. In detail, it has been found that rabbits exposed to 100% oxygen for 64 h exhibited a marked decrease in phosphatidylcholine synthesis and cell lipid content followed by a recovery to normal patterns and subsequently supranormal levels beginning 3 days postexposure [74]. The same group [114] showed that intratracheal surfactant substitution significantly diminished the progression of hyperoxic injury. In rats exposed to 85% oxygen for 72 h, increased levels of phosphatidylcholine and SP-A were found in lung lavages [126]. It was concluded that hyperoxic lung injury is not due to intraalveolar decreases of these two major surfactant components. However, in another animal study DPPC was decreased and the PG:PI ratio was markedly lower than baseline values after 4–5 days exposure to 100% oxygen. Longer periods of exposure resulted in a further drop of DPPC values [95]. Pulmonary oxygen toxicity does not seem to be consistently related to changes of surface tension measured in lung lavage fluids of exposed animals [1].

In conclusion, hyperoxic lung injury may be associated with alveolar type II cell changes in surfactant biosynthesis. However, different studies have found partly conflicting results, and the way in which hyperoxic lung injury contributes to quantitative and functional changes of alveolar surfactant is still poorly understood. It should also be remembered that the oxidant attack of oxygen

is not limited to type II cells or surfactant, and thus, some of the described phospholipid changes may not exclusively reflect surfactant abnormalities.

Others

Cigarette smoke is a complex mixture of particles and gases. A reduced yield of phospholipids from BAL fluids of smokers compared with nonsmokers has been described [41]. This difference was interpreted to be partly due to lower lavage fluid recovery from smokers related to their known tendency to bronchoconstriction. Additionally, it was thought to reflect the enhanced phagocytosis activity of alveolar macrophages [21]. Another group found no such quantitative differences between smokers and nonsmokers but described a decreased phospholipid/protein ratio in smokers [108]. In rats exposed to cigarette smoke, a decrease of surfactant material in lung lavages was found. Additionally, a progressive injury of alveolar type II cells was observed over time as determined by electron microscopy, indicating that type II cells and therefore possibly the surfactant system may be one of the targets of cigarette smoke in the peripheral lung [102]. In vitro studies showed that smoke particles but not the gas phase of cigarette smoke interacted with a surface film of surfactant and altered its surface active properties in such a way that the maximum surface area was reduced, but the minimum surface tension was increased [68]. This may possibly contribute to altered mechanical properties of the lungs of smokers. In conclusion, only a few studies have so far investigated the possible impacts of cigarette smoke on the surfactant system. Thus, our knowledge about the effects of this important pulmonary toxicant is still very fragmentary and awaits further investigations.

Smoke generated from the burning of polyurethane foam has been shown to increase significantly the total phospholipid content of lung lavages from rats after short-term exposure [134].

Diesel exhausts were shown to induce pulmonary phospholipidosis in rats [37]. In another study, short-term exposure of rats to 6 mg/m^3 diesel exhaust resulted in an increased labeling index in type II cells and enhancement of whole lung DNA synthesis [195]. Additionally, lavage phospholipid values were increased, and there was evidence of reversible alterations of fatty acid and phospholipid metabolism.

Hydrogen sulfide is an irritant gas with toxic effects on the respiratory tract. An animal study suggests that higher doses impair the ability of surfactant to lower surface tension. However, this does not seem to be due to a direct effect of hydrogen sulfide on surfactant material but due to surfactant inhibitors in the pulmonary edema fluid induced by hydrogen sulfide [53].

Dusts, especially those with a high fibrogenic potential, seem to stimulate the production of surfactant [55]. Silica (usually quartz dust) inhalation leads to a striking increase in the alveolar surfactant phospholipid and SP-A content [31, 90]. Recently, the accumulation of SP-D has also been reported [29]. Morphologically, these observations are accompanied by type II cell hypertrophy and hyperplasia [90, 119]. The lungs of silica-exposed animals share common features with alveolar proteinosis in man so that they may be used as animal models of this disease. Asbestos inhalation provokes a very similar accumulation of surfactant material in the alveolar space [38, 55].

The heavy metal *cadmium* is a known pulmonary toxicant. The main sources of human exposure are cigarette smoke, automobile emissions, and metal-processing plants. In rats, the inhalation of cadmium chloride led to an early decrease of phospholipids in lung lavage, accompanied by an increase in tissue phospholipids. After 4 days, levels of lavage phospholipids then markedly increased above normal values [16]. In vitro studies with alveolar type II cell cultures exposed to cadmium chloride demonstrated inhibition of surfactant secretion, while cadmium alone had no such effect [40].

Paraquat, a commercially important herbicide, has marked toxic effects on the lung, particularly on the alveolar type II cell. In vivo and in vitro studies have shown that the synthesis of surfactant phospholipids decreases after exposure. However, it is not yet clear whether this effect on surfactant production is the primary cause for paraquat-associated respiratory failure. A major problem in the clinical treatment of paraquat poisoning is the synergistic toxicity of high oxygen mechanical ventilation [55].

Surfactant and pulmonary disease

The number of available studies on surfactant changes in human lung diseases is still limited. However, with further improvement of investigative tools and increasing interest in possible clinical implications, it should be expected that such studies will prosper in the near future. The main material for surfactant studies in man is BAL because it is available at a low risk to the patient and gives access to all alveolar surfactant components. Nev-

ertheless, this method has its limitations. BAL does not give direct insight into cellular changes of alveolar type II cells and methods of quantification of the obtained epithelial lining fluid are limited by the complex nature of fluid dynamics during the procedure [92, 111]. Also, not all phospholipids in BAL fluid must necessarily be surfactant phospholipids. They may in part stem from airway epithelial cell secretions [93] or from cell membranes (alveolar cells, but also leucocytes, macrophages, etc.), which seems particularly important to consider in inflammatory lung diseases (e.g., [148]). Nonetheless, the first clinical studies have shown that quantitative changes of surfactant components in different lung diseases can be found in comparison with healthy controls. Normal values for SP-A in human BAL are found in the range of about 0.5–3 μg/ml lavage fluid ([62, 116, 142] and own unpublished data). Normal total phospholipid levels seem to vary considerably among individuals and also among laboratories [61, 76, 77, 81, 116, 143, 154]. Additionally, chromatographic determination of the distribution of individual BAL phospholipids (Fig. 1) is often used to describe surfactant abnormalities. Recently, simpler enzymatic methods were recommended for phospholipid analysis in BAL fluids [48]. However, at present, only phosphatidylcholine and phosphatidylglycerol may be quantified by this method. Another frequent approach is to investigate the biophysical properties (ability to lower surface tension) of the obtained surfactant material.

Adult respiratory distress syndrome

Various conditions such as severe trauma, major surgical procedures, burns, sepsis, acute pancreatitis, and aspiration pneumonia are capable of inducing this form of acute lung injury. It may rapidly progress to respiratory failure and continues to have a high mortality of around 50%–65% [36, 45] with hardly a change over the years in spite of many improvements in modern intensive care medicine. ARDS may be triggered or aggravated by high oxygen mechanical ventilation which is necessary in many of these patients. Surfactant changes are thought to play an important role in the pathogenesis of this condition. However, it should be emphasized that ARDS is a severe, multifactorial disease in which surfactant is only one piece of the puzzle.

Serious disturbances of surfactant phospholipid composition and surfactant function as well as a reduced SP-A content in the BAL have been described in animal models [104, 168] and in man [11, 61, 143, 144], while the total phospholipids have not consistently been found to be reduced. The pathogenesis of these changes seems complex and is still only partly understood. Again, some of the reported phospholipid changes may not be directly related to surfactant abnormalities but may be caused by other mechanisms like breakdown of cell membranes.

One of the major mechanisms leading to surfactant disturbances in ARDS is probably connected to a massive fluid and protein accumulation in the alveolar compartment. Especially in the early stages of ARDS, the increased permeability of the alveolocapillary barrier leads to noncardiogenic pulmonary edema with high concentrations of plasma-derived proteins. Edema fluid and coagulated proteins block the alveolar air spaces and impair normal gas exchange [36, 161]. Several lines of evidence suggest that many of these plasma-derived proteins also have a strong potential for inactivation of surfactant material [71, 97, 161]. In detail, this has been experimentally demonstrated for fibrinogen [46], fibrin monomers [160] albumin [73], and even hemoglobin [72]. These results suggest that in ARDS a major problem is probably not only the postulated deficit of alveolar type II cell function but also a relative deficit of functionally intact surfactant material due to massive protein inactivation. It remains to be investigated whether or not the protein inactivation of alveolar surfactant is, to a minor degree, also relevant to other pulmonary diseases.

Another possible mechanism of surfactant inactivation in ARDS is that surfactant phospholipids may be degraded by phospholipase A_2, an enzyme which is probably involved in ARDS caused by pancreatitis or sepsis [35, 82]. Furthermore, it has been shown that *E. coli* endotoxin reduces surfactant synthesis in vitro [103] and in vivo [130].

Many authors have called for clinical studies to investigate the benefit of surfactant substitution therapy in ARDS [36, 100, 112, 125], but there are still numerous problems to be solved like dosage, timing, and delivery method [71] which dampen the enthusiasm for patient trials. Much of the optimism is certainly due to the fact that ARDS shares common features with IRDS in which surfactant replacement is on the verge of becoming a standard therapy. Additionally, animal studies (e.g., [101]) and human case reports [85, 100, 127, 152] support the hope for a beneficial effect of surfactant replacement therapy in ARDS. The first controlled clinical trials are presently under way. Even if beneficial effects on survival can be demon-

strated, a significant mortality will probably remain, since the cause of death in ARDS is not invariably related to respiratory failure [121].

Idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a progressive fibrosing lung disease of unknown origin which involves alveolar epithelial injury and alveolar type II cell proliferation [91]. Total phospholipids in BAL were found to be reduced, with decreases of PG and DPPC and an increase in PI [76]. Another study [154] reported similar findings in 15 untreated patients. Total phospholipids in BAL were less than half that of controls, with raised percentages of PI and lowered PG. The severity of these changes correlated with more advanced histopathologic fibrosis. In 28 patients (including the 15 patients of the former study), the SP-A content of BAL was reported to be significantly lower than in normal controls [116]. In 32 patients with untreated IPF, the PG level was lowered, and its increase after the commencement of steroid therapy seemed to indicate clinical improvement [81]. These studies suggest that surfactant studies may be of clinical value to assess the prognosis and proper management in IPF [107].

Sarcoidosis

Sarcoidosis is a generalized granulomatous disease of unknown origin which frequently involves the lung. In 3 patients, total phospholipids in BAL were not significantly decreased, and changes in phospholipid composition were not found [76]. In partial agreement with these observations, no significant changes in total BAL phospholipids were found in 12 untreated patients with sarcoidosis [154], but there was an increase in the PG:PI ratio. If confirmed, these findings could be of interest as a clinical tool to separate sarcoidosis from IPF in the differential diagnosis of fibrosing lung diseases. Another study of 13 untreated patients described a decrease of DPPC in BAL [12]. In 8 untreated patients with active sarcoidosis, our group found raised SP-A levels in BAL in comparison with healthy controls [63]. Further studies are necessary to confirm surfactant changes in pulmonary sarcoidosis and to evaluate their role in this disease and their potential in the differential diagnosis of fibrosing lung disorders.

Hypersensitivity pneumonitis

The known immunoregulatory role of surfactant makes this pulmonary disease an interesting object of surfactant studies. However, only a few reports have so far addressed the role of surfactant in hypersensitivity pneumonitis (HP). In a recent study, 3 untreated patients with HP are mentioned whose SP-A values in BAL were lower than in normal subjects [116]. By contrast, our own preliminary data from BAL fluids of 8 patients with untreated, active HP show higher SP-A values than controls [63]. Also, the SP-A content of alveolar macrophages (obtained by BAL) as assessed by immunocytochemistry was elevated in untreated HP patients in comparison with healthy controls [54].

Another recent study demonstrated that acute immune lung injury in guinea pigs is augmented in animals with partial surfactant depletion while surfactant replacement ameliorated the parameters of lung injury [153]. This prompted a somewhat optimistic comment that surfactant replacement might be useful in the therapy of cell-mediated immune diseases of the lung [151].

Pneumonia

It is an attractive hypothesis that surfactant abnormalities may play a role in the pathogenesis of pneumonia and/or that surfactant changes occur as a consequence of alveolar infection. As an example, viral infection could damage alveolar surfactant, facilitating the secondary invasion of bacteria. As yet, only a few studies have investigated these questions, so that our knowledge of the role of surfactant in pneumonia is still rather incomplete.

In patients with bacterial pneumonia, changes in the fatty acid composition of BAL phospholipids have been described [11]. In an animal model of *Pneumocystis carinii* pneumonia, increased amounts of total phospholipids and decreases in the percentage of PC were observed [162]. It was hypothesized that these findings contribute to the altered lung mechanics and respiratory distress in this disease. However, it should be stressed once more that phospholipid changes may not necessarily reflect true surfactant abnormalities. In 22 patients with acquired immunodeficiency syndrome (AIDS)-related pneumonia (mostly *P. carinii* pneumonia), a marked increase of SP-A in BAL was reported in comparison with 21 healthy controls [142]. Phospholipid analysis was not done, and it remained unclear whether the observed changes were primarily related to human immunodeficiency virus (HIV) infection or to pneumonia. Further studies of the reactions and the potential role of the surfactant system in bacterial or viral invasion of the alveolar space are certainly necessary and may be awaited with interest.

Alveolar proteinosis

This is a rare disease in which for unknown reasons the alveolar type II cell synthesizes and secretes excessive amounts of abnormal surfactant material. Lungs of silica-exposed animals share common features with human alveolar proteinosis (see above), but there is no evidence that dust exposure has a role in the pathogenesis of this disease in man. A typical finding is the accumulation of tubular myelin-like multilamellated structures in the alveoli [78]. The BAL fluid is characterized by increased content of total phospholipids with a relative decrease in PG and an increase in PI [77]. Diagnosis is usually made histologically but may also be made by the demonstration of excessively high SP-A levels in BAL or simply in sputum [113]. Further studies of surfactant material and alveolar type II cells of these patients may possibly help to identify the cause of this condition, which is presumably related to a disturbance of the normal type II cell regulation.

Radiation injury

Radiation pneumonitis and subsequent fibrosis are known problems after radiotherapy of thoracic organs. Animal studies have shown that the number of lamellar bodies in type II cells drops dramatically immediately after radiation and that this is accompanied by an increase in lavage surfactant content [40]. In vitro studies by the same group demonstrated that this is a direct effect of radiation on type II cells and that these cells exhibit changes which may indicate a switch of phospholipid synthesis to cell membrane repair after radiation damage. These experimental findings indicate that radiation may lead to massive surfactant secretion from type II cells early after exposure followed by a sharp drop in further surfactant synthesis.

In 4 patients with pleural mesothelioma, hemithorax irradiation caused protracted accumulation of proteins in the alveolar epithelial lining fluid which may inhibit the surface activity of surfactant. No significant changes in total phospholipid content were found, but PG, PI, PC, and SP-A levels were decrased, while the sphingomyelin concentrations were markedly increased [62]. However, the raised sphingomyelin levels in this study probably originate from other sources than alveolar surfactant. The changes were most evident 4 months after the completion of radiotherapy. Unfortunately, immediate or early effects of radiation were not investigated. Further work will be necessary to determine the role of surfactant abnormalities in the pathogenesis of radiation pneumonitis.

Drug-induced pulmonary disease

Drug-induced pulmonary disease (DIPD) is often accompanied by histological changes of alveolar type II cells like dysplasia and proliferation [25, 26]. Therefore, it is reasonable to expect changes of the type II cell surfactant production in drug-induced lung injury. However, only a few drugs which are potentially able to induce DIPD have so far been investigated in this respect.

Polychemotherapy has in one report been described as inducing decreased phosphatidylcholine levels and increased phosphatidylglycerol levels in BAL of patients with bronchial carcinoma [156].

Bleomycin is an antineoplastic drug which has a known capability to induce fibrosing lung disease. In animal studies, bleomycin lung injury is frequently used as a model of pulmonary fibrosis [25]. Bleomycin induces proliferation of type II cells and giant intracellular lamellar bodies in mice. In rats with bleomycin lung disease, the BAL after days 14 and 30 revealed increased amounts of total phospholipids, with increased percentages of PC and PI, while that of PG was decreased. These changes coincided with an altered lung compliance [173]. Another study described an initial decrease of total phospholipids after 4 days and a subsequent 2.5-fold increase over control animals on days 21 and 28 [80]. The percentage of PG was reduced, and that of PI was increased. SP-A levels did not change throughout the experiment. From these results, a rather general conclusion was drawn that SP-A is insensitive to lung injury and repair. Decreased BAL phospholipids were also found in the early phase of fibrosis in hamsters. The surface-active properties of surfactant were inhibited and lung pressure-volume curves deteriorated [133].

In conclusion, bleomycin apparently leads to a decrease of total phospholipid values within the first days of lung injury, followed by an increase above normal values with a decreased PG:PI ratio. It remains to be confirmed that these observations adequately and specifically reflect the injury of alveolar type II cells. It seems surprising that SP-A, a more specific secretory product of alveolar type II cells than phospholipids, did not change in the one study cited above. The conclusion that SP-A is insensitive to lung injury is not convincing, since changes in SP-A levels have been reported in idio-

pathic pulmonary fibrosis and other lung disorders.

It has been hypothesized that an increase of alveolar surfactant material may contribute to the pathogenesis of pulmonary fibrosis by activating alveolar macrophages which in turn stimulate fibroblasts [39]. However, there is no experimental support to this idea, and from a clinical point of view, this hypothesis appears doubtful since most patients with alveolar proteinosis do not tend to develop pulmonary fibrosis.

Amphiphilic drugs like amiodarone, propranolol, chloramphenicol, and chlorpromazine may interact with pulmonary phospholipids and thus surfactant phospholipids, causing pulmonary phospholipidosis. A proposed mechanism is that normal phospholipid degradation is impaired by binding to the drugs. Inhibition of phospholipases may also be involved [87]. It seems reasonable to suspect surfactant abnormalities in many other drug-induced lung disorders because DIPD is often associated with morphological alterations of type II cells. As an example, we recently observed morphological changes of alveolar type II cells in a case of acute mesalazine alveolitis [187]. Subsequent analysis of the BAL fluid of this patient revealed an increase of SP-A content approximately 10-fold above healthy controls.

Others

Byssinosis is a lung disease observed in cotton workers. Clinically, patients present with fever, flulike symptoms, and bronchoconstriction. Lipopolysaccharides from gram-negative bacteria found in respirable cotton dusts are thought to be responsible for this disease. A recently published in vitro study suggests that cotton extracts cause biophysical alterations of the lung surfactant [30]. It is hypothesized that these effects play a part in the pathogenesis of byssinosis.

BAL from *lung transplants* of 11 dogs were recently investigated with the principle aim of finding surfactant phospholipid changes specific to infection or rejection [89]. This differential diagnosis represents one of the major problems in the treatment of lung recipients. However, the data obtained in this study were essentially inconclusive. The optimism that surfactant abnormalities specific to rejection or infection will be found in the future seems somewhat questionable because both are inflammatory processes with presumably similar responses of alveolar type II cells. Another recent study [96] determined DPPC levels in BAL of excised dog lungs during hypothermic storage (4° C for 24 h) and after left lung transplantation (6 dogs, 24-h postoperative observation period). A decrease of DPPC levels was found in both situations. However, in a second group of 6 transplanted dogs receiving L-carnitine infusions pre- and postoperatively, DPPC levels and oxygen tension were higher postoperatively than in the group not treated with carnitine. It was concluded that the drop in DPPC levels reflected ischemic damage to alveolar type II cells and that carnitine (a cofactor for fatty acid transport into mitochondria) improved surfactant synthesis and therefore pulmonary gas exchange in the transplants. However, analysis of other BAL phospholipids and surfactant-specific proteins is lacking in this study, and it remains to be confirmed that carnitine infusions really have such in vivo effects. A current review of this subject [128] outlines some of the possible perspectives of surfactant analysis and treatment in lung transplantation in more detail. Certainly, much more work has to be done to assess the usefulness of surfactant studies or even surfactant replacement therapies in lung recipients.

Surfactant therapy

As outlined before, surfactant therapy may prove to have beneficial effects on the course of ARDS and is now being investigated in clinical trials. Presently, there is no convincing evidence to suggest that such a treatment may also be of use in other adult lung diseases.

Surfactant substitution

A number of surfactant preparations are now in use, and some of them are already marketed for the treatment of IRDS. Their composition and therefore their properties vary considerably, and it is not yet clear which preparation will be the best considering efficacy, safety, availability, and price. It seems possible that different surfactants will in the future prove optimal for different indications, thus perhaps leading to a variety of specifically designed preparations.

Natural surfactants

Bovine surfactant preparations (e.g., Survanta, Surfactant-TA, Alveofact) are organic solvent extracts of minced cow lungs and contain phospholipids in a natural composition plus SP-B and SP-C but no SP-A. In a randomized controlled trial of a bovine surfactant preparation (single dose) for the prevention of IRDS [51], it could be demon-

strated that the survival rate without bronchopulmonary dysplasia was significantly improved. Furthermore, there was a tendency to a better overall survival rate and a reduction in total time of mechanical ventilation. A single-dose regimen of Survanta reduced the severity of respiratory distress and the frequency of pneumothorax but not the mortality in another randomized controlled study of IRDS [79]. Survanta has also been reported to improve lung recoil but not arterial blood gases in a rabbit lung model of ARDS [101].

Surfactant-TA in a randomized controlled trial has been shown to diminish the amount of respiratory support necessary in premature infants with IRDS [49]. Another similar study demonstrated the reduction of intracranial hemorrhage and bronchopulmonary dysplasia in infants surviving IRDS [47]. In an anecdotal report of 2 cases of ARDS, Surfactant-TA was also reported to have beneficial effects, although the dose was unusually low [127].

Porcine surfactants (e.g., Curosurf) are organic solvent extracts from minced porcine lungs with a composition comparable to bovine surfactants. Beneficial effects of Curosurf have been described in 3 patients with ARDS [152] and in a series of children with severe IRDS [166].

Also, natural surfactants from amnion fluid or BAL have been used. A serious drawback of all natural surfactants is their limited availability and their high prices.

Synthetic surfactants

ALEC is a simple preparation of only two phospholipids, DPPC and PG (in a weight proportion of 7:3).

Exosurf (or Exosurf Neonatal) is a mixture of DPPC, hexadecanol, and tyloxapol and also does not contain surfactant-specific proteins. In a recently published, large, multicenter trial in infants with IRDS [106], Exosurf in a two-dose regimen was shown to reduce mortality and perinatal morbidity. However, in a sheep model of ARDS, aerosolized Exosurf failed to demonstrate a beneficial effect [200].

Presently, great efforts are being made to produce synthetic surfactants which resemble natural surfactants more closely. The genes of SP-A, B, and C have been cloned so that these proteins can be produced by methods of recombinant DNA technology [158]. Surfactant phospholipids can easily be produced by chemical synthesis. Thus, different surfactant preparations can now be designed and studied in vitro and in vivo. These synthetic surfactants will have the advantage of high quality and nearly unlimited availability, which is an important prerequisite for pharmacological trials and subsequent clinical use on a larger scale. However, several remaining issues will have to be solved, e.g., the optimal composition of such "designer surfactants." Probably, only the phospholipids DPPC and PG will be necessary in conjunction with surfactant proteins to guarantee full surfactant efficacy [158]. Another issue which is presently debated is whether or not SP-A is a necessary component of synthetic surfactant preparations. Bovine and porcine surfactant preparations without SP-A have already been shown to be effective, and it is feared that the addition of SP-A may increase the immunogenicity and impair the stability of the preparation. However, the addition of SP-A enhances the biophysical activity and increases the resistance of surfactants against inhibitory proteins in vitro, which seems an important aspect, especially when treating ARDS [179].

Dosage

Most data on surfactant dosage in adults are derived from animal studies or clinical trials of IRDS treatment (e.g., [27, 47, 58, 79, 166]). Usually, a single dose or a two-dose regimen is preferred over repeated surfactant instillations. An adequate single dose for the treatment of ARDS is thought to be in the range of 50–300 mg phospholipids/kg body weight [36, 71, 83, 85, 100]. Alternatively, for example, a cumulative total dose of 4 g has been used [152].

Potential side-effects of surfactant replacement therapy

Surfactant preparations containing proteins should be expected to have a potential for sensitization of a patient to foreign proteins. Data from children with IRDS treated with exogenous surfactant indicate that circulating surfactant-anti-surfactant immune complexes frequently occur [117]. However, many IRDS patients without substitution therapy also seem to have such circulating immune complexes [167], and negative effects have not yet been observed. A recently published study [19] demonstrated IgM antibodies to surfactant specific proteins in patients with severe IRDS and showed that the antibody occurrence decreased after surfactant treatment. It was concluded that IRDS can lead to a leak of surfactant-specific proteins into the circulation and that surfactant treat-

ment may reduce this leak by reducing the lung damage.

Another issue is that exogenous surfactant could interfere with endogenous surfactant synthesis and secretion. Indeed, in vitro evidence on surfactant regulation (see above) would suggest that surfactant substitution could have such unwanted effects, e.g., by feedback inhibition of type II cells. However, a recent in vivo study in rabbits [129] has shown that this was not the case. On the contrary, administration of different surfactant preparations tended to stimulate endogenous surfactant synthesis and secretion.

In conclusion, several studies and existing clinical experience suggest that surfactant substitution therapy is not associated with serious risks. However, possible long-term effects are not yet known, and further studies should continue to monitor patients for potential side effects of surfactant treatment.

Pharmacologic agents

Pharmacologic improvement of surfactant abnormalities or deficits in human lung diseases, especially in ARDS, would be of considerable clinical value. Despite some encouraging in vitro and animal studies, no clinical studies have yet convincingly demonstrated the usefulness of theoretically promising pharmacologic agents. One of the problems in ARDS is probably that successful pharmacological substances would require very strong stimulatory effects on alveolar type II cells to overcome not only the alveolar surfactant deficit but also the inhibitory effects of exudated proteins in the alveoli.

Steroids are known to interfere with many of the mechanisms thought to be involved in ARDS. Their actions include beneficial effects on surfactant synthesis. However, a number of large clinical trials have not been able to prove a clinical benefit of steroid therapy in ARDS (for review see [50]). This is not necessarily due to a failure of steroids to enhance surfactant synthesis but may simply reflect the multiple disturbances associated with ARDS. Steroids are frequently and with some success used in mothers at risk of premature delivery to prevent IRDS (for review see [124]).

Beta-agonists are able to enhance surfactant synthesis and secretion from alveolar type II cells in vitro. These agents are also used to suppress premature labor in mothers and possibly accelerate fetal lung maturation as a beneficial side effect [124, 125]. However, no clear evidence has so far been presented to support such in vivo effects of beta-agonists.

Ambroxol, a drug which is primarily marketed as a mucolytic agent, seems to enhance surfactant production and secretion [32, 147] and has been reported to be useful for the prevention and treatment of IRDS (for review see [125]). We were not able to find studies investigating a potential use of ambroxol in ARDS.

Conclusions

This review has attempted to summarize briefly the present knowledge on the pulmonary surfactant system and has tried to outline some of the available information which may in the future become relevant to clinical pulmonary medicine. After more than 60 years of research, the surfactant system of the human lung has not yet become part of routine diagnostic or therapeutic considerations in adult pulmonary medicine. However, with growing knowledge from basic research, surfactant studies are beginning to give us some new insights into the mechanisms involved in various lung diseases and in the degree of involvement of alveolar type II cells. Thus, a variety of possible perspectives have now arisen, ranging from diagnostic to therapeutic implications and preventional aspects.

It does not seem likely that surfactant analyses will in the future be used primarily to arrive at a specific diagnosis of a disease since there are probably not many conditions which feature characteristic surfactant abnormalities. However, present evidence fosters speculations that surfactant studies may prove useful in giving some information about the activity, intensity, and perhaps the duration of some pulmonary diseases or pollutant exposure, and they may be found helpful in the differential diagnosis of fibrosing lung disease. Furthermore, it can be speculated that surfactant studies may help to monitor the effects of therapies and to assess the prognosis of various lung diseases. However, much more work has to be done to investigate these hypotheses, and possible useful results will have to be weighed against the established clinical tools.

Therapeutic perspectives are at present mainly focussed on ARDS. Here, the first results of clinical trials will soon be available and are awaited with interest. Other indications of surfactant therapy are not yet clearly visible and remain highly speculative. However, the known role of the surfactant system in pulmonary host defense mechanisms and local immunomodulation will continue to stimulate clinical interest in its role in inflammatory and immunologic disorders of the lung.

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