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REVIEW

Pulmonary surfactant in health and human lung diseases: state of the art

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ABSTRACT: Pulmonary surfactant is a complex and highly surface active material composed of lipids and proteins which is found in the fluid lining the alveolar surface of the lungs. Surfactant prevents alveolar collapse at low lung volume, and preserves bronchiolar patency during normal and forced respiration (biophysical functions). In addition, it is involved in the protection of the lungs from injuries and infections caused by inhaled particles and micro-organisms (immunological, non-biophysical functions).

Pulmonary surfactant can only be harvested by lavage procedures, which may disrupt its pre-existing biophysical and biochemical micro-organization. These limitations must always be considered when interpreting *ex vivo* studies of pulmonary surfactant.

A pathophysiological role for surfactant was first appreciated in premature infants with respiratory distress syndrome and hyaline membrane disease, a condition which is nowadays routinely treated with exogenous surfactant replacement. Biochemical surfactant abnormalities of varying degrees have been described in obstructive lung diseases (asthma, bronchiolitis, chronic obstructive pulmonary disease, and following lung transplantation), infectious and suppurative lung diseases (cystic fibrosis, pneumonia, and human immunodeficiency virus), adult respiratory distress syndrome, pulmonary oedema, other diseases specific to infants (chronic lung disease of prematurity, and surfactant protein-B deficiency), interstitial lung diseases (sarcoidosis, idiopathic pulmonary fibrosis, and hypersensitivity pneumonitis), pulmonary alveolar proteinosis, following cardiopulmonary bypass, and in smokers.

For some pulmonary conditions surfactant replacement therapy is on the horizon, but for the majority much more needs to be learnt about the pathophysiological role the observed surfactant abnormalities may have.

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Pulmonary surfactant components and their dysfunction

Pulmonary surfactant is a complex and highly surface active material composed of lipids and proteins which is found in the fluid lining the alveolar surface of the lungs. Surfactant plays a vital role in pulmonary physiology. Its major biophysical functions are to prevent alveolar collapse at low lung volume and to preserve bronchiolar patency during normal and forced respiration, and its major nonbiophysical, immunological, functions are the protection of the lungs from injuries and infections caused by inhaled particles and micro-organisms.

A pathophysiological role for surfactant was first appreciated in premature infants with respiratory distress syndrome (RDS) and hyaline membrane disease, a condition which can nowadays be treated by means of exogenous surfactant replacement. Various other lung diseases are associated with surfactant abnormalities, and in some of these diseases replacement therapy is on the horizon. In this article, the data on the human surfactant system in health and in various disease conditions are reviewed and an overview of potential dysfunctions is given.

The composition and structure of pulmonary surfactant

Pulmonary surfactant is heterogeneous with respect to biochemical composition, morphological organization and specific biophysical functions [1]. Biochemically, pulmonary surfactant is composed of approximately 90% lipid and 10% protein, the latter representing the four surfactant-associated proteins surfactant protein (SP)-A, SP-B, SP-C and SP-D, as well as a large number of other, mostly serum-derived, proteins. A schematic illustration of these components and their relative sizes is given in figure 1.

The majority of pulmonary surfactant lipids are phospholipids. The most abundant phospholipid, phosphatidylcholine, is largely disaturated dipalmitoylphosphatidylcholine (65%), which plays an essential role in decreasing surface tension. Pulmonary surfactant also contains a relatively large portion of phosphatidylglycerol. Studies suggest that, in surfactant, phosphatidylglycerol can be replaced by another negatively charged phospholipid, namely phosphatidylinositol, without affecting the surfactant's properties of lowering the surface tension at the air–water interface from ~70 $mN \cdot m^{-1}$ at a pure water–air

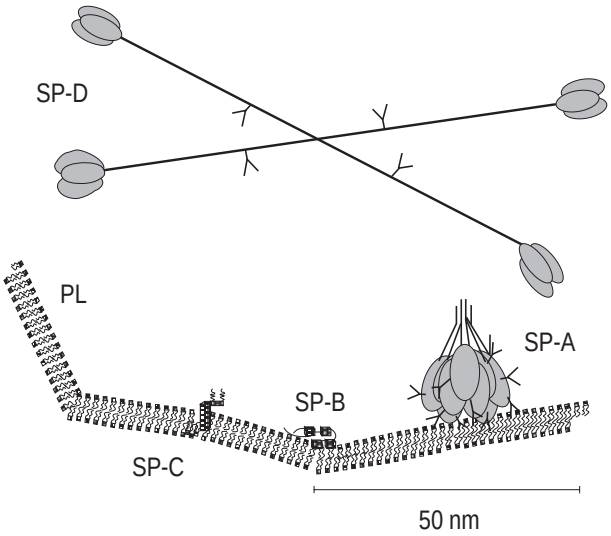

Fig. 1. – Schematic structure and relative size of the principal components of pulmonary surfactant. The phospholipids (PL) are shown in the form of a bilayer, omitting the various other structural forms of organization demonstrated by electron microscopy, *e.g.* vesicles, sheets, tubular myelin, and lamellar bodies. Surfactant protein (SP)-A is shown as an octadecamer with a bouquet-like appearance, and SP-D is depicted as a dodecamer with a cross-like structure. The carbohydrate recognition domains of these two proteins are shown as globular formations. SP-B is shown as a monomer, although a significant amount of dimers are present in the airspaces. SP-C is depicted as an integral membrane monomer. PL: phospholipids.

interface to approximately 0–1 $mN \cdot m^{-1}$ during expiratory compression [1]. Little is known about the role of the other lipid components, of which cholesterol is the most abundant (approximately 10% by mass), the other neutral lipids occurring in trace amounts only.

The most abundant SP by weight is SP-A. The SP-A monomer (molecular weight approximately 32 kDa) is a glycoprotein with three distinct structural domains [2, 3]. A long stretched collagenous domain is connected *via* a linking region (possibly responsible for the binding of phospholipids) to a globular region. This region contains a calcium-dependent carbohydrate recognition domain (CRD) which is able to bind both lipids and type II cells, as well as other structures (*e.g.* surfaces of micro-organisms). A complex oligosaccharide is also attached to this region of the SP-A molecule. Because of their mixed collagen-like and globular structure, such molecules are called collectins. The fully processed and secreted form of SP-A consists of 18 SP-A monomers (octadecamer or six trimers), organized by means of covalent disulphide bridges and noncovalent interactions in the shape of a bouquet of tulips (fig. 1). The two genes for human SP-A are located on chromosome 10 and are expressed in alveolar type II cells, bronchiolar Clara cells and airway submucosal gland cells. SP-A and SP-B (see below) both have a role in the conversion of endogenous surfactant into tubular myelin. SP-A accelerates the adsorption of surfactant phospholipids at the air–water interface, stimulates the defence system which depends on macrophages [4], reduces the inhibitory effect on surface activity of the nonsurfactant proteins within the alveolar space and possibly plays a role in the regulation of surfactant homeostasis, since it inhibits surfactant secretion and increases the uptake of surfactant by type II pneumocytes [5, 6].

SP-D is the second hydrophilic surfactant protein and also a collectin [3, 4, 7]. The collagen-like domain of SP-D is much larger than that of SP-A and is attached directly, without a connecting region, to the CRD domain. The molecular weight of the SP-D monomer is approximately 43 kDa. The native SP-D found in the lungs consists of 12 SP-D monomers, three of which are joined to form a trimer. Four trimers form a cross-shaped molecule (fig. 1), as demonstrated by electron-microscopic investigations. This cross-like structure (width of the molecule approximately 92 nm) may bind to bacterial lipopolysaccharide (LPS) and to cell surfaces, forming larger networks of cells or bacteria. In addition, a receptor which binds SP-D independent of its CRD domain has recently been identified on alveolar macrophages [8]. SP-D is also expressed in type II cells and in Clara cells, the gene being located on chromosome 10. The majority (70%) of SP-D is found dissolved in the watery surfactant residue, whereas SP-A, SP-B and SP-C are almost entirely found in association with lipids. SP-D is able to bind phosphatidylinositol and ceramides but not much is known about its influence on the regulation of surfactant homeostasis. Recently, however, disturbances of surfactant metabolism have been reported in SP-D knock-out mice. SP-D does not play a role in the biophysical functions of surfactant.

Intra-alveolar SP-B is a hydrophobic, positively charged molecule with a molecular weight of approximately 8 kDa. SP-B is coded for by a gene on chromosome 2, which is expressed in the lung by type II cells and Clara cells. A large preprotein is processed intracellularly to form the active SP-B molecule (fig. 1). SP-B is found mainly in the form of a dimer in the alveolar space, with two SP-B molecules linked to each other *via* disulphide bonds. The main function of SP-B is to accelerate the formation of a surface active film composed of phospholipids at the air–water interface by means of an increase in the adsorption rate by a factor of >150. This effect is further accelerated by the presence of calcium ions such that mixtures of phospholipids and SP-B display almost the same biophysical properties as whole lung surfactant. SP-B in conjunction with SP-A and calcium ions is also involved in the formation of tubular myelin. SP-A is found at every vertex of the lattice structure of these aggregates and determines the distance by which the lipid lamellae which are associated with SP-B are separated.

SP-C is the only surfactant protein which is expressed exclusively by type II cells in the mature lung. The human gene is found on chromosome 8 and SP-C, too, is translated as a larger preprotein and processed intracellularly. The active molecule is a very hydrophobic polypeptide to which two palmitoyl groups are attached *via* covalent bonds (molecular weight 4 kDa) (fig. 1). The main function of SP-C is to maintain the biophysical surface activity of the lipids. This occurs through an acceleration of the rate of adsorption at the air–water interface as well as through an increase in the resistance of surfactant to inhibition by serum proteins or by oedema fluid. SP-B and SP-C also increase the uptake of phospholipids into type II pneumocytes. SP-C stabilizes the surface activity of the surfactant film during the expansion and compression involved in breathing.

Biophysical functions of pulmonary surfactant (table 1)

The notion that surface tension is more important than tissue elastic forces for the retractive force of the lungs at all levels of inflation was first expressed by NEERGAARD [9] in 1929. The surface tension of the alveolar air–water interface provides this retractive force opposing lung inflation. The law of Laplace illustrates that the difference in pressure between the airspace and the lining (ΔP) depends only on the surface tension (T) and the radius of the alveoli ($\Delta P=2\ T/r$). The presence of surfactant in the fluid film can lower air–water surface tensions to near zero values (table 1). This ensures that the alveolar space remains open during the whole respiratory cycle, thus preventing intrapulmonary shunts resulting in inadequate oxygenation of the blood, and this also leads to reduced work of breathing.

Increasing evidence suggests that surfactant is needed not only in the alveolar part of the lung but also in the bronchioli through which air is conducted to the alveoli [10–12]. *In vitro* and *in vivo* studies have shown that a lack of surfactant leads to closure of the small cylindrical airways. In addition to this, the presence of phospholipases, proteases and exuded plasma proteins, in inflamed airways might severely disrupt the functional ability of surfactant to keep the conducting airways open [13].

Low surface tension is also important for ensuring that a net fluid flow is directed from the alveolar space into the interstitium [14]. This mechanism is of particular importance in the alveoli, because of their small diameter. In such areas, with a relatively high surface tension, a thicker fluid film may develop. Thus a well-functioning surfactant keeps the alveoli clear of liquid while also maintaining a thin fluid film. A lack of surfactant, conversely, leads to the accumulation of oedema fluid in the airspace.

Lastly, pulmonary surfactant is believed to play a role in the physical removal of particulate material from the alveoli and small airways by means of the displacement of particles into the hypophase and improvement of mucociliary clearance.

The molecular details of surfactant dysfunction are largely unknown. Some of the mechanisms which may lead to impaired surfactant function in pathological states are listed in table 2 and will be referred to when the individual diseases are discussed.

The functions of surfactant in host defence

The phospholipid components in large abundance under normal conditions (in neonates, phosphatidylcholine, phosphatidylglycerol and phosphatidylinositol) have been shown to suppress various lymphocyte and macrophage immune functions, whereas SP-A and SP-D have been demonstrated to activate several immune cell functions (table 1) [3, 4]. However, there is as yet no information available on the *in vivo* relevance of these findings.

SP-A specifically interacts with alveolar macrophages and increases the intensity of their respiratory bursts, migration, chemotaxis and complement-dependent and independent phagocytosis. While SP-A stimulates the formation of cytokines and immunoglobulins by lymphocytes, the surfactant lipids inhibit lymphocyte proliferation and immunoglobulin production. SP-A binds to LPS, group A streptococci, pneumococci, *Staphyloccus aureus, Mycobacterium tuberculosis, Haemophilus influenzae* type A, influenza A virus, herpes simplex virus type 1, candida and *Pneumocystis carinii*. Specific binding of SP-A to carbohydrates such as asialo-GM2, Galactosylceramide and gp 120, amongst others also takes place [3, 4]. SP-A also binds to specific receptors on type-II cells and is probably involved in the regulation of surfactant secretion and reuptake.

For SP-D there are no functions known that are related to the biophysical activity of surfactant. This molecule may be of great importance for the nonadaptive defence system of the lung. SP-D has specific binding sites on alveolar macrophages, can induce a "respiratory burst", and stimulates their phagocytotic activity. SP-D also binds to polymorphonuclear granulocytes, LPS, *Escherichia coli, Pseudomonas aeruginosa*, Influenza A virus and *P. carinii.* The precise overall roles played by SP-A and particularly SP-D in pulmonary host defence have yet to be elucidated [4].

Extracellular surfactant metabolism

After synthesis by type II pneumocytes, surfactant is secreted into the alveolar space. This process of exocytosis is regulated by various stimuli [15, 16] and dependent on ontogenesis [17]. In the alveolar space and in the presence of calcium, SP-A and SP-B, the highly surface active

Table 1. – Functions of pulmonary surfactant

Biophysical functions of surfactant
Prevents collapse of the alveoli and lungs during expiration
Supports inspiratory opening of the lungs
Prevents lung oedema formation by balancing hydrostatic filtration forces
Stabilizes and keeps small airways patent
Improves mucociliary transport
Translocates particles <6 μm into the hypophase of the epithelial lining fluid
Facilitates removal of particles and cellular debris from the alveoli into the large airways by lowering surface tension during end-expiration
Immunological, nonbiophysical surfactant functions
Phospholipids suppress the proliferation, immunoglobulin production and cytotoxicity of lymphocytes
Phospholipids inhibit endotoxin-stimulated cytokine (TNF, IL-1, IL-6) release from macrophages
SP-A and SP-D modulate the phagocytosis, chemotaxis and oxidative bursts of macrophages
Neutralization of endogenous mediators like radicals and reactive oxygen species
SP-A and SP-D opsonize various micro-organisms for easier phagocytosis
Binding and capture of bacterial toxins by SP-A and SP-D

TNF: tumour necrosis factor; IL: interleukin; SP: surfactant protein.

Table 2. – Potential mechanisms leading to impaired biophysical surfactant function in the lungs

Reduced amount of whole surfactant complex
Altered proportions of individual surfactant components (*e.g.* PC, DPPC, PG, PI, SP-A, SP-B, SP-C)
Increased amounts of "nonsurfactant" phospholipids (*e.g.* PE, PS, LPC)
Damage caused by lipolytic or proteolytic degradation
Oxidative degradation or inactivation of surfactant components
Lack of functionally active surfactant fraction (*e.g.* tubular myelin, large aggregate forms)
Impaired enzymatic conversion of large into small surfactant aggregates
Presence of large amounts of inhibitory compounds in the alveolar and bronchiolar airspaces (*e.g.* fibrinogen, amino acids)

PC: phosphatidylcholine; DPPC: dipalmitoyl-PC; PG: phosphatidylglycerol; PI: phosphatidylinositol; SP: surfactant protein; PE: phosphatidylethanolamine; PS: phosphatidylserine; LPC: lyso-PC.

tubular myelin is formed. From these structures, lipids can rapidly adsorb to the air–water interface and form a surfactant film. It is not yet clear whether the film is composed of a molecular monolayer or of several layers of phospholipids. When the surfactant film is compressed and decompressed during breathing, the nonsaturated phospholipids and protein components are squeezed out, leading to an enrichment of dipalmitoylphosphatidylcholine and so to a reduction in the surface tension to very low levels. Surfactant vesicles, in both uni- and multivesicular form, are created within the aqueous hypophase. The smaller vesicles are taken up preferentially by the type II pneumocytes and reutilized for surfactant synthesis. Under normal conditions, approximately 50% of the surfactant present in the alveolar space is in the form of functionally active large aggregates (LAs), and approximately 50% in the form of small surfactant vesicles (small aggregates (SAs). This ratio is established in the neonatal period, during the first 24 h of life, and can be changed in pathological states [18]. Although an enzymatic activity appears to be involved in these processes, the exact sequence of individual surfactant forms are still not clearly understood.

Techniques for the recovery of surfactant from the lungs

Pulmonary surfactant, found in the alveolar space, can only be harvested by lavage procedures, using a bronchoscope or a catheter and blind suctioning. During this procedure, the normally air-filled airspaces which are covered by a very thin film of epithelial lining fluid are flooded with saline. This process disrupts the pre-existing biophysical and biochemical organization of this microenvironment and may generate surfactant forms that do not exist *in vivo* and mix together forms that are separated *in vivo*. In addition, the fluxes of fluid and solutes between the interstitial or vascular compartment and the alveolar space introduce some major uncertainties that make precise estimation of the amount of epithelial lining fluid sampled and the dilution from the procedure itself impossible [19]. This is not an insurmountable limitation in studies of the surfactant system in health and under various disease conditions, but this limitation must always be considered when interpreting *ex vivo* studies of pulmonary surfactant.

For bronchoalveolar lavage (BAL) the bronchoscope is wedged in segmental or subsegmental bronchi, thus including the airway surfactant material of some 15–18 generations of bronchi and bronchioli into the total lavage sample. However, the majority of this airspace material is thought to derive from alveolar surfactant which has been transported by ciliary beating and other mechanisms. SP-A and SP-D are also produced within the airways. Therefore, it appears reasonable to separately analyse the sequential BAL aliquots, *i.e.* to separate at least the first and the following pooled samples. However, this has rarely been performed in studies of human surfactant. Whereas the use of a bronchoscope as opposed to blind suctioning is not expected to make much difference (no direct comparisons are available), the total amount and the size of the aliquots of lavage fluids instilled appear to be of great importance. In children <20 kg body weight (bw), often 3 or 4 aliquots of 1 mL·kg bw^{-1}, and, in persons >20 kg bw, 20 mL aliquots up to a total of 3 or 4 mL·kg bw^{-1} have been used for BAL. Others have used 40–60 or 100 mL aliquots in adults. In adults, no differences in differential cell counts are observed with these volumes [20]. When a lower volume is instilled, the more proximal airspaces are more likely to be sampled. For routine use, for all age groups, a total volume of 4 mL·kg bw^{-1} is proposed. It should be instilled in aliquots of 1 mL·kg bw^{-1} and the initial (bronchial) aliquot can be separated from the three successive (alveolar) aliquots. Lastly, even differences between different regions of the lungs may exist. Therefore, the sampling site should be consistent and indicated in the methods [21].

When the cells are separated from the lavage fluid, centrifugation forces of much $>200 \times g$ should be avoided in order to prevent some of the larger aggregated forms of surfactant (*e.g.* tubular myelin) being lost to the cell pellet. Importantly, the lavage fluid should not be frozen before processing the cells. The lavage supernatant may be analysed as such or separated further by differential centrifugation into various fractions (fig. 2). Unfrozen material is preferred; if this is not possible, it should be indicated. A surfactant-rich pellet (LAs) is generated by centrifugation at $28{,}000–73{,}000 \times g$. A number of groups use $40{,}000 \times g$ [18, 23–25]. The supernatant obtained from this centrifugation step is the SA fraction of the surfactant. A somewhat more purified surfactant fraction can be obtained by differential density gradient centrifugation [26, 27], but these methods have been used rarely for lavage samples from humans. Although not all biochemical and biophysical surfactant markers have been investigated, relatively good agreement has been demonstrated for some parameters between density gradient centrifugation and the more simple centrifugation procedures [22].

Material sampled by bronchial lavage differed in biochemical composition from that sampled by BAL, but was similar to sputum [28, 29]. The latter has also been used as

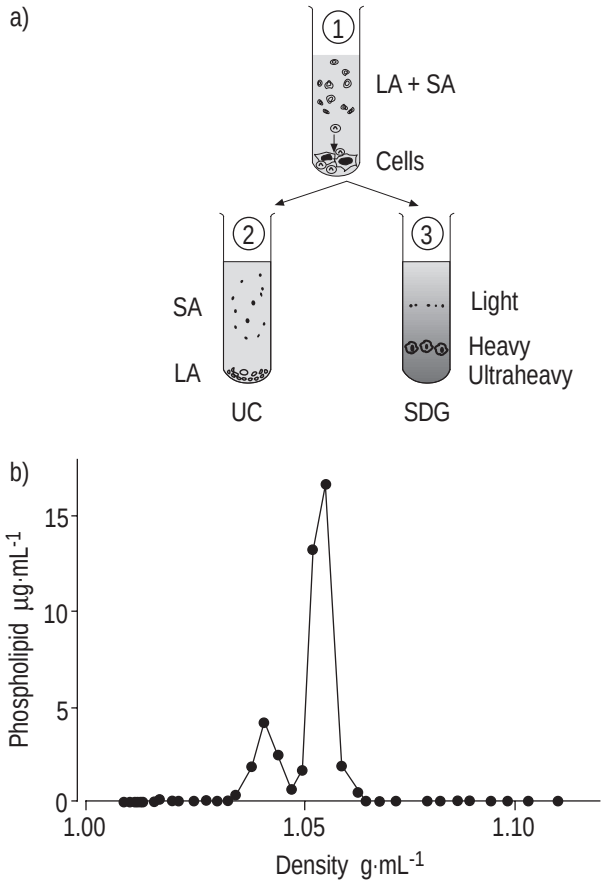

Fig. 2. – Preparation of surfactant from bronchoalveolar lavage fluid. a) After removal of the cells at low speed ($200 \times g$) to prevent the large surfactant aggregates (LA) from sedimenting, LA and small surfactant aggregates (SA) are separated by means of either ultracentrifugation (UC) at $40,000 \times g$ or, on the basis of their density, sucrose (SDG) or other density gradient centrifugation. b) Sucrose density gradient centrifugation profile. The ratio of SA/LA recovered with the two methods is comparable (author's own unpublished results and additional information from VELDHUIZEN *et al.* [22]).

a noninvasive source of material, however sputum is a mixture of surfactant from the alveoli as well as inflammatory cells and material secreted from mucous and other glands in the air space. It is, therefore, not easy to separate a fraction of surface active material from sputum [29].

For neonates or infants on ventilators low volume lavage procedures (approximately 1 mL·kg bw^{-1}) are routinely used and appear to sample rather distal airspaces [18, 30], as demonstrated by the biochemical surfactant profile and the morphological demonstration of lamellar bodies, but the exact area of the lavage is not known. Recently it was shown that, in neonates, there were no differences in the biochemical composition of the secretions with respect to a small collection of markers irrespective of whether or not additional fluid was instilled [31]. A direct comparison of some biochemical markers showed greater similarities between newborn tracheal aspirates and bronchial lavages than BALs [28]. Similar to BAL, no good marker for dilution by the lavage procedure exists [32].

It is very important to realize that none of the many studies reviewed in this paper used identical methods for harvesting surfactant from the lungs and analysis of its properties. Therefore, all comparisons and conclusions must be made with great care and have to be related to their own controls. Also, BAL fluid recovery must not only be given but should also be considered when calculating the results, as this may change completely the conclusions to be drawn [33]. Reasonable technical recommendations for the lavage procedure are awaited to allow better standardization (Task Force of the European Respiratory Society). Lastly, the interpretation of BAL data in functional terms is most difficult, since the relevance of subtle changes in the quantity of alveolar lining fluid components is just beginning to be explored.

Status of pulmonary surfactant in humans

A large amount of data with relevance to the human lung surfactant system under normal and various pathological conditions has been collected, using approaches which differed to a greater or lesser extent with respect to patient selection and methods used. Only those studies reporting data in appropriate units, *e.g.* expressed per lavage volume recovered, were included in the analysis. However, in order to allow an estimate of the order of magnitude of the parameter, the means or medians of the major biochemical and biophysical surfactant parameters were collected and the means calculated (table 3). Valuable additional information not fitting into this format is given in the text. For the sake of clarity, the various diseases were grouped into certain categories, knowing that there is substantial overlap. Instead of reporting the numerical results of the individual studies, the data are summarized in table 4 by indicating the qualitative changes observed. Each symbol represents the result obtained in comparison with the appropriate control group.

Healthy controls and smokers

No systematic and large scale studies of the pulmonary surfactant system in healthy adults are available. However, as there is a wide range of variation even within normal subjects, each lavage study should analyse its own control groups for comparison. Most of the variation is likely to be caused by the different methods used to obtain the lavage fluids, prepare the surfactant and analyse its composition. An appropriate meta-analysis cannot be performed on these heterogeneous studies. Generally, in the lavage supernatant obtained from healthy adults phosphatidylcholine and phosphatidylglycerol together make up approximately 80% of total phospholipids and the surfactant-specific proteins represent <10% of total protein (table 3). The minimal surface tension varies widely and several studies reported values in healthy control subjects well above 0 mN·m^{-1} [23, 37, 47]. These differences may, in part, relate to the different methods used for lavage and sample preparation, as low minimal surface tensions were obtained with complete natural surfactants, but not with lipid extracts, by some of these groups [29]. Issues related to the technical differences between the various pulsating bubble surfactometers are currently being addressed in a European multicentre quality control trial of various laboratories operating a surfactometer.

In healthy children and neonates, it is not possible, for ethical reasons, to perform lavage procedures solely to obtain representative data. However, lavages may be

Table 3. – Surfactant in bronchoalveolar lavage fluid from healthy persons

Component	No. of studies	Content or activity
Total protein mg·mL^{-1}	13	0.09±0.03 (0.04–0.15)
Total phospholipid mg·mL^{-1}	22	0.04±0.03 (0.01–0.13)
Phospholipid class % total		
Phosphatidylcholine	23	68.7±9.3 (53.1–83.8)
Phosphatidylglycerol	19	12.6±4.7 (8.3–27.4)
Phosphatidylinositol	17	4.1±3.3 (1.2–13.5)
Phosphatidylethanolamine	21	5.3±4.9 (0.3–21.0)
Phosphatidylserine	16	2.3±1.6 (0.0–5.7)
Sphingomyelin	20	3.3±2.6 (0.8–8.3)
Lysophosphatidylcholine	17	1.0±1.1 (0.0–4.5)
Surfactant proteins (SP) μg·mL^{-1}		
SP-A	12	4.5±4.8 (0.8–15.0)
SP-B	4	4.9±7.0 (0.7–15.3)
SP-C	0	ND
SP-D	2	1.1±0.3 (0.9–1.3)
Minimal surface tension mN·m^{-1}		
Bubble surfactometer	5	9.6±10.5 (0–23)
Wilhelmy balance	3	24.3±13.6 (9–35)

Data are presented as mean±SD with range in parentheses, and were calculated from 33 studies [21, 23, 25, 34–63] in which the results were expressed as concentrations in the volume recovered. These studies used relatively small numbers of subjects (14.0±8.2, range 4–50). The experiments with the pulsating bubble surfactometer were performed at various phospholipid concentrations (2.7±1.3 mg·mL^{-1}, range 1.8–5) and values obtained after >3 min were used for calculations.

performed in all age groups during anaesthesia for elective surgery for other reasons in children without pulmonary diseases. Concentrations of SP-A and total phospholipid appear to be age-dependent [64]; however, in that study, the number of individuals was rather low and, for technical reasons, the amount of lavage fluid instilled per syringe was only increased with weight in children weighing <20 kg. Above that weight, *i.e.* from approximately 8–10 yrs onward, multiple aliquots of 20 mL were used. The nonlinearity associated with this technical modality may have contributed to this result.

A very early study on BAL fluid from smokers showed reduced levels of total phospholipid [65], whereas in later studies these were normal [44, 49, 50] or even increased [46]. The markedly reduced level in the study of FINLEY and LADMAN [65] may be explained by the lower recovery of BAL fluid in heavy smokers, which returned to normal with cessation. Overall the phospholipid profile did not alter very much; two studies demonstrated increased fractions of phosphatidylethanolamine [46, 50], whereas one did not [49]. The levels of the surfactant proteins SP-A and SP-D were reduced [44]. In addition, the surface activity was impaired [60, 66]. The functional relevance of these findings in smokers are not yet clear. Reduced levels of SP-A and SP-D might be associated with impaired innate host defence [4], and thus contribute to the greatly increased rates of respiratory tract symptoms present in smokers, especially with the increased mortality from influenza and pneumonia [67]. Importantly, smokers cannot be included in groups of healthy controls in studies on BAL.

Obstructive lung diseases

The potential role of pulmonary surfactant in obstructive airway disease has recently been reviewed in detail [68]. Unfortunately, there is not yet much human data available clearly supporting a significant pathophysiological role for a deficient surfactant system in obstructive lung disease (table 4).

Asthma. SAHU and LYNN [69] characterized the lipid and fatty acid composition of lavage fluids in great detail; unfortunately, they did not have sufficient material from healthy volunteers for comparison. In children, lavage levels of phosphatidylcholine were reduced [87]. Recently, it was reported that, during an acute asthmatic attack, the surface activity of sputum is reduced and that it recovers with improved clinical condition [70]. Segmental allergen challenge in asthmatics results in functionally impaired surfactant which cannot maintain the patency of the small bronchiolar airways [88]; this was mainly caused by increased protein leakage into the airspaces. In stable asthmatics, SP-A was found to be reduced (table 4) [62].

Bronchiolitis. A deficiency in SP-A, dipalmitoylphosphatidylcholine and surfactant function was demonstrated during acute viral bronchiolitis in infancy, induced by respiratory syncytial virus (table 4) [71].

Chronic obstructive pulmonary disease. In nonasthmatics chronic obstructive pulmonary disease (COPD) patients who were smokers, a marked (6–7-fold) decrease in total phospholipid in BAL fluid was found with almost no changes in phospholipid composition [89]. Unfortunately, cigarette smoking, which is a major cause of COPD, itself induces the same changes (see above), thus making it impossible to differentiate between the two conditions on the basis of the available data. Also, normal phospholipid composition, in COPD, has been reported (table 4) [39].

Lung transplantation. In animal experiments the role of surfactant in the preservation of lungs during storage before transplantation, reduction of reperfusion injury and graft function after lung transplantation have been investigated for a long time, but only recently have data become available for the human system. In adult lung transplant recipients pulmonary surfactant activity was impaired irrespective of episodes of infection or rejection [72]. The ratio of SAs to LAs was increased and a reduced content of SP-A has previously been reported [73]. No correlations of surface activity with pulmonary function data or time after transplantation were observed. Thus, a persistent impairment of biophysical surfactant properties was found which may contribute to graft dysfunction. The potential benefit of exogenous surfactant therapy needs to be assessed in these patients. In summary, there is increasing evidence for significant contributions of surfactant disturbances to the pathology of obstructive lung diseases. These are likely to be related to biophysical impairment of surfactant function, especially in the small airways. In addition, decreased levels of SP-A suggest altered lung collectin function in these diseases. Many more data on humans are needed to fully evaluate these long-standing and intriguing hypotheses.

Table 4. – Surfactant recovered from bronchoalveolar lavage in humans with lung diseases

Disease	Protein	Phospho-lipid	Phospholipid class							Protein			Surface tension	[Ref]
			PC	PG	PI	PE	PS	SPH	LPC	SP-A	SP-B	SP-D	γ_{min}	
Smoker	==	====↑	==↓	==	==	↑↑=	==	===↑	=	↓		↓	=	[44, 46, 49, 50, 60]
Obstructive lung disease														
Asthma	↑	=	=							↓			↑	[62, 69, 70]
Bronchiolitis			↓↓							↓			↑	[71]
COPD		=	=	=	=	=	=	=	=					[39]
Lung transplantation	↑	=								↓			↑	[72, 73]
Infection and suppurative lung disease														
Cystic fibrosis	=	==	↓↓	↓	↑	=	=	↑	=	↓↑	==		↑=	[23, 47, 69]
Pneumonia	↑	↓=	==	↓↓	↑↑	==	==	↑↑	==	↓↓	=		↑	[25, 34, 39]
Pneumonia + ARDS	↑	↓	=	↓	↑	=	=	↑	=	↓	=		↑	[25]
AIDS + related pneumonia	↑↑	=	=	↑						↑				[53, 59]
No HIV, pneumocystis+										=				[61]
HIV+, pneumocystis+		↓							=	↑				[61, 74]
HIV+, no pneumocystis+		=							=	↓				[61, 74]
HIV+, pulmonary involvement	↑	↓	↓					=	=					[37]
HIV+, no pulmonary involvement	=	↓	↓					=	=	↑				[37, 75]
Acute lung injury and lung oedema														
ARDS	==↑↑↑	=====↓↑	↓↓↓↓↓==	↓↓↓↓↓↓=	↑↑↑↑↑=	↑↑↑↑==	===↑↑	↑↑↑↑↑=	====↑↑	↓↓↓	=↓		↑↑↑=	[25, 38, 39, 52, 54–56]
Hydrostatic lung oedema	↑	↑	↓	=	↑	↑	=	↑	=					[52]
Cardiogenic lung oedema	↑	=	=	=	=	=	=	=	=	=	=		=	[25]
Disease specific to neonates and infants														
RDS	===	↓↓↓	=↓	↓↓	=	=↑	=↑	=↑		↓↓			↑	[76–80]
BPD	=		↓											[35]
SIDS	↓	↓↓	↓	=		↑	↑	↑					↑	[80, 81]
Interstitial lung disease														
Sarcoidosis	=	===↓	====↓	===	==	===↑	===	===	==	=↑		=		[36, 40, 43, 48, 58, 62, 82]
Idiopathic pulmonary fibrosis	↑	↓↓↓↓=↑	======	==↓↓↓↓	↑↑↑	======	====↑	===↑↑	===	↓=		=		[34, 36, 40, 43, 45, 48, 51, 58]
Exogen allergic alveolitis	=	=↑	=↓	==	=	==	=	=	=	↑↑				[46, 48, 82, 83]
Interstitial pneumonia with collagen disease												=		[43]
Silicosis		↓	=	=		↑	=	=	=					[36]
Asbestosis		=								↑				[63]
Miscellaneous lung disease														
Pulmonary alveolar proteinosis		=↑	===↑↓	=↓↓	==↑	====	==↓	==↑↑	==↑↑	↑				[21, 39, 41–43, 57]
Eosinophilic granuloma		=	=	=	=	=	=	=	=					[40]
Microlithiasis	↑	↑	=	↑	=	=	↑	=	=	=				[84]
Irradiation of the thorax	↑↑	↑	↓↓	↓	=	=	=	↑		↓			↑	[85, 86]

Surfactant parameters are shown as unchanged (=), significantly increased (↑) or decreased (↓), as determined by the primary study in relation to the appropriate control group. Each symbol represents the data from one study. Not all studies measured all parameters. Data from a total of 34 studies in which the results were expressed as concentrations in the volume recovered were used. Other studies in which the results were reported as ratios compared to protein or other variables were excluded. These studies used relatively small numbers of subjects (14.0±8.2, range 4–50). The experiments with the pulsating bubble surfactometer were performed at various phospholipid concentrations (2 $mg \cdot mL^{-1}$, range 0.76–28.5) and values after >3 min were used for calculations. PC: phosphatidylcholine; PG: phosphatidylglycerol; PI: phosphatidylinositol; PE: phosphatidylethanolamine; PS: phosphatidylserine; SPH: sphingomyelin; LPC: lysophosphatidylcholine; SP: surfactant protein; γ_{min}: minimum surface tension; COPD: chronic obstructive pulmonary disease; ARDS: adult respiratory distress syndrome; AIDS: acquired immune deficiency syndrome; HIV: human immunodeficiency virus; RDS: respiratory distress syndrome; BPD: bronchopulmonary dysplasia; SIDS: sudden infant death syndrome.

Infections and suppurative lung diseases

Cystic fibrosis. Bronchial lavage studies in cystic fibrosis (CF) patients demonstrated an extremely decreased phosphatidylcholine content [90] and an increased mole fraction of arachidonic acid among the phospholipids [91]. The results are very similar to those reported for tracheobronchial surface active material obtained from sputum [29]. Although, the percentage of phosphatidylcholine was reduced, the concentration of SP-A was increased. The minimal surface tension of CF secretions was similar to that of secretions from adult patients with tracheostoma [29]. Compared to normal children, the surface activity of bronchial surfactant was worse in children with CF [92]. A recent study, using a lavage technique that very probably recovers mainly bronchial material in addition to alveolar surfactant, did not find any differences between very young healthy children with stridor and CF patients of a comparable age. However, another group of CF patients who were currently suffering from infection and inflammation (bacteria, increased interleukin-8 and lavage fluids neutrophils >50% of total cells) also had increased SP-A levels (table 4) [47]. This study suggested that there is no primary abnormality of bronchial surfactant in CF and that the ongoing endobronchial inflammation results in (secondary) surfactant abnormalities.

Studies on BAL fluid from somewhat older CF patients who had a chronic airway disease found severe alterations even in the alveolar compartment (table 4) [23, 69]. Impairment of surfactant function was mainly due not to inhibition by serum or other exuded compounds, but rather appeared to be related to a reduced concentration of SP-A and surface active phospholipid [23]. The reasons for the reduction in SP-A concentration may include altered recovery of lavage fluid from damaged airspaces, binding to mucus, reduced production or increased proteolytic degradation.

In summary, in CF, functional and biochemical surfactant abnormalities develop with progressing disease; this is supported by correlations between surfactant parameters and clinical or lung function data [23]. Additional studies which are more carefully related to the actual clinical presentation of the patients are needed.

Chronic bronchitis. Changes similar to those observed in CF have been reported in chronic bronchitis, but no good controlled studies are available [92, 93].

Pneumonia. Changes in pulmonary surfactant during bacterial pneumonia have been noted for a long time [94], but data from human subjects is scarce (table 4). Generally SP-A concentration was found to be reduced [25, 34, 95] and SP-B unaltered. In children with pneumonia, the level of phosphatidylcholine in lavage fluid was reduced [87]. Changes in the phospholipid profile appeared to depend on the type of pneumonia, being most pronounced in interstitial pneumonia [25, 39]. Surfactant in these diseases also had the worst surface activity in comparison to other severe lung diseases [25]. The fatty acid composition of the phospholipids was changed, palmitic acid (16:0) being significantly reduced [96]. These relatively consistent data support the view that functional surfactant abnormalities are associated with pneumonia. Almost all of the potential mechanisms leading to impaired surfactant function are likely to be involved to varying degrees (table 2). Altered surfactant composition during the course of pneumonia may be of especial functional relevance in critically ill patients needing mechanical respiratory support. The results from the first interventional studies are described below.

Acquired immune deficiency syndrome related lung disease. In patients with human immunodeficiency virus (HIV) and *P. carinii* pneumonia, a reduction in BAL fluid total lipids to approximately 50% was observed (table 4) [74]. This appeared to be mainly due to a decrease in phosphatidylcholine levels. In the lavage fluids an increased phospholipase A_2 activity was also noted. This increase in lipolytic activity, up to 30-fold, might be one of the mechanisms responsible for the decreased amount of total phospholipid in pneumonia (table 2). The lack of a concomitant increase in lysophosphatidylcholine and free fatty acid concentrations may be accounted for by rapid metabolism of these compounds [74]. In addition, further mechanisms, *e.g.* a reduced production of surfactant by alveolar type II cells, may operate (table 2). The exact pathophysiological relevance of increased levels of phosphatidylglycerol and cholesterol [59] are not yet precisely known. Others have also demonstrated increased percentages of phosphatidylglycerol (measured together with phosphatidylethanolamine) [37]. Interestingly, this is in contrast to most other conditions with perturbation of the surfactant system, like pneumonia, adult respiratory distress syndrome (ARDS), interstitial lung disease and, also, the immature lung (table 4). Similarly and very consistently, SP-A levels were increased in AIDS-related pneumonia [53, 75]. However, a decreased SP-A level was characteristic of HIV status itself in the absence of *P. carinii.* Indeed HIV-positive patients with pneumocystis had significantly higher SP-A levels than HIV-positive patients without [61]. Those patients who underwent BAL after 21 days of therapy for pneumocystis, and showed a complete resolution of the infection, showed a significant drop in their SP-A concentrations at follow-up lavage [61]. The relationship between BAL SP-A concentration and the amount of pneumocystis in these patients may be related to SP-A binding to pneumocystis in the airspaces [3] or to alterations in surfactant protein homeostasis with HIV infection. The increased attachment of *M. tuberculosis* to alveolar macrophages in the presence of BAL fluid from HIV-infected individuals, was identified as being caused by SP-A [75]. Thus, SP-A is believed to mediate the first critical step in the establishment of a tuberculosis infection in HIV-infected patients. Increased levels of SP-A in the presence of pneumocystis might, therefore, explain the increased risk of tuberculosis, even before there is a significant loss of CD4 lymphocytes [97].

In summary, the data clearly show specific abnormalities in the lipid and protein components of surfactant in HIV. It is tempting to speculate that, especially, interference with the host defence functions that are attributed to SP-A may be of pivotal relevance to the numerous pulmonary insults associated with progressive HIV infections. However, much more data from humans are needed to understand the relationship between surfactant components and cellular elements like lymphocytes, alveolar macrophages and alveolar epithelial cells and the various regulatory mediators released.

Acute lung injury/adult respiratory distress syndrome and pulmonary oedema

Pulmonary surfactant in ARDS is characterized by a decrease in the percentage of phosphatidylcholine [38, 52, 54–56] and phosphatidylglycerol in total phospholipids [25, 38, 39, 54–56], decreased concentrations of SP-A [25, 38, 56] and reduced surface activity [25, 38, 56], whereas the percentage of phosphatidylinositol in total phospholipids [25, 38, 52, 54–55] is increased (table 4). The changes in phospholipid profile observed in patients with sepsis-associated ARDS were very similar to those in patients with trauma-induced lung injury [54, 56]. A close inverse correlation between the phosphatidylcholine concentration and respiratory failure score [55] or arterial oxygenation [98] was observed. The ratio between SAs and the more surface active LAs was significantly increased in patients with ARDS in comparison to non-ARDS patients [24]. Although such alterations in surfactant were not observed in all ARDS patients, surfactant abnormalities are thought to contribute significantly to lung dysfunction, as demonstrated by successful trials of exogenous surfactant administration (see below). Very early, PETTY and coworkers [99, 100] had reported increased film compressibility, but normal minimum surface tension in patients with ARDS. It is very likely that several if not all of the mechanisms listed in table 2 are involved in the pathogenesis of the observed changes. The huge leakage of various plasma proteins into the lungs with consequent biophysical inactivation of the surfactant is of major importance. This was demonstrated by recombination experiments using proteinaceous supernatants from BAL samples from patients with ARDS. These markedly and dose-dependently inhibited surfactant function, in contrast to those from normal controls [25]. Subsequently, surfactant synthesis, surfactant secretion and other impairments in alveolar type II cell function appear to be of additional major importance.

Patients at risk of ARDS, *e.g.* after trauma and hypotension, multiple blood transfusions, sepsis, pancreatitis, near drowning or other insults [38], have already demonstrated decreased levels of total phospholipids and SP-A, increased lysophosphatidylcholine and a significantly altered surface activity. In addition to these findings in a study investigating sequential changes in surfactant parameters, the ratio of SAs to LAs was elevated and the static compliance of the respiratory systems was inversely related to minimal surface activity [101]. Thus, during the early clinical disease course with merely ARDS predisposition, profound alterations of the endogenous surfactant system are present. Whether these early abnormalities may be used as specific predictors of outcome is questionable as several other lung diseases exhibit similar changes. Overall, a knowledge of these biochemical and biophysical surfactant abnormalities in ARDS and their consequences such as atelectasis formation, loss of compliance, ventilation–perfusion mismatch, and lung oedema formation have resulted in several successful therapeutic approaches. The precise role of a new therapeutic modality, *e.g.* exogenous surfactant substitution, is currently being defined in clinical trials (see below). In addition, the impact of the surfactant abnormalities on host defence mechanisms, chronic inflammatory responses and repair processes including the generation of residual lung fibrosis are just beginning to be unravelled [3, 4].

In patients with hydrostatic pulmonary oedema, significantly reduced amounts of phospholipid were recovered by BAL. The phospholipid pattern was changed similarly to that noted in ARDS, except that the levels of phosphatidylserine, phosphatidylinositol and lysophosphatidylcholine were unaltered [52]. Although no assessments of functional surfactant activity were made, the authors hypothesized that the magnitude of the alterations alone was not sufficient to cause prolonged respiratory failure. In contrast, except for elevated total protein concentration, others did not find any differences with respect to phospholipid composition, SP-A and SP-B levels, and surface activity in their patients with cardiogenic lung oedema (table 4) [25]. SHIMURA *et al.* [102] noted increased levels of SP-A in sputum and aspirated airway secretions in patients with cardiogenic pulmonary oedema, ARDS and clinically stable congestive heart failure.

These data are in line with those reported for patients at risk of ARDS and support the view that secondary abnormalities of the surfactant system may develop very rapidly and early on in acute lung injury and pulmonary oedema. The functional relevance of such alterations needs to be tested in clinical trials aimed at correcting surfactant abnormalities or, better still, preventing their emergence.

Surgical procedures involving extracorporeal membrane oxygenation and surfactant function

Procedures which involve extracorporeal membrane oxygenation and hypothermia, *e.g.* for cardiac surgery, may induce an acute lung injury. Although rare in adults (<2%), the frequency increases in high risk groups, such as infants of <1 yr of age [103], older patients and with increased duration of extracorporeal membrane oxygenation and hypothermia. The lung injury is mainly initiated by shear forces and from contact of the venous blood with the nonphysiological surfaces of the extracorporeal circuit, resulting in activated platelets and polymorphonuclear granulocytes, mediator release and activation of the complement and kallikrein–kinin systems [104]. Infants with congenital cardiac lesions who were already undergoing mechanical ventilation because of respiratory failure and who were operated on with the support of a heart–lung machine, were subjected to lavage before and 1 h after cardiopulmonary bypass. The intervention increased the SA/LA ratio significantly, indicating a reduced amount of the surface active LA fraction; unfortunately no more direct assessment of the functional state of the surfactant was made [105]. Serial small-volume bronchial lavages were analysed in infants <1 yr of age who were operated on with the support of a heart–lung machine [106]. In agreement with the study of MCGOWAN *et al.* [105], GRIESE *et al.* [106] found impaired surfactant function as indicated by a deterioration in surface activity from day 0 to day 3 after bypass. The levels of total protein, phospholipid, SP-A and SP-B were increased on day 0 and 1 after bypass and then returned to the range of the normal control group [106]. These data suggested that there was a significant functional impairment of the surfactant activity that was not compensated for by a concomitant increase in SP-A and SP-B levels.

The most likely mechanism involved was surfactant inactivation by means of leakage of proteinaceous oedema fluid into the airspaces. In contrast to these findings, MARCATILI *et al.* [107] described reduced amounts of total phospholipid in BAL fluids 24 h and 8 days after surgery using extracorporeal circulation in adults. They also observed alterations in the phospholipid composition (decreased phosphatidylglycerol and increased phosphatidylinositol and sphingomyelin concentrations). All these changes were reported to be prevented by treatment with ambroxol. However, due to the very limited number of subjects (five in each of the two groups), the data must be interpreted very cautiously and further studies are necessary to precisely define the role of ambroxol.

In a heterogeneous group of infants with respiratory failure, SP-A level was decreased [108]. After being put on extracorporeal support (without hypothermia), the SP-A concentration recovered towards normal values with time. Lung compliance was also increasing; unfortunately, no other measurements on the surfactant system were made [108]. These data suggest that the lungs are able to recover despite ongoing insult from extracorporeal membrane oxygenation.

In summary, the available data clearly support the view that in high risk groups, such as infants, during extensive extracorporeal support and hypothermia, functional and biochemical disturbances to the surfactant will occur. Future studies should include additional control groups, *e.g.* patients also undergoing a cardiac operation but without extracorporeal support or hypothermia, to more precisely assign the potential different effects of these interventions.

Diseases specific for neonates and infants

Neonatal respiratory distress syndrome. AVERY and MEAD [109] were the first to directly document functional pulmonary surfactant deficiency in the watery lung extracts of infants dying from neonatal RDS (hyaline membrane disease). This was confirmed by several other investigators [110–118]. Immunohistochemical studies demonstrated a lack of SP-A in infants dying before 48 h of life and intense staining of proliferating type II cells for SP-A in those surviving >48 h [119].

In neonates with RDS, the most striking and consistent finding is a lack, or a greatly reduced amount, of phosphatidylglycerol [76, 78, 80, 120] in addition to increased surface tension [120, 121] and decreased amounts of total phospholipid and SP-A (table 4) [77, 79]. In contrast to most other diseases investigated, studies in neonates have primarily used tracheobronchial aspirates or small-volume lavages instead of BAL. This approach appears to be valid, although, as discussed above, the compartment that is sampled is likely to be somewhat more proximal in the lung.

Unfortunately, a large number of studies cannot be directly compared with these data or those obtained by BAL because the data are merely expressed as ratios of other parameters of the samples. However, some important features may be derived from these studies, *e.g.* an acceleration of pulmonary surfactant maturation in stressed pregnancies after prolonged rupture of the membranes and treatment with isoxuprine, and after treatment with corticosteroids or a delay in pregnancies with maternal diabetes and hypothyroidism [122, 123]. More detailed analyses have been performed on dipalmitoylphosphatidylcholine and its fatty acid composition in order to monitor the maturation of the surfactant system in RDS [124–126]. It is not clear whether the observed differences in phospholipid composition may differentiate infants with RDS with surfactant deficiency from those with transient tachypnoea of the newborn [127] or not [128]. The sensitivity of phosphatidylglycerol or of the lecithin/sphingomyelin ratio in predicting RDS was high (90–100%), but the specificity was relatively low (50–95%) [129]. Prenatal dexamethasone treatment had no effect on the concentration of surfactant phospholipids, but improved the surface activity of surfactant isolated from airway specimens, decreased the amount of, and inhibition by, nonsedimental proteins and increased the responsiveness to exogenous surfactant treatment [130]. Postnatal dexamethasone treatment had similar effects [131], and SP-D levels were also shown to be increased [132]. The lack of SP-A in infants with RDS increases their susceptibility to surfactant inhibitors [129, 133]. With recovery from RDS, the amount of SP-A [77, 134, 135] and the hydrophobic surfactant proteins increased [135]. The SP-A in infants with RDS exhibited a lesser degree of post-translational modifications than that from controls [134].

The complex changes occurring during the postnatal course in infants with RDS and exogenous surfactant administration have been used to estimate the surfactant half-life and turnover times of pulmonary surfactant components [76, 78, 126].

Taken together, these data give a detailed picture of the pulmonary surfactant system in neonates with RDS, showing decreased concentrations of total phospholipids, dipalmitolphosphatidylcholine, phosphatidylglycerol and SP-A, a reduced surface activity and the modulation of surfactant by various influences. The functional biophysical relevance of an impaired surfactant system is immediately demonstrated by surfactant substitution, as described below. Issues regarding the host defence aspects of surfactant in this age group are currently being addressed in ongoing studies.

Meconium aspiration syndrome. Although various *in vitro* and animal studies suggest surfactant dysfunction after meconium aspiration and surfactant administration appears to be of benefit (see below), no biochemical or functional data from human neonates have yet been presented.

Congenital diaphragmatic hernia. In infants with congenital diaphragmatic hernia, a primary surfactant deficiency is unlikely; however, a secondary surfactant deficiency after respiratory failure may be involved [136]. Thus, surfactant substitution might be of help in this condition.

SP-B deficiency. SP-B deficiency is a genetic disorder which occurs in (mature) newborns with severe respiratory distress at birth. Despite extracorporeal membrane oxygenation [137], glucocorticoids and exogenous surfactant substitution [138, 139], this condition leads to death within the first year of life. BAL reveals a lack of SP-B and abundant aberrant pro-SP-C. Immunohistological studies of lung tissue show quantitative and qualitative abnormalities of SP-A and SP-C [140]. The

ratio of phosphatidylcholine to sphingomyelin is reduced. Various mutations, including a mutation on chromosome 2 (121ins2), result in the same histological picture, *i.e.* an alveolar proteinosis. One infant, however, with the typical clinical picture of congenital alveolar proteinosis syndrome, had an abundance of SP-B [140]. Currently, lung transplantation represents the only treatment option [141]. Recently, transient SP-B deficiency has been reported in a term infant with severe respiratory failure [142]. These data show another example where analysis of the pulmonary surfactant system has resulted in the definition of new disease entities which are associated with a clearer definition of treatment options and prognosis.

Nosocomial infection in ventilated preterm neonates. Long after resolution of neonatal RDS, deterioration of respiratory function in ventilated premature infants during severe nosocomial infection is often observed. Gram-positive *Staphylococcus epidermidis* is the principal organism isolated from these extremely immature infants who suffer from relative immunodeficiency. During this period, the total amount of phospholipids recovered was decreased, in particular the content of phosphatidylcholine in the surfactant SA fraction was reduced [18]. A concomitant increase in lysophosphatidylcholine suggested increased activity of phospholipases during this type of hospital-acquired pneumonia in extreme neonates with relative immunosuppression. There were no other changes in the phospholipid composition. The surface activity of the surfactant recovered in the LA fraction was reduced during the peak of infection and returned towards normal levels afterwards; a close correlation with respiratory support, expressed as the oxygenation index, was observed [143]. The impaired surface activity was not explained by leakage of serum proteins into the airspaces. Unfortunately, no measurements of SP-A were made.

The data suggest secondary functional and biochemical surfactant abnormalities during sepsis and severe nosocomial infection of the lungs in these immature neonates. Although very difficult to carry out, more studies with the appropriate control groups are necessary, as well as controlled and prospective trials of the effect of exogenous surfactant therapy during such episodes.

Chronic lung disease of prematurity or bronchopulmonary dysplasia. The only available study suggests reduced levels of phosphatidylcholine, but no functional measurements have been performed so far (table 4) [35].

Sudden infant death syndrome. Surfactant isolated from infants who died of sudden infant death syndrome (SIDS) contained a reduced amount of phospholipid and had a composition that was altered to a similar degree to that found in RDS, except that the phosphatidylglycerol content was not decreased (table 4) [80, 81]. In a prospective study, a reduced content of dipalmitoylphosphatidylcholine was similarly found and appeared to be related to the presence of bacterial organisms with reported phospholipase A_2 activity, and not to other factors investigated [144]. In addition to these biochemical data, several studies have found consistent functional surfactant abnormalities, resulting in high minimum surface tensions and impaired hysteresis loops (table 4) [81, 145, 146]. Similar observations were made in two infants with recurrent cyanotic episodes [147]. In contrast, others found unchanged pressure–volume characteristics in whole lungs from infants who died of SIDS [148].

Taken together, these data strongly suggest primary or secondary surfactant abnormalities in infants dying of SIDS. Future studies assessing the genetics of pulmonary surfactant components in population based studies [149] might be helpful in identifying the subgroup at increased risk of SIDS

Interstitial lung diseases

Sarcoidosis. The majority of studies on patients with sarcoidosis do not suggest derangements in surfactant phospholipids [36, 40, 58, 62]. Only one of five studies showed a slightly decreased phosphatidylcholine content and an elevated level of phosphatidylethanolamine [48]. No measurements of surface activity have been reported. Whereas VAN DE GRAAF *et al.* [62] found unchanged levels of SP-A, HAMM *et al.* [82] reported increased SP-A and total protein. SP-D levels were unchanged (table 4) [43]. Although it is likely that a closer consideration of the disease state might reveal a more specific picture, based on the data reported, sarcoidosis does not appear to be a lung disease associated with major abnormalities of pulmonary surfactant.

Idiopathic pulmonary fibrosis. Several studies have shown reduced amounts of total phospholipid recovered from BAL fluid in patients with idiopathic pulmonary fibrosis (IPF) in comparison to normal volunteers [36, 40, 48, 58]. Others found slightly increased [51] or unchanged levels [45]. No correlations with the state of the disease were made. In addition, the percentage of phosphatidylglycerol [51] was reduced (table 4). In one study, the content of SP-A was unchanged [34], whereas it was reduced in another [51]. In a second study, these authors also showed that the reduction in SP-A predicted survival [150]. Thus, it is very likely that the surfactant alterations are specific for the disease state. The level of SP-D was in the range of normal controls [43].

In summary, IPF is associated with secondary alterations to the biochemical composition of pulmonary surfactant. In addition to a reduction in the total phospholipid, the phosphatidylglycerol fraction is decreased, whereas phosphatidylinositol is increased. Decreases in SP-A were predictive of survival. The value of SP-A in indicating outcome at a potentially reversible phase of the disease must be determined in future studies. The roles surfactant components may play in immunomodulation, especially during early disease states, need to be addressed.

Hypersensitivity pneumonitis. In acute hypersensitivity pneumonitis, also called exogenic or extrinsic allergic alveolitis, the total phospholipid concentration was unchanged [48] or increased [46], whereas the principal surfactant phospholipid phosphatidylcholine was reduced. There were no alterations to the other phospholipids. SP-A concentration was increased in BAL fluid [82, 83]. One month after treatment, SP-A levels were unchanged, although all patients were clinically improved [83]. Also, in alveolar macrophages, SP-A content was increased [151]. However, these data are difficult to interpret as it has been shown that SP-A antibodies detect blood group

A antigenic determinants and the blood group distribution in these patients is not known [152]. Although the pathophysiological role of the increased SP-A levels in this condition is unclear, it is very likely that, in addition to the known immunological consequences of the changes in surfactant lipids in hypersensitivity pneumonitis (see below), the immunomodulatory functions of SP-A are also of relevance. Future studies will have to clarify the exact modulatory role of SP-A to give new insights into the mechanisms of this disease and to open new therapeutic approaches. As in other interstitial lung diseases, no assessments of the surface activity of the surfactant material recovered have yet been reported (table 4).

Other interstitial lung diseases. In asbestosis, SP-A level appeared to be increased (table 4) [63]. In patients with silicosis, the total phospholipid recovered was reduced [36]. This finding is somewhat unexpected because rat animal models of silica-induced lung injury lead to alveolar proteinosis. Among other potential explanations, differences in the causative agent (complex natural silica dust *versus* purified silica slurry) or different disease states, which have unfortunately not been characterized very well, may be responsible for some of the changes.

Pulmonary alveolar proteinosis

Pulmonary alveolar proteinosis (PAP) is characterized by abundant periodic acid–Schiff (PAS)-positive material that fills the alveolar spaces. This material mainly represents pulmonary surfactant phospholipids and protein components. PAP is a heterogeneous group of diseases which are divided into a congenital form (SP-B deficiency, see *Diseases specific for neonates and infants*), paediatric forms and adult forms. For the paediatric forms of PAP, which are at least 10 times less frequent than the adult forms, no biochemical surfactant analysis is yet available in the literature. A male infant with PAP who presented with failure to thrive and atrophy of the intestinal villi and developed respiratory symptoms 2 months later has recently been observed by the author. This combination of atrophy of the villi and paediatric PAP may explain the failure to thrive often observed in other infants with PAP. Therapeutic BALs were performed on each side, one week apart. In the lavage fluids, the phospholipid concentration was increased 10–50-fold, total protein approximately 3-fold, and SP-B approximately 10–50-fold. The phospholipid composition (phosphatidylcholine 74%, phosphatidylglycerol 7%, phosphatidylinositol 5%, phosphatidylethanolamine5.7%,phosphatidylserine2.9%, sphingomyelin 1.5% and lysophosphatidylcholine 1.2%), concentrations of SP-A and SP-D and the surface activity (minimum surface tension=3 $mN \cdot m^{-1}$ at 3 $mg \cdot mL^{-1}$ phospholipids) were normal. The course in this child has been favourable for 3 yrs, not necessitating further whole lung lavage (unpublished results).

The surfactant system in adult PAP is relatively well characterized [21, 39, 41–43, 57]. The phospholipid composition of the PAS-positive material is typical of pulmonary surfactant, with minor variations which are found regularly. The percentage of phosphatidylglycerol is decreased, whereas sphingomyelin and lysophosphatidylcholine are increased (table 4). Unfortunately, there are almost no data on the biophysical properties of surfactant from PAP patients, which appears not to be reduced much [39, 57]. In an early outstanding paper, the lipid composition and *in vivo* synthesis of lipids in adult patients with PAP was described [57]. Similarly, AKINO and coworkers [41, 153, 154] have collected detailed information on the biochemical nature of the surfactant lipids [155] and surfactant proteins from PAP patients. Two oligomeric forms, alveolar proteinosis protein (APP)-I, consisting of large SP-A multimers of 70–90 μm in size, and APP-II, hexameric SP-A particles, were isolated and investigated regarding their effects on isolated type II epithelial cells [156, 157]. Recently, DOYLE *et al*. [21] described a great variety of immunoreactive SP-A isoforms, which differed widely among various patients, suggesting further heterogeneity of PAP patients at the level of the surfactant proteins. Increased SP-D (table 4) and SP-C content [158] are also characteristic of adult PAP. The high content of SP-A in sputum has been proposed as a means of noninvasive diagnosis of PAP [159].

Besides PAP of idiopathic origin, both the paediatric and the adult forms of PAP may be associated with infections (*M. tuberculosis, P. aeruginosa*, cytomegalovirus, herpes simplex virus, *P. carinii*, aspergillus, candida, *etc*.), haematological malignancies and immunodeficiency states [160–163]. Recently, impaired secretion of granulocyte-macrophage colony-stimulating factor has been reported to be the cause of a single case of a female with PAP [164]. The surfactant abnormalities in acute silicosis may be related to these alveolar lipoproteinoses (see above). Generally, in PAP, synthesis and secretion of surfactant appear to be intact; however, they are not balanced by adequate reuptake and removal of surfactant, which consequently accumulates in the airspaces.

Miscellaneous lung diseases

Surfactant abnormalities have been reported for some other rare, lung diseases, such as eosinophilic granuloma [40] and pulmonary alveolar microlithiasis (table 4) [84]. Unfortunately, lavages are often performed in these rare diseases but are seldom analysed with respect to pulmonary surfactant. Detailed surfactant analysis may lead to a broader understanding of the pathophysiology of some of these pulmonary diseases, which may have very similar clinical presentation.

Toxic effects on the surfactant system

A wide range of compounds exert toxic effects on the pulmonary surfactant system [165]. These have been almost exclusively explored in *in vitro* studies or in animal experiments. Well known are the oxidant gases (oxygen, ozone, nitrogen dioxide), inhaled particles (silica, metallic dusts containing nickel or cadmium, organic compounds from cotton, flax, hemp or other LPS-containing sources) or gases (chloroform, halothane, diesel exhaust) and systemically delivered substances such as drugs (bleomycin, combinations of anticancer drugs, the antiarrhythmic agent amiodarone, the anorectic agent chlorphentermine, clofibrate) or chemicals like the herbicide paraquat or *N*-nitroso-*N*-methylurethane.

However, in humans, it is not possible to relate the clinical impact of these agents unequivocally to their effect on the surfactant system. This has to do with the fact that most of the compounds have a broad range of effects (*e.g.* bleomycin results in subacute interstitial lung disease, pulmonary infiltrates or eosinophilia, bronchiolitis obliterans, acute permeability oedema and enlargement of the mediastinal lymph nodes) [66] and that multiple mechanisms of lung injury often result in similar surfactant changes (*e.g.* high inspired oxygen, lung injury from mechanical ventilation, pneumonia). There is no clinical entity in which a specific toxic effect on the surfactant system is the sole or principal manifestation of disease. In addition, species-specific differences, the dependency on specific disease states and on the developmental stage make a direct transfer of these data to humans impossible. Interpretation of the scarce data in humans on the toxic effects on pulmonary surfactant must consider this.

In amiodarone-induced pulmonary toxicity, only small changes in lavage phospholipid content were observed between patients with or without evidence of developing lung injury. However, the study was very much hampered by its design and the small number of patients investigated [167]. Following combination chemotherapy (methotrexate, doxorubicin, cylophosphamide, lomustine) for non-resectable lung cancer, in BAL fluid, the percentages of phosphatidylcholine and palmitic acid decreased and that of phosphatidylglycerol increased [168]. These results are difficult to interpret as other factors such as the lung cancer itself and other therapeutic- or disease-associated complications may interfere. Irradiation, both from external sources and from inhalation of nuclides such as plutonium-239 oxide, results in rapid and pronounced changes to type II pneumocytes and pulmonary surfactant. HALLMAN *et al.* [85] studied the BAL fluid from four patients with pleural mesothelioma before, during and at monthly intervals, up to 4 months after hemithorax irradiation (70 Gy) (table 4). The concentration of sphingomyelin increased 9-fold and saturated phosphatidylcholine and phosphatidylglycerol concentrations decreased approximately 4-fold and the SP-A concentration 7-fold and the surface activity was also much reduced. After radiotherapy, the soluble protein content increased 23-fold and reflected the composition of serum. The strong correlations between all of these biochemical parameters and vital capacity implied a role for surfactant defects in causing the progressive injury associated with irradiation of normal lung tissue [85]. Whereas total phospholipid concentration was almost constant in the former study, sequential lavages in a single patient who had undergone bone marrow transplantation and who had idiopathic interstitial pneumonitis after fractionated whole body irradiation (10 Gy total body dose, 8 Gy lung dose) showed increasing amounts of phospholipid being recovered from this patient over time [169]. A decrease in the concentration of phosphatidylcholine at 6–8 weeks and 3 months after radiotherapy was also observed in a larger study of 30 patients. Although analysis of the BAL fluid predicted the degree of radiation pneumonitis, computed tomography scans were superior for scoring radiation-induced lung injury [86].

In summary, it is likely that changes in pulmonary surfactant metabolism and function similar to those reported from animal experiments also occur in humans and contribute to overall injury. However, many more studies are necessary in order to assess their actual contribution in clinical conditions and to investigate the impact of designed exogenous surfactant supplementation.

Pathophysiological consequences related to impaired pulmonary surfactant and ways of their assessment

The pathophysiological impact of deviations in the biophysical and biochemical surfactant parameters assessed *ex vivo* in patients with lung diseases is very difficult to estimate directly. There are several reasons for this. Firstly, the pulmonary surfactant system has a large functional reserve before decompensation occurs. Secondly, there may be large local inhomogeneity within the lungs [21], which may be difficult to detect. Thirdly, there appears to be a high level of redundancy which compensates for specific defects with alternative biochemical compounds, *e.g.* substitution of phosphatidylinositol for phosphatidylglycerol [1]; similarly, the adaptive host defence will take over, if the surfactant-associated innate host defence mechanisms are overwhelmed. Fourthly, changes in lung mechanics may be related to a large number of factors other than the surface activity of pulmonary surfactant, which may also be relevant. Lastly, the sensitivity and specificity of only a few of the potential variables (*e.g.* phosphatidylglycerol, lecithin/sphingomyelin (L/S)-ratio, SP-A) are known for only some specific disease processes [77, 129]. Without doubt, an impaired surfactant system will be functionally deficient, but the tools to precisely diagnose this in a non-invasive manner are currently lacking.

The potential biophysical and immunological consequences that may be associated with specific pulmonary disease processes can be envisaged as an impairment of the nonbiophysical surfactant functions listed in table 1. These have been mainly derived from animal experiments and *in vitro* investigations. However, in assessing their relevance under clinical conditions in humans, the approach to be chosen depends on the question to be answered. If a deficiency is assumed, only interventional clinical trials in which the substitution of the lacking components are assessed, appear useful. If a surplus of stimulatory or regulatory activity is assumed, selective blockade or removal of the specific compound(s) may be helpful. Potential problems associated with this approach relate to difficulties in administering the correct component at the correct concentration, targeting the specific region in the lungs and competing with surfactant inactivators present in the lungs. Lastly, great care must be taken in selecting the appropriate variable, and monitoring the success of the procedure. Many more investigations in the field of the assessment of the pathophysiological consequences of dysfunctional surfactant are needed.

Trials of exogenous surfactant substitution – proof of a role of impaired pulmonary surfactant in disease states

Obstructive lung diseases

Asthma. A pilot study on the inhalation of a natural surfactant (Surfactant TA (Surfacten) (Tanabe, Tokyo,

Japan), 10 mg in adults), conducted as a double-blind, placebo-controlled trial showed improved respiratory function parameters in the 10–30% range during an acute asthmatic attack [170]. In another study, nebulization of a similar surfactant (Alveofact (Boehringer, Ingelheim, Germany), 100 mg in children) did not alter airflow obstruction or bronchial responsiveness to histamine in clinically stable patients [171]. Thus, there may be a dependency on disease activity that determines the response. Further studies with more subjects are needed, as well as a solution to the other major problem, that of delivering sufficient surfactant by inhalation into the lungs. Segmental challenge and rescue using surfactant delivered through a bronchoscope may be the approach needed to clarify the role of surfactant in asthma and other obstructive lung diseases.

Bronchiolitis. In a randomized study, 20 infants with severe bronchiolitis were treated with mechanical ventilation with and without intratracheal instillation of a porcine surfactant (50 mg·kg bw^{-1}) [172]. The amount of respiratory support necessary, the duration of mechanical ventilation and the length of stay in the intensive care unit were significantly reduced in the group with surfactant treatment. Larger and more rigorously controlled trials are necessary to establish this intervention in such infants.

Infectious and suppurative lung diseases

Cystic fibrosis. In another double-blind, placebo-controlled trial on the inhalation of a bovine surfactant (Alveofact, 120 mg in adults) in patients with moderate-to-severe CF, no improvements in lung function parameters or oxygenation were observed [173]. This was probably related to the administration of rather small doses of exogenous surfactant, caused by the limitations of current nebulizer technology.

Stable chronic bronchitis. A prospective, multicentre, randomized, double-blind, parallel group, placebo-controlled comparison of a 2-week treatment with aerolized synthetic surfactant (Exosurf, (GlaxoWellcome, Hamburg, Germany) 200–1,000 mg·day^{-1}) gave improved pulmonary function test results and *in vitro* sputum transportability with surfactant inhalation [174].

Pneumonia. Surfactant replacement appeared to be of benefit in selected cases. Selective intrabronchial instillation of surfactant *via* a flexible bronchoscope in an adult patient with lobar Gram-negative pneumonia resulted in a small improvement in oxygenation [175]. Similar improvements have been seen in HIV-infected infants with *P. carinii* pneumonia [176, 177] or pneumonia caused by Respiratory syncytial virus [178].

Acute lung injury/adult respiratory distress syndrome

The alterations to surfactant in ARDS are thought to contribute significantly to lung dysfunction. In various case reports successful surfactant replacement has been demonstrated [179, 180]. In addition, there have also been systematic trials of exogenous surfactant administration. Whereas the aerosolized synthetic surfactant Exosurf had no significant effect on 30-day survival, duration of mechanical ventilation or physiological lung function [181], its instillation in two patients was reported to rapidly improve respiratory function [182]. The natural surfactant Survanta (Beractant) (Abbot, North Chicago, IL, USA) (up to 4 doses of 100 mg·kg bw^{-1}) significantly decreased the inspiratory oxygen fraction 5 days after endotracheal instillation and the mortality rate showed a trend towards reduction (19% *versus* 44% in the control group, p=0.075) [183]. Bronchoscopic surfactant administration (Alveofact, 300 mg·kg bw^{-1}) immediately improved gas exchange and oxygenation significantly [184]. A smaller amount (50–60 mg·kg bw^{-1}) appeared less effective [185]. Aerosolized administration of the artificial surfactant artificial lung-expanding compound (ALEC (Pumactant) (Britannia Pharmaceuticals, Redhill, Surrey, UK)), containing only phosphatidylcholine and phosphatidylglycerol, produced no clinical improvement [186]. In some cases of infants and children with ARDS, exogenous surfactant application was associated with improved gas exchange [187–189]. A retrospective chart review of 18 children with ARDS treated with 69 endotracheal applications of a bovine surfactant found a 40% higher probability of survival in responders to therapy than in nonresponders [190]. Randomized, blinded studies are lacking.

Diseases specific to neonates and infants

Neonatal respiratory distress syndrome. The first successful trial of exogenous surfactant administration in humans was reported by FUJIWARA *et al.* [191]. This therapy has significantly improved outcome in premature infants at risk of RDS. Currently, more than half of the very low birthweight infants in North America and Europe receive surfactant treatment. The numerous clinical trials from Europe and the USA have recently been reviewed [192, 193]. The doses, methods of administration and timing of treatment regimens have been optimized and different preparations directly compared. Natural surfactants appear to be more efficacious than synthetic preparations, which currently lack SP-B and SP-C.

Meconium aspiration syndrome. Natural surfactant preparations may have a role in the management of severe meconium aspiration syndrome, as demonstrated by two recent trials [194, 195]. However, there is not a good response in all infants treated and further investigation is warranted.

SP-B deficiency. In the congenital form of pulmonary alveolar proteinosis, *i.e.* SP-B deficiency, exogenous surfactant therapy was without significant effect, utilizing a natural surfactant preparation also containing SP-B [138].

Neonates with severe respiratory failure due to congenital pneumonia, neonatal sepsis/pneumonia syndromes or congenital diaphragmatic hernia. Experience from numerous small series or case observations indicates improvement of gas exchange in some but not all neonates

to an extent that is much smaller than that found in neonates with RDS (*e.g.* [196–199]). In a randomized, double-blind placebo-controlled trial the use of a bovine surfactant significantly decreased the need for extracorporeal membrane oxygenation in the treatment of term neonates with respiratory failure. Thus, particularly in the early phase of respiratory failure, exogenous surfactant (4×100 mg·kg bw^{-1}) may be of benefit [200]. Several case reports suggest improvement of respiratory function by means of surfactant treatment in neonates with congenital diaphragmatic hernia [201–203]. These infants have very hypoplastic lungs but do not have a primary surfactant deficiency [136]. Treatment before and after surgical repair has been tried. For all these heterogeneous clinical conditions, well-planned, multicentre prospective trials are necessary to assess the value of exogenous surfactant therapy.

Other lung diseases

Lung injury after cardiopulmonary bypass. ALEC was also used in an unsuccessful attempt to improve the respiratory status after cardiopulmonary bypass [204], whereas nebulized exogenous natural surfactant (30 mg·kg bw^{-1}) appeared promising [205]. A case of successful treatment with nebulized synthetic surfactant (Exosurf) was reported for reperfusion injury after single lung transplantation [206].

Respiratory failure due to near-drowning. If administered early after near-drowning, exogenous surfactant was reported to be of some benefit, but randomized studies have not yet been performed [207, 208].

Future aspects in surfactant therapy

The first generation of therapeutic surfactant preparations, that are currently used in clinical practice, consists either of lipid extracts of natural, nonhuman surfactants containing the lipid components, SP-B and SP-C of whole surfactant (Surfacten, Survanta, Infasurf (calf lung surfactant extract, CLSE, or bovine lipid extracted surfactant, bLES) (Rochester, New York, NY, USA), Alveofact, Curosurf (Chiesi Farmaceutici, Parma, Italy)) or of synthetic, completely protein-free mixtures of phosphatidylcholine, tyloxapol and hexadecanol (Exosurf). The next generation of surfactants will be composed of defined lipids and hydrophobic proteins or peptides.

Such a surfactant containing 2% recombinant SP-C (containing phenylalanine instead of cysteine at positions 4 and 5 of the human SP-C sequence, and isoleucine instead of methionine at position 32 [209]) is currently being tested in a European clinical trial with adult ARDS patients. Other surfactants contain designed synthetic hydrophobic peptides (*e.g.* KL4), which have also been successfully used in neonates [210]. These approaches were reviewed recently [211]. The enrichment of first generation surfactants with the hydrophilic SP-A successfully increased the resistance of the preparation to inactivation by oedema fluid [212].

These new developments will supply surfactants that are biophysically more active and hopefully also less expensive, in order to allow the application of sufficient amounts into the larger lungs of adult patients. Much more needs to be learnt before surfactant or its components can be used with respect to their immunomodulatory actions. Such an application might offer new therapeutic options for some of the various lung diseases listed.

Observations of immunological consequences of impaired pulmonary surfactant

Both a large number of *in vitro* studies with isolated surfactant components from normal lungs and data from SP-A knock-out mice have led to the suggestion that *in vivo* surfactant is involved in pulmonary host defence [4]. On the one hand, it is believed that SP-A and/or SP-D bind to or opsonize inhaled pathogens or other environmental particles. This enhances their preferential interaction with phagocytes. After phagocytosis and killing, in some but not all cases, the activated cells produce various cytokines in order to involve other cells, including lymphocytes and lung epithelial cells. Additionally, SP-A and SP-D directly modulate cellular function [4]. It is not completely clear whether these surfactant proteins preferentially suppress or enhance the alveolar immune responses [213]. On the other hand, the surfactant lipids phosphatidylcholine and phosphatidylglycerol appear to downregulate or suppress lung immune cell function [4]. Many more data are still needed to substantiate and detail the *in vivo* relevance of such effects in the lungs under normal conditions.

Till now, only a few studies have been performed, on lungs under pathological conditions, characterizing the potential immunological consequences of aberrant surfactant with respect to specific lung diseases. In the BAL fluid of patients allergic to pollen, the distribution of SP-A oligomers was analysed [214]. In comparison to healthy control subjects, patients allergic to birch pollen had much less of the large octadecameric forms of SP-A and an increased proportion of the smaller dodecameric and hexameric or trimeric forms [214]. As described above, SP-A is a complex molecule comprising up to 18 polypeptide chains (octadecamer). Depolymerization of these chains leads to a loss of binding capacity for carbohydrate-rich structures, associated with losses or alterations of biological function.

In children with asthma, both SP-A and SP-D were found to inhibit house dust mite allergen-induced histamine release in a dose-dependent manner [215]. In addition, these two proteins inhibited phytohaemagglutinin and housedust mite allergen-induced proliferation of peripheral blood mononuclear cells in children with stable asthma and in control subjects. Only a very small suppression (<25%) was observed in activated lymphocytes derived from asthmatic children with acute attacks [215]. These data suggest that SP-A may be involved in both the early phase of allergen provocation and the late phase of bronchial inflammation which is dominated by lymphocytes. Further *ex vivo* experiments are necessary to substantiate such intriguing potential roles for surfactant components in more detail.

In normal subjects, total alveolar fluid and its lipid extracts usually suppress T-cell proliferation in a concentration-dependent manner. This is significantly altered in interstitial lung diseases [48]. In acute hypersensitivity

pneumonitis, both total alveolar fluid and its lipid extract enhanced the proliferation of T-cells. The authors suggested that an imbalance of the surfactant phospholipid composition and not changes in the total lipid content were likely to be responsible. Increases in sphingomyelin with reduced proportions of phosphatidylcholine and phosphatidylglycerol were believed to play a major role [48]. In another study surfactant isolated from hypersensitivity pneumonitis patients failed to completely inhibit the mitogen-induced proliferation of lymphocytes which was already partly suppressed by alveolar macrophages [216]. Similarly, the altered surfactant composition in hypersensitivity pneumonitis was hypothesized to account for this lack of T-cell immunosuppressive activity and might be responsible for the observed alveolitis. Interestingly, in sarcoidosis (stage 2 of the chest radiography classification) and IPF, the normal suppressive effect of alveolar fluids on T-lymphocyte proliferation was lost only in total BAL fluid and not in the lipid extracts of these fluids [216]. This suggested that components other than those extracted into the lipid fraction were responsible.

As alveolar fluid or various surfactant fractions contain large numbers of different compounds, disease-specific alterations of the immunomodulatory properties of surfactant are only now beginning to be unravelled. Up to now, experimental approaches have mainly involved the *in vitro* exposure of cells to the whole, weakly-defined preparations. Specific blockade of certain components of these mixtures, *e.g.* by antibodies or antagonists, will aid the identification of potential candidates. The ultimate proof will be dependent on studies at both the phenotypic and the genetic level. For various pulmonary diseases specific mutations and/or associated genetic polymorphisms will be identified [149] and lead to a better understanding of lung pathophysiology.

Conclusions

Analysis of the pulmonary surfactant system in humans yields a deeper understanding of lung physiology in health and disease and may open new approaches to the treatment of pathological conditions. Currently, the only means of recovering surfactant *ex vivo* from the lungs is *via* the lavage technique. This process disrupts the pre-existing biophysical and biochemical structural organization and may introduce a significant bias. Thus, strictly standardized methods are necessary for the maximal control of potential confounders and the obtainment of reproducible results. For the sake of comparison, all studies analysing pulmonary surfactant should at least include data on total protein and phospholipids, expressed per mL of BAL fluid recovered. Until more information on normal reference values is available and a more uniform standardization of the techniques used is established, all studies must include a defined population of subjects for control and comparison purposes. The data obtained so far suggest the existence of both functional and biochemical surfactant abnormalities in a wide range of lung diseases. Methods of estimating the significance of the contribution of these abnormalities to the specific disease processes in question need to be developed urgently. Except for respiratory distress syndrome in the premature infant, where surfactant deficiency has been unequivocally demonstrated and exogenous surfactant substitution is now part of the routine clinical management, the contribution of surfactant therapy is currently under investigation in a variety of disease states. Besides their role in regulating surface activity, the role that surfactant components may also play in the local immune regulation of the lungs is just beginning to be unravelled.

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