SURFACTANT SYSTEM IN LUNG INJURY 16

Patricia L. Haslam

The search for therapeutic agents to improve the clinical outcome in adult patients with the acute respiratory distress syndrome (ARDS) has focused on early intervention. A variety of approaches have been attempted using methods that modulate the components of the systemic inflammatory response, but none has led to a major breakthrough. However, early intervention is difficult to achieve for the majority of patients with ARDS who present with the syndrome fully established. Consequently, therapeutic approaches for rescue therapy also need to be developed. This requires a knowledge of events associated with progression and prognosis. The development of ARDS is characterized by damage to the pulmonary vascular endothelium. This is followed by leakage of proteins from the damaged vessels and extravasation of activated neutrophils causing interstitial inflammation and edema. Damage to the alveolar epithelium then occurs, resulting in accumulation of proteins and neutrophils within the alveolar spaces. These changes within the alveoli have important pathophysiological consequences. In particular, they cause dysfunction of the pulmonary surfactant system which amplifies the injury (Figure 16.1).

Pulmonary surfactant dysfunction can lead to alveolar instability, flooding and collapse, and in these respects the catastrophic acute lung injury that occurs in ARDS shows many similarities to the infantile syndrome (IRDS). This can develop in premature neonates because of surfactant deficiency due to immaturity of the lungs at the time of birth [1]. The lungs fail to inflate adequately, resulting in alveolar flooding and collapse. Various types of exogenous surfactant have been developed for treatment of IRDS and are of established benefit [2,3]. This has provoked interest in the potential for surfactant replacement therapy in ARDS [4]. However, while surfactant deficiency is the primary etiologic factor in IRDS, alterations in the surfactant system in ARDS develop as a secondary consequence of lung injury. The mechanisms involved and the precise approaches to therapy in the two disorders therefore differ in a number of respects. The aim of this chapter is to describe the composition and functions of normal human pulmonary surfactant, and to review the alterations which develop in ARDS. The current status of exogenous surfactant therapy in ARDS is also reviewed.

NORMAL PULMONARY SURFACTANT

ROLE IN REGULATION OF ALVEOLAR SURFACE TENSION

Pulmonary surfactant is a complex, highly surface active material that spreads to cover the alveolar epithelial surfaces of the lungs [5]. Phospholipids account for about 85% of the composition, the main component being dipalmitoylphosphatidylcholine (DPPC), which is also the main surface active com-

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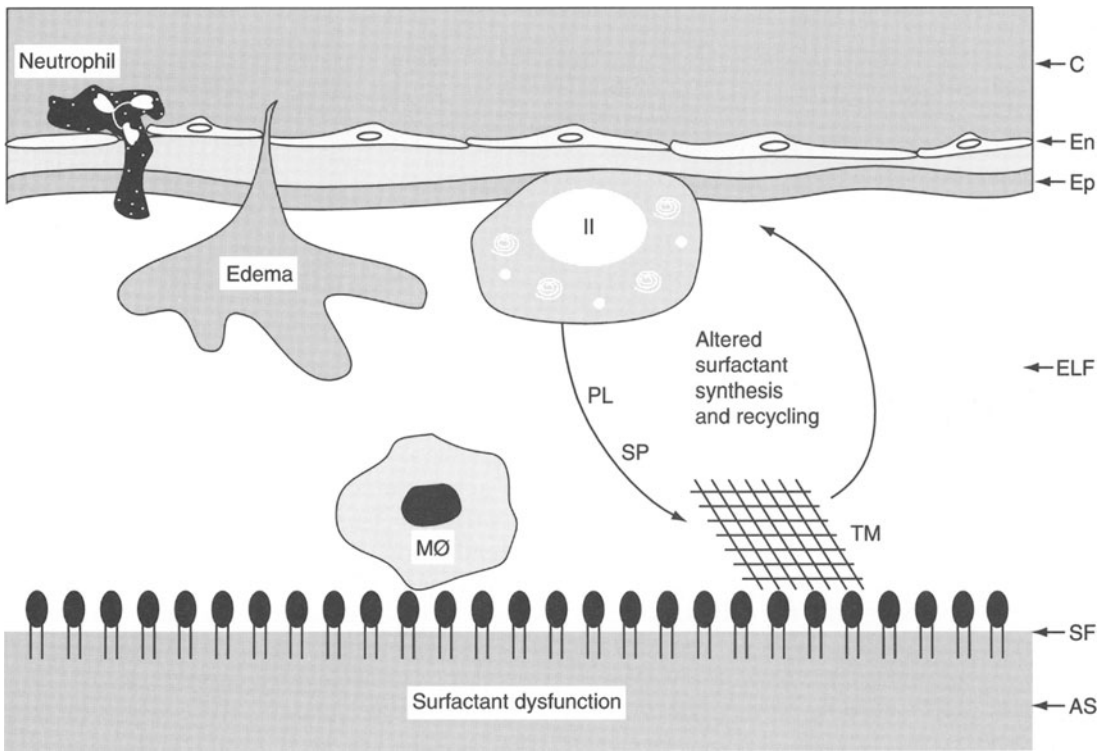


Figure 16.1 Events that lead to dysfunction of the pulmonary surfactant system in patients with ARDS. C = capillary; En = endothelium; Ep = epithelium; ELF = epithelial lining fluid; SF = surface film indicating phospholipid monolayer; AS = alveolar space; II = Type II cell; PL = phospholipid; SP = surfactant-specific apoprotein; MØ = macrophage; TM = tubular myelin.

ponent. In addition, the surfactant system contains about 8% proteins, 5% neutral lipids and 2% carbohydrates [1,4–6]. These components interact together in a highly organized manner to form a monolayer film of phospholipid over the surface of the alveolar epithelium. This film plays an important role in lung function by generating a 'film-pressure' which opposes and reduces the surface tension of the alveolar epithelial lining fluid to maintain it at near zero throughout the whole cycle of breathing. This counteracts the differences in pressure between alveolar spaces and tissues which would otherwise cause the alveoli to flood and the lungs to collapse, especially at low lung volumes when the intra-alveolar pressures are very low. There is still controversy as to the exact mechanism by which surfactant stabilizes the alveoli and prevents flooding and collapse, and the various hypotheses proposed have been reviewed in detail elsewhere [7]. The conventional and most widely accepted hypothesis is the 'bubble' model. This assumes that the alveoli are lined with a continuous layer of liquid, and that they behave like bubbles in terms of their inherent stability. Mathematically, the collapsing pressure (Δp) of a bubble is related to the surface tension (γ) and the radius of curvature (r) by the law of Young and Laplace $\Delta p = 2\gamma/r$. Bubble stability is established when the air inside has been compressed until its pressure is raised above the pressure outside by an

amount (Δp) which opposes further collapse. Equilibrium is established when Δp equals the recoil pressure of the bubble ($2\gamma/r$). Because alveoli are networks of interacting 'bubbles' of differing sizes that change during breathing, Clements *et al.* [8] proposed that the alveoli might remain stable despite the variation in their radii if surfactant is able to progressively vary surface tension to decrease bubble recoil ($2\gamma/r$), then further reduce surface tension as the bubble starts to collapse until $2\gamma/r$ of each bubble is the same for all. In this way a whole range of bubbles of widely differing diameters can be brought to equilibrium with the same external air source and, hence, with each other.

The 'bubble' hypothesis is based on the view that a 'continuous' layer of fluid lines the alveolar surfaces. However, alternative theories suggest that the fluid lining is 'discontinuous' with dry and wet patches [7]. The 'water repellent model' postulates that the fluid is confined to 'pools' and 'pits' from which it can tension dry patches of the alveoli by pulling in excess epithelial membrane as 'pleats' [9]. The gas transfer surface is kept essentially dry by water repellency induced by a monolayer of surfactant directly adsorbed on to the epithelial surface. The 'pools' assume a convex profile and act as 'corner pumps' to return fluid to the interstitium. If flooding continues, the 'pools' will link to form the 'bubble' model, but only in a pathological and not the physiological state [9].

The monolayer film of surfactant phospholipid is able to modulate surface tension because of the highly polar nature of the molecules (Figure 16.2). This causes them to align at the air–liquid interface, with their hydrophilic phosphatidyl head groups in the liquid and their hydrophobic fatty acid tails in the air space. The compression of these molecules during expiration generates the increasing film pressure which opposes, and reduces, the force of surface tension at the air–liquid interface. As the radii of the alveoli

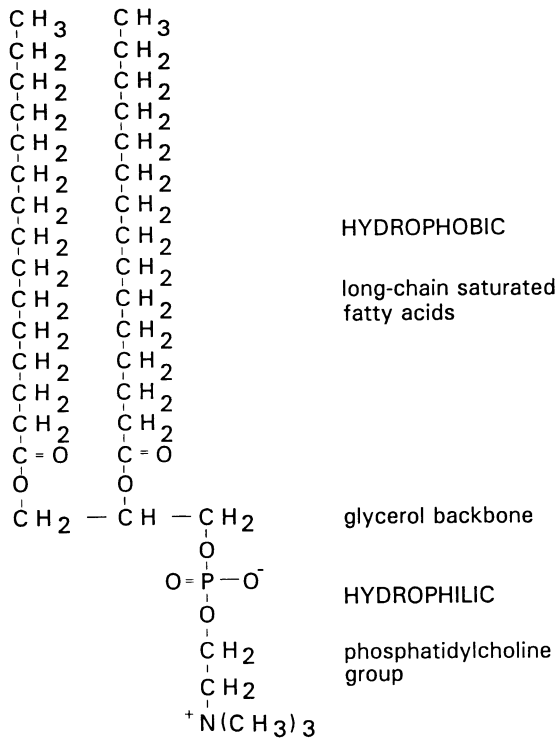


Figure 16.2 Chemical structure of dipalmitoylphosphatidylcholine (DPPC) showing the highly polar nature of the molecule.

increase on inspiration the pressure between the molecules decreases, allowing the surface tension to increase. The ability of pulmonary surfactant to lower surface tension under dynamic compression *in vitro* can be assessed in a variety of ways, but problems of reproduciblity can arise because the measurements are very sensitive to variability in concentration and contamination [10]. The two methods most commonly used are the Langmuir–Wilhelmy balance [11] and more recently the oscillating bubble apparatus of Enhörning [12]. Thus, Clements used a modified Wilhelmy balance to confirm the hypothesis that pulmonary surfactant was capable of lowering surface tension when the film it formed was compressed into a smaller area [11]. Both methods can generate similar information, but the oscillating bubble

Table 16.1 Surfactant-specific apoproteins

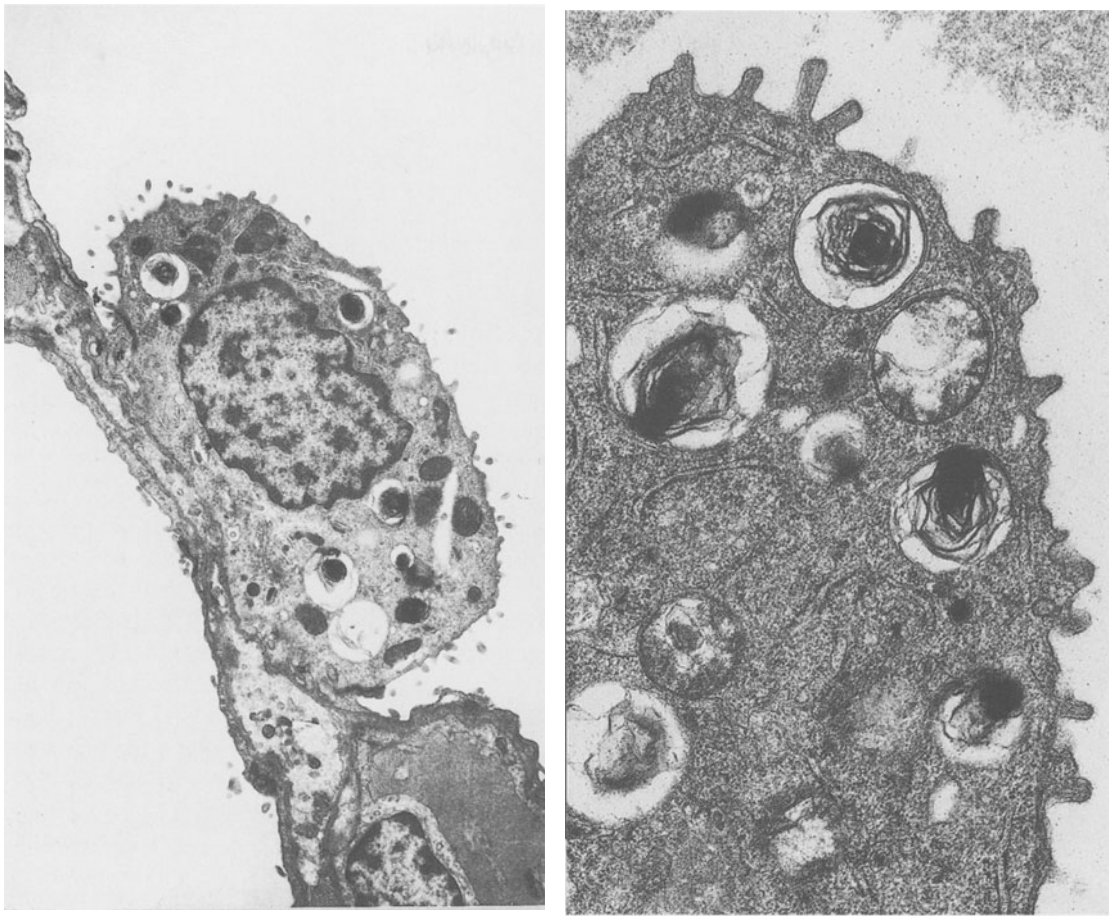
Apoprotein	*Biochemical properties*	*Molecular weight (kDa)*	*Selected references*
SP-A	Hydrophilic glycoprotein	26–36	23–25
SP-B	Hydrophobic protein	7.5–9	28–33
SP-C	Hydrophobic protein	3.5	28–33
SP-D	Hydrophilic glycoprotein	43	26,27

method has the advantage that surface tension measurements can be continuously determined between maximum and minimum bubble size for very small volumes of surfactant material (25 μL). This makes it a convenient method for clinical studies of pathological events which disturb the biophysical function of the surfactant system.

BIOCHEMICAL COMPOSITION

Clinical studies of the composition, as well as the function, of human pulmonary surfactant in health and disease have been facilitated by the technique of bronchoalveolar lavage (BAL) which enables components to be sampled from the air spaces of the lungs of humans with safety [13,14]. BAL is conducted using a standardized procedure, by instilling and aspirating aliquots of physiological saline (typically, 4 × 60 ml in adults) via a fiberoptic bronchoscope placed into a defined segment of the lung, usually the right middle or lateral segment of the right lower lobe [15]. The recovered BAL samples are examined to determine the number of cells present, then spun immediately at low speed ($\leq$ 300 g) for 10 minutes at 4°C to separate the cells from the cell-free supernatant fluid. For lipid studies it is essential to remove rapidly cells which might otherwise contaminate the surfactant components in the supernatants with cell derived lipids and proteins. High centrifugation forces must not be used because, apart from inducing cell damage, these can sediment and cause loss of aggregates of surfactant phospholipids and proteins. To evaluate surfactant phospholipid composition, lipids are extracted from the cell-free BAL fluids using conventional methods, for example chloroform–methanol extraction. The main phospholipid classes are separated and quantified by thin layer or high pressure liquid chromatography [5,16,17].

On average, phosphatidylcholine (PC) accounts for 73% of the phospholipid in lavage samples from normal, healthy, non smoking human volunteers, and it is mostly in the form of saturated DPPC which is highly surface active. Phosphatidylglycerol (PG) is the second major phospholipid component, accounting for on average 12% of the phospholipid, while the remainder include the minor components phosphatidylinositol (PI), phosphatidylethanolamine (PE), phosphatidylserine (PS), sphingomyelin (SM), cardiolipin, and lysophosphatidylcholine, none of which usually account for more than 3% of the total phospholipid. By contrast, phospholipids in lung tissue and blood plasma contain lower proportions of PC (mean < 50%) with a higher content of unsaturated fatty acids, and much lower proportions of PG (< 2%). Lung tissue also contains higher proportions of PE (mean 17%), and both lung tissue and blood plasma contain higher proportions of SM (mean 11% and 24% respectively). In pathological situations, components from damaged tissue and exudation of plasma into the alveolar spaces can contaminate the surfactant system [18]. This can result in abnormal elevations in PE and SM (as well as in proteins and other tissue or plasma-derived components) in BAL samples. Cigarette smoking must be taken into account in clinical studies of surfactant,

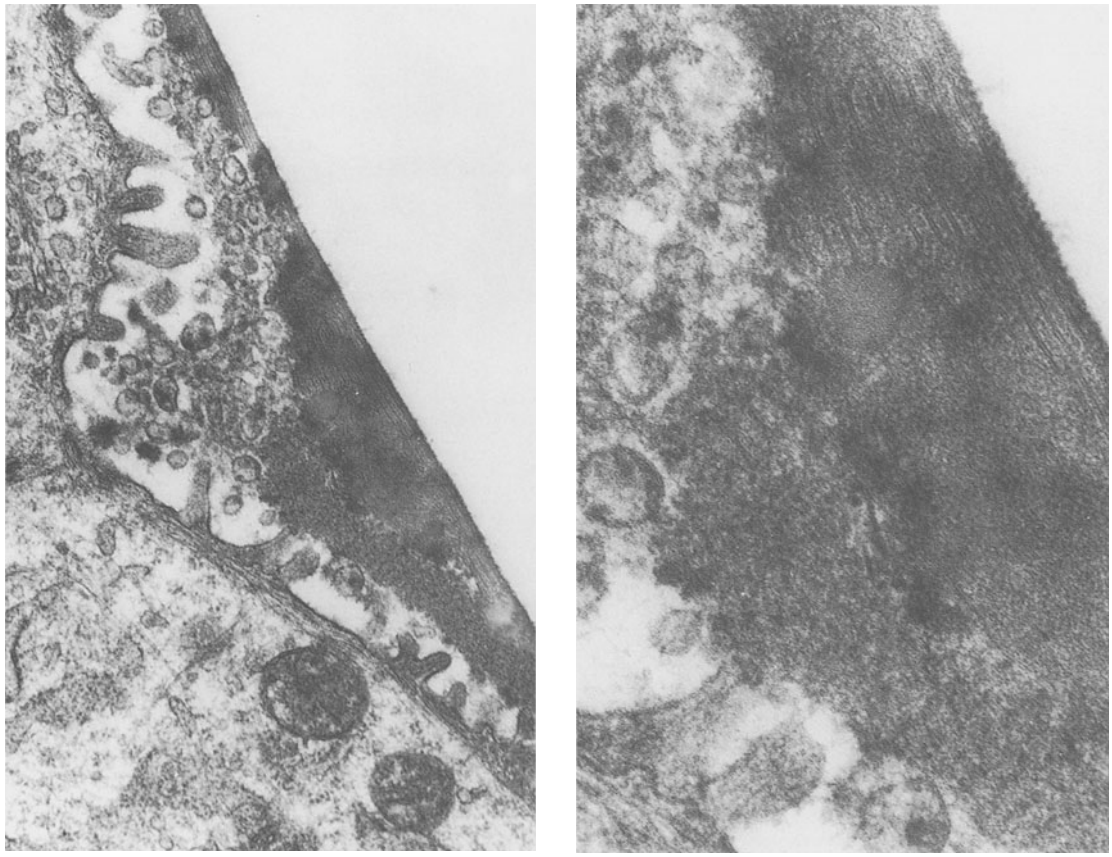


(a) (b)

Figure 16.3 (a) Electron micrograph showing the ultrastructural appearance of a human type II alveolar epithelial cell (×6500). (b) Higher power view of the cytoplasm of the cell showing the characteristic lamellar bodies containing phospholipids (×19 500). (By courtesy of Ann Dewar, National Heart & Lung Institute, London, UK.)

because BAL samples from apparently healthy cigarette smokers contain significantly higher proportions of PE and SM compared with healthy nonsmoking volunteers [19]. This indicates that smoking related tissue damage is sufficient to have a measurable effect on surfactant composition. The functional consequences have not been investigated. However, pulmonary vascular permeability is increased in cigarette smokers compared with nonsmokers [20]. Dysregulation of surface tension due to surfactant alterations could be relevant to this observation.

Apart from phospholipids, about 2% of the proteins in normal alveolar lining fluid are specific to the surfactant system. These are termed the surfactant apoproteins. They have been studied less than the phospholipids because they have been discovered more recently [6,21,22]. Four surfactant-specific proteins have been identified including the



(a) (b)

Figure 16.4 (a) Electron micrograph showing the membranes and part of the cytoplasm of two human alveolar epithelial cells covered by a layer of alveolar lining fluid containing surfactant. The lung was fixed *in situ* to preserve the surfactant layer. A large tubular myelin aggregate composed of phospholipids and apoproteins is demonstrated (×32 000). (b) Higher power view of the surfactant layer showing the tubular myelin aggregate together with smaller aggregates in the liquid hypophase (×85 500). (By courtesy of Ann Dewar, National Heart & Lung Institute, London, UK.)

higher molecular weight hydrophilic glycoproteins Sp-A [23–25] and Sp-D [26,27], and the low molecular weight hydrophobic proteins Sp-B and Sp-C [28–33] (Table 16.1). These proteins, as well as the phospholipids, are essential for the efficient functioning and turnover of the surfactant system. The main phospholipid and specific protein components are synthesized and secreted by the type II alveolar epithelial lining cells. After synthesis, the phospholipids are stored within the cytoplasm of the cells in specialized organelles termed lamellar bodies (Figure 16.3), from which they are released on to the alveolar epithelial surfaces. The surfactant-specific proteins are transported separately to the cell surface in multivesicular bodies, although some Sp-A may be present in lamellar bodies. The phospholipids and proteins aggregate extracellularly to form lattice structures known as tubular myelin [34–38] (Figure 16.4). These aggregates are the most highly surface active fraction of surfactant and they aid the spreading of phospholipid to form a

monolayer film over the surface of the epithelial lining. Sp-A is the major surfactant apoprotein and plays a very important role in tubular myelin formation by interacting with phospholipids, Sp-B, Sp-C and calcium ions [35,36,39,40]. Sp-B and Sp-C play a further essential role in enhancing the adsorption and spreading of the monolayer of phospholipid at the air–liquid interface of the lung lining fluid [29,30,41]. Sp-B also promotes the squeezing out of impurities and 'exhausted' phospholipids from the lipid monolayer [30]. Sp-A then regulates phospholipid recycling to type II cells for resynthesis [42] and it plays a role in regulating secretion [43]. There is also evidence that Sp-A plays a role in host defense by enhancing the phagocytosis and killing of micro-organisms by alveolar macrophages [44]; and recent evidence suggests that Sp-D may play a similar role in host defense [45]. Compared to phospholipids, the importance of the apoproteins in the surfactant system has been recognized relatively recently and this is an active field of current research. In particular, it is hoped that synthetic surfactants containing recombinant surfactant apoproteins or their functional analogs plus synthetic phospholipids, now being developed for therapeutic use [46,47], will provide an advance over current artificial surfactants containing phospholipid because they should more closely mimic the natural product.

SYNTHESIS AND TURNOVER

The synthesis and turnover of surfactant components is a highly dynamic process [48,49]. This is necessary because the cycles of contraction and expansion which occur during breathing have an adverse effect on the surface phospholipid which is continuously expelled from the monolayer and replaced with newly synthesized material [50]. Ultrastructural examination of lungs fixed to preserve surfactant show the presence of small aggregates distinct from the larger aggregates of tubular myelin [51] (Figure 16.4). These small aggregates have poor surface activity and are thought to represent spent phospholipid. Some of the material expelled from the monolayer is recycled back to type II alveolar epithelial cells for resynthesis, while some is phagocytosed by alveolar macrophages and removed [48,52,53]. Phospholipid recycling to type II cells is aided by the main surfactant specific protein Sp-A, which can interact with phospholipid and attach to Sp-A receptors on the surface of the type II cells [42,54]. Sp-A also appears to play a role in modulating the release of phospholipid by type II cells [43,55]. The highly dynamic nature of synthesis and turnover of surfactant components means that the system is susceptible to disturbance not only due to contaminants, degradative processes or loss by increased diffusion across the damaged epithelial and endothelial barriers, but also by any interference with synthesis or turnover.

MECHANISMS OF SURFACTANT DYSFUNCTION

Surfactant deficiency caused by immaturity of type II alveolar epithelial cells is the primary etiological factor in IRDS [56]. The levels and relative proportions of the two major phospholipids PC and PG are markedly reduced, while the levels and proportions of the phospholipids PI and SM are increased [1] (Figure 16.5). In fetal lung, PI is produced preferentially to PG from the common precursor CDP diacylglycerol, but in full term infants there is a switch to preferential production of PG prior to birth. The risk of surfactant deficiency is higher with increasing prematurity and, while IRDS occurs in about 20% of infants born at 30–32 weeks gestational age, the incidence is 60–80% in those of 26–28 weeks gestational age [57,58]. Fetal lung immaturity also results in a deficiency in apoprotein components of surfactant [59]. The lungs fail to inflate adequately because of the primary lack of phospholipid and apoprotein components, and there is alveolar flooding

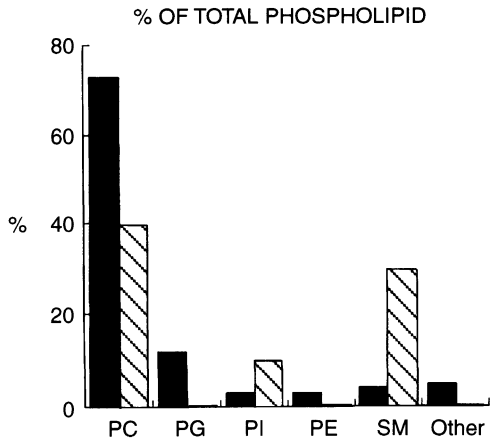


Figure 16.5 Phospholipid compositional alterations in IRDS. ▨ = IRDS; ■ = normal.

due to leakage of proteins from the plasma into the alveolar spaces. Pulmonary edema then provides a secondary mechanism which further promotes surfactant dysfunction. This occurs because protein contamination can interfere with the surface tension lowering properties of surfactant in a dose dependent manner.

A variety of plasma proteins and proteins such as fibrin degradation products generated by the coagulation processes involved in edema formation have been shown to inhibit surfactant function *in vitro*. Different proteins vary in their inhibitory effects, and in general fibrinogen and fibrin monomers are more inhibitory than serum proteins such as hemoglobin and albumin at comparable doses [60–63]. The levels of inhibition induced by protein are highly dependent on the concentration of surfactant as well as the amount of inhibitory protein. At low surfactant concentrations, plasma proteins significantly inhibit adsorption of the surface film of phospholipid, resulting in an increase in the equilibrium surface tension. However, at higher surfactant concentrations, adsorption is not impaired by the presence of plasma proteins even when their levels are increased proportionately [61,62,64]. Indeed, in experiments using the pulsating bubble surfactometer to study the effects of plasma derived proteins on the dynamic surface tension lowering ability of lung surfactant, a fourfold increase in surfactant phospholipid concentration to 2 mg/ml reduced the inhibitory effect of even a 20-fold increase in the concentration of inhibitory proteins compared with the marked inhibition observed using a surfactant concentration of 0.5 mg/ml and protein concentrations of 10 mg/ml [64]. *In vitro* experiments have also shown that different formulations of therapeutic surfactant differ markedly in their sensitivity to the effects of inhibitory plasma proteins [65–67]. This is also indicated by *in vivo* studies in experimental models [68,69]. It is clear that such differences may be of considerable importance in selecting a therapeutic formulation appropriate for use in different pathological situations. It is of interest that addition of surfactant apoproteins to various surfactant lipid preparations has recently been shown to increase the resistance of these preparations to plasma protein inhibition [67,70–72]. The possible mechanisms proposed to explain how contaminating proteins might inhibit surfactant function have been reviewed recently [73]. These include physicochemical interactions with surfactant phospholipids or apoproteins, interference by insertion into the intact surface film, or by competition with surfactant molecules for space at the air–liquid interface during adsorption.

Protein contamination due to increased endothelial and epithelial permeability resulting in intra-alveolar edema is probably the main mechanism by which surfactant dysfunction initially occurs in ARDS [4,74]. As the syndrome progresses, surfactant dysfunction will be amplified if damage to type II alveolar epithelial cells results in a quantitative deficiency of surfactant components due to impaired synthesis, secretion and turnover [75]. It has been proposed that additional mechanisms may also contribute to surfactant damage in ARDS. Phospholipases with the

capacity to disrupt surfactant function have been found in BAL samples from patients with ARDS [76]; and increased phospholipase A_2 activity correlating with decreased PC levels has been reported in patients with respiratory failure associated with acute pancreatitis [77–79]. In patients with sepsis, it has been suggested that secretion of phospholipases by bacteria should also be considered [80]. Oxidants released by activated neutrophils, monocytes and macrophages are thought to play a role in the pathogenesis of ARDS [81–83], and lipid peroxidation products have been shown to be capable of interfering with the normal surface activity of surfactant *in vitro* [84]. The saturated phospholipids which form the major part of the phospholipid in the surfactant system are resistant to oxidation, but tissue damage may release lipids containing unsaturated fatty acids which can provide a substrate. Activated neutrophils also produce proteases which are capable of damaging the major surfactant apoprotein Sp-A *in vitro*, implying that they might induce such damage *in vivo* in ARDS [85]. This situation might be promoted if the major inhibitor of neutrophil elastase in the air spaces of the lung, α_1-antiprotease, is rendered inactive due to oxidation of methionine in the active site of the inhibitor [81].

Thus, it is clear that surfactant dysfunction is subject to wide variation dependent upon many contributory factors. However, when pathological situations arise where there is an abnormally low available pool of functional surfactant together with increased alveolar protein contamination, there is a high risk of failure of surfactant to regulate alveolar surface tension.

SURFACTANT ALTERATIONS AS A CONSEQUENCE OF LUNG INJURY

ARDS

The risk factors which can precipitate ARDS in previously healthy individuals, and the initial mechanisms involved are described in detail elsewhere (Chapter 2). Risk factors which affect the lungs directly include infectious and aspiration pneumonias and smoke inhalation, while indirect risks include sepsis, sepsis syndrome, multiple blood transfusions, drug overdose, disseminated intravascular coagulation, trauma or multiple fractures, major surgery (including that needing cardiopulmonary bypass) and many other risks [86–89]. These initial insults, by mechanisms not fully understood, may result in increased pulmonary vascular capillary permeability to plasma proteins leading to massive pulmonary edema [90,91]. The prevalence of ARDS in relation to different risk factors and time scale of development of the injury is variable, presumably reflecting differences in the exact mechanisms involved [86]. It is conceivable that risks which directly affect the lungs may lead to epithelial damage and amplified surfactant dysfunction more rapidly than systemic risk factors.

Evidence of surfactant dysfunction

Pulmonary surfactant dysfunction probably first arises during the course of ARDS as a consequence of protein contamination due to edema. It is likely to be enhanced when damage to type II epithelial cells becomes sufficient to result in a deficiency of surfactant components (Figure 16.1). The surfactant dysfunction contributes to the development of the life threatening acute respiratory failure, which in many cases progresses to multiorgan failure and death. The syndrome was described by Ashbaugh *et al.* in 1967, who also first reported that fluid recovered from minced postmortem lung tissue had a reduced ability to lower surface tension *in vitro* (minimum surface tension $>$ 20 dyne/cm) compared with normal values ($<$ 10 dyne/cm) suggesting surfactant dysfunction [92]. This was confirmed by Von Wichert and Kohl, who also showed that lung extracts from patients who died of ARDS had

a decreased DPPC content [93]. BAL studies of the composition and surface activity of surfactant from patients with ARDS provided further evidence that the lung injury causes disturbances to the surfactant system [76]. Thus, lavages from postmortem lungs of patients with ARDS have an abnormally increased surface compressibility compared with those from control lungs [94]. Static pressure–volume curves obtained from the intact specimens also showed that the ARDS lungs exhibited lower volumes and decreased compliance compared with normals. The BAL samples from the ARDS lungs had a high protein content, and the authors concluded that this may have been responsible for the decreased compliance recorded for the whole lungs. Studies were subsequently performed on the phospholipid composition and surface activity of surfactant recovered from BAL samples from living patients with a variety of pulmonary diseases including ARDS, pneumonia, malignancy and chronic obstructive lung disease [75]. The surfactant was purified from the BAL samples by sucrose density gradient centrifugation and the pellets solubilized in organic solvents to remove contaminating protein prior to measuring the surface tension lowering ability using a Wilhelmy balance. Despite these steps, the samples from patients with ARDS had little or no surface tension lowering activity compared with normal controls. There was no significant difference in total phospholipid, but the fractional content of PC and PG was reduced and SM increased compared to normal. In ARDS associated with trauma the alterations in surfactant composition and function in BAL were then shown to correlate with the severity of ARDS as assessed by respiratory failure scores [95]. No quantitative abnormality in total phospholipid in BAL was identified, but the fractions of PC and PG were reduced compared with normal controls, and PC was lower in patients with high compared with low respiratory failure scores. Surfactant function assessed by the Wilhelmy balance also showed a correlation between increasing minimum surface tensions, declining hysteresis areas and the degree of respiratory failure in these patients. In a subsequent study of trauma complicated by sepsis this complication was shown to be associated with further depletion of alveolar phospholipids [96]. These workers also investigated the time course of development of surfactant abnormalities in ARDS associated with trauma, the relationship with levels of protein contamination, and the severity of respiratory failure [97]. A rapid increase in alveolar protein load (demonstrated within the first 48 hours) was followed by progressive decreases in PC and PG and increases in PI, PE and SM. Declining surfactant function correlated with levels of protein leakage, decline in PC, and the severity of respiratory failure assessed by the ARDS score. These observations were confirmed in a prospective study employing serial BAL samples [98]. Plasma protein leakage was more evident than alterations in PC over the first week of the injury. A progressive decrease in PC (percent of total phospholipid) then became increasingly apparent with increasing duration of the injury in patients with a high ARDS score. More recently, the surface tension-lowering ability of the surfactant fraction of BAL samples has been shown to be decreased in patients at risk of developing ARDS, although not to the same extent as in patients with the established syndrome [99].

Although studies of surfactant composition and function in patients with ARDS are relatively few compared with work in experimental models of acute lung injury (see below), they support the view that surfactant abnormalities contribute to the pathophysiology of ARDS. They indicate that protein contamination initially plays the major role, and that alterations in phospholipid composition also develop at a later stage in the course of severe lung injury. Our own observations in a series of patients with severe ARDS associated with a range of risk factors are con-

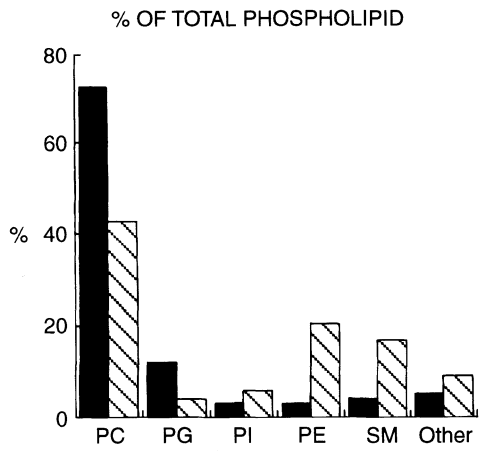


Figure 16.6 Phospholipid compositional alterations in ARDS. ▨ = ARDS; ■ = normal.

sistent with these conclusions (Figure 16.6). We have also observed notable increases in the nonsurfactant phospholipid SM in the BAL samples from our patients, indicating the presence of contaminants from necrotic tissue or dead inflammatory cells, as has been suggested by Hallman *et al.* [75].

Mechanisms implicated

Many mechanisms are likely to contribute to protein contamination and compositional alterations in surfactant in ARDS. The total protein content of the alveolar lining fluid may be the combined result of plasma leakage, tissue damage, products generated by activation of the humoral clotting, fibrinolytic and complement systems, plus products from inflammatory cells. The alterations in phospholipid composition may be due to the combined effects of damage to type II alveolar epithelial cells, altered synthesis as a result of type II regeneration and hyperplasia, contamination with phospholipids from plasma or necrotic cells, interference with phospholipid recycling from the air spaces due to type II cell dysfunction, or disturbance in the normal mechanisms of clearance of surfactant by alveolar macrophages or via the mucociliary escalator. Damage or dysfunction of type II alveolar epithelial cells may also explain the reductions in the levels of the surfactant-specific proteins Sp-A and Sp-B reported in patients with ARDS, and the reduced levels of Sp-A in patients at risk of ARDS [99]. Type II cells are the main source of surfactant-specific proteins, and monitoring their levels should provide a sensitive indicator of damage to type II cells because of their specificity to the system. Although *in vivo* studies (supported by evidence from transgenic models) have shown that Sp-B and Sp-C, but not Sp-A, are essential for maximal surface tension lowering efficiency of surfactant [31–33, 70], reductions in Sp-A may have an adverse effect *in vivo*. Sp-A contributes in tubular myelin formation and it also regulates phospholipid recycling to type II cells, which may be important to 'kickstart' the normal dynamic process of phospholipid turnover after injury. Phospholipid recycling and regeneration is essential to maintain normal lung function throughout cycles of compression and expansion. Sp-A also plays an important role in host defense, as described below, and may help to protect patients with ARDS from nosocomial infections.

Protein contaminants

There have been many investigations of total protein in BAL samples from patients with ARDS, but few attempts to identify the range of proteins present. In one of the most detailed [100], the authors explored whether the normal size-selective process that restricts the passage of large molecules from the plasma to the alveolar lining fluid is destroyed by the alveolar-capillary injury of ARDS. They determined the relative concentrations in plasma and BAL of total protein and of seven individual proteins: α_1-antitrypsin (54 000 mol.wt), albumin (68 000 mol.wt), transferrin (90 000 mol.wt), haptoglobin (100 000 mol.wt), ceruloplasmin (150 000 mol.wt), α_2-macroglobulin (820 000 mol.wt) and IgM (900 000 mol.wt). The mean total

protein in BAL in eight patients with ARDS was greater than 12 times the level detectable in 11 normal volunteers and three patients with cardiogenic pulmonary edema; and the high molecular weight proteins immunoglobulin M and α_2-macroglobulin were present at greater than 90 times the normal or cardiac edema concentrations. For the seven proteins studied, the distribution coefficients of BAL concentrations versus log molecular weights increased hyperbolically in normals but were flat in ARDS patients. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis indicated the presence of the full spectrum of plasma proteins in BAL from ARDS, but not normals. This evidence supports the proposal that lung injury in ARDS is associated with a loss of size restriction of the alveolar-capillary barriers so that plasma proteins are distributed more equally between plasma and alveolar lining fluid than in the normal lung. Fowler *et al.* have also demonstrated increases in high molecular weight plasma derived proteins in ARDS lavage fluids [101], including fibrinogen (340 000 mol.wt), which is one of the most potent protein inhibitors of surfactant function [60]. Evidence that proteins from activated inflammatory cells (notably neutrophils and possibly macrophages) also contribute to the increases in total protein in BAL in ARDS patients comes from observations that both collagenase and neutrophil elastase are present in increased amounts in lavage samples from patients with ARDS [102,103]. Apart from interfering in adsorption and function of the surface film of surfactant, proteases have the capacity to degrade surfactant apoproteins *in vitro* [85]. It is possible that such mechanisms may operate *in vivo* if there is deficiency of antiproteases. The acute phase C-reactive protein, which is generated in increased amounts during inflammatory processes, including ARDS, also has the capability to impair the surface tension lowering ability of surfactant *in vitro* [104].

Protein inhibition of surfactant function is reversible in the sense that functional surfactant can be recovered by centrifugation to separate it from the soluble proteins [105]. This approach obviously cannot be exploited for therapeutic purposes *in vivo*, but attempts to increase the relative surfactant concentration to reduce the inhibitory effect of proteins represent one of the major aims of surfactant therapy in ARDS. The therapeutic formulations and doses to achieve this effect may, however, be variable according to the *in vivo* situation. Approaches to therapy are discussed in greater detail in the separate section below.

Phospholipid alterations

The exact mechanisms resulting in the alterations in phospholipid composition seen in patients with ARDS are unclear (Figure 16.6). The reasons for the more pronounced decreases in the second major surfactant phospholipid component, PG, compared with the major phospholipid, PC, are also unknown. A decrease in PG with a relative increase in PI, as observed in some patients, may reflect the pattern of phospholipid production by regenerating type II alveolar epithelial cells, which *in vitro* show a pattern of phospholipid synthesis similar to that of fetal lung [106,107]. Direct damage to type II cells may also contribute to the reductions in levels and proportions of PG and PC. However, proportionate reductions relative to the total phospholipid present may arise due to increased phospholipid contamination of alveolar lining fluid from necrotic cells and edema fluid. Increases in SM and PE in the lavage samples of ARDS patients provide evidence that this mechanism is also involved [75,97]. The functional consequences of decreases in the second major phospholipid component of surfactant PG in adult lungs are unknown, because PG is not essential for the normal biophysical function of surfactant *in vitro* if it is replaced by PI [108,109]. Decreases in the proportions and levels of the major surfactant phospholipid PC, however,

parallel the severity of ARDS *in vivo* [97,98]. Contamination of the surfactant system with phospholipids from necrotic cells and edema may explain partly why there have been contradictory reports on surfactant phospholipid pool size in patients with ARDS. Some workers have reported no changes in total phospholipid levels in BAL from ARDS patients compared with controls [75,95], while others have reported decreases [96,98] or increases [110]. This variability may be partly because of a lack of recognition of the contribution of contaminants to the total phospholipid pool, compounded by the lack of accurate markers to assess dilution due to the BAL procedure, and poor standardization of methods for recovery and measurement of phospholipids which are subject to technical variation. These considerations make it difficult to interpret studies of surfactant pool sizes in humans and explain why surfactant dysfunction, including deficient production of surfactant, can occur without an apparent reduction in the total phospholipid pool size.

Experimental models have been used to obtain more standardized information on surfactant pool size, composition and function during the course of acute lung injury, but these models have the limitation that they cannot provide an exact representation of the complexity of the clinical situation. However, they have generated much useful information about the early events involved in the pathogenesis of acute lung injury, and have enabled a number of new therapeutic approaches, including the use of therapeutic surfactants, to be explored.

EXPERIMENTAL MODELS OF ACUTE LUNG INJURY

Many experimental models of acute lung injury have been developed which exhibit the histological and pathophysiological features of ARDS [111]. A detailed description is beyond the scope of this chapter, but they have been of great importance in elucidating the wide range of mechanisms involved in pathogenesis [4,74,90]. Different insults are used to induce the injury and, as in the clinical syndrome, the damaging mechanisms are variable. A number of the models closely mimic certain of the clinical risk factors. They have confirmed that functional deficiency of surfactant develops as a common consequence of severe acute lung injury, and that the reasons for the dysfunction vary dependent upon the nature of the injury. Surfactant replacement therapy has improved gas exchange and reduced the severity of the injury in several of these models, as described in the following section.

SURFACTANT REPLACEMENT THERAPY

THERAPEUTIC SURFACTANTS

The recognition that pulmonary surfactant deficiency is the primary etiological factor in the pathogenesis of IRDS [1] led to the development of a variety of exogenous surfactant preparations, which are of established benefit both for prophylaxis and for rescue therapy in premature infants [2,3]. Initial attempts at therapy employed pure DPPC, which had by then been identified as the major surface active phospholipid in pulmonary surfactant [112]. The results were very disappointing [113,114] and almost discouraged further trials. It was then discovered that DPPC alone is not suitable for use in therapy because additional components are required to enable it to adsorb rapidly and spread to form a surface film, and to stabilize the film during repeated cycles of expansion and compression [115]. The breakthrough in therapy came when Enhörning and Robertson used natural surfactant extracts from the lungs of adult rabbits to treat premature rabbit pups and showed a marked improvement in lung function [116,117]. This was confirmed in models using other species [118]. In 1980, Fujiwara *et al.* reported the first successful treatment of newborn infants with respiratory distress

syndrome [119]. They achieved striking improvements in arterial oxygenation using a saline extract of minced bovine lung enriched with synthetic lipids. Therapeutic surfactants obtained from mammalian sources are termed 'natural' surfactants, but the methods of preparation usually result in some 'modification' compared with normal pulmonary surfactant. A range of 'natural' and 'modified natural' surfactants from mammalian sources have been developed for therapeutic use in IRDS and shown to be efficacious [2]. These include extracts from minced bovine lung [119–124], from porcine lung [125–129], calf lung lavages [130–138] and surfactant recovered from human amniotic fluid [139–141]. Surfactant recovered from alveolar lavage or amniotic fluid is similar to the natural material, consisting mainly of surface active phospholipid, together with both hydrophobic and hydrophilic proteins. The 'modified natural' surfactants are lung minces or lavages that have been extracted with organic solvents to remove hydrophilic proteins. They contain mainly phospholipids and hydrophobic surfactant apoproteins, and are usually reconstituted in physiological saline before use. The lung tissue extracts contain nonsurfactant lipid contaminants from cell membranes, and they are often supplemented with additional components, for example synthetic DPPC, to optimize the surface activity [119,120].

Because of concerns about the risk of infection, potential immunogenicity of proteins and the limited supply of material from natural sources, artificial surfactants were also developed for therapeutic use in IRDS [3]. Two are in current use, and both contain synthetic phospholipid and are protein free. The artificial surfactant Exosurf consists of synthetic DPPC, to which is added the alcohol hexadecanol, and the mucolytic agent tyloxapol to aid liquefaction and adsorption of the surface film of phospholipid. These components are mixed in weight proportions 30.5:1.5:1 and suspended in 0.1M sodium chloride at a DPPC concentration of 13.5 mg/ml [142–146]. The other artificial surfactant used for treatment of IRDS is ALEC (Artificial Lung Expanding Compound; Britannia Pharmaceuticals, Redhill, Surrey, UK), which is a 3:1 mixture of synthetic DPPC and PG prepared from egg [115]. It was initially used as a dry powder [147] but is now prepared for clinical use as a 100 mg/ml suspension in saline [148–150].

The consensus of many clinical trials of surfactant therapy in IRDS is that prophylactic treatment using 'natural', 'modified natural' or artificial surfactants can reduce mortality by up to 50% [1–3]. Moreover, 'natural' or 'modified natural' surfactants can also reduce mortality by up to 40% when used as rescue therapy in infants with established IRDS [1,2]. New therapeutic surfactants are still being developed because of the recognition of the importance of the apoproteins in surfactant function. Attempts are being made to produce synthetic surfactants which more closely resemble the natural material [46,47]. The genes for the surfactant-specific apoproteins have been cloned [151–155] and recombinant apoproteins have been produced [156]. Synthetic analogs of apoproteins have also been developed [157–159]. It is hoped that mixtures of synthetic phospholipids and recombinant apoproteins or their analogs will be more efficacious than current protein-free artificial surfactants. The advantage of synthetic materials is that they can be produced in large quantities, without risk of infection, and at lower cost than the natural products. A summary of some of the main types of exogenous therapeutic surfactants currently used in IRDS, and synthetic products in development, is given in Table 16.2.

EXPERIENCE IN EXPERIMENTAL MODELS OF ACUTE LUNG INJURY

The benefit of surfactant replacement therapy in IRDS has raised much interest in its poten-

Table 16.2 Therapeutic surfactants used in IRDS and synthetic products in development

Main groups	*Main components*	*Examples of preparations*	*Selected references*
Natural or modified natural surfactants	Phospholipids/neutral lipids/proteins	Human amniotic fluid	139–141
		Lung tissue extracts:	
		Surfactant-TA (bovine)	119,120
		Survanta (bovine)	121–124
		Curosurf (porcine)	125–129
		Lung lavage extracts:	
		Infasurf (bovine)	130–133
		CLSE (bovine)	134–136
		Alveofact (bovine)	137,138
Artificial surfactants	DPPC/hexadecanol/tyloxapol	Exosurf	142–146
	DPPC/PG	ALEC	147–150
Synthetic 'natural' surfactants	Phospholipids/Recombinant apoproteins or analogs	Phospholipids/rSpC	46
		Phospholipid/KL_4	47

rSpC, recombinant SpC.

tial for treatment of other disorders where surfactant dysfunction is a contributory factor, particularly ARDS. However, because the causes of surfactant dysfunction in ARDS differ from those in IRDS and vary during the course of the syndrome, the optimal approaches to therapy are likely to differ and need to be defined. Important questions yet to be resolved are the following.

1. What is the optimal time to commence therapy after the patient has encountered the risk factor triggering ARDS?
2. What is the optimal formulation of therapeutic surfactant and will this be influenced by the risk factor?
3. Is the optimal formulation likely to differ at different stages in the development of ARDS?
4. What is the optimal method of delivery?
5. What is the optimal dose?
6. Are multiple doses likely to be required and for what duration?
7. Is anti-inflammatory therapy also needed to reduce the risk of damage to the instilled material by products of inflammation such as oxidants and proteolytic enzymes?
8. Can replacement therapy also promote endogenous surfactant synthesis?

These questions have not yet been answered definitively, but the results of surfactant therapy in experimental models indicate that it can reduce the severity of injury and improve oxygenation.

The models of ARDS in which a beneficial effect of exogenous surfactant administration has been demonstrated (Table 16.3) include acute lung injury induced by saline whole lung lavage in guinea pigs and other species [160–166], influenza A pneumonia and other infections in mice and rats [167–169, 111], injection of antilung serum in guinea pigs [170], vagotomy in rabbits [105], *N*-nitroso-*N*-methylurethane injection in rats and rabbits [171–172], prolonged hyperoxia in rabbits and baboons [173–177] and hydrochloric acid instillation in rabbits [178–179].

Saline lung lavage

Repeated *in vivo* whole lung lavage causes acute lung injury by removing most of the alveolar lining fluid, including the surfactant. This results in reduced lung compliance and severe hypoxemia ($Pa_{O_2} < 60$ mmHg (5 kPa)) despite ventilation with 100% oxygen. Lachmann *et al.* conducted one of the first studies

Table 16.3 Experimental models of ARDS in which a beneficial effect of exogenous surfactant administration has been demonstrated

Model	*Species*	*Surfactant*	*Delivery*	*Reference*
Saline lung lavage	Guinea pigs	Porcine modified natural	Instilled	160–163
	Rabbits	Sheep (164) or porcine (165) modified natural	Instilled	164,165
	Sheep	Bovine modified natural (Survanta)	Instilled and aerosolized	166
Infections				
Influenza-A pneumonia	Mice	Bovine modified natural	Instilled	167
Sendai virus pneumonia	Rats	Bovine modified natural	Instilled	168
Pneumocystis carinii pneumonia	Rats	Bovine modified natural	Instilled	109
Streptococcus pneumonia	Mice	Bovine modified natural	Instilled	111
Antilung serum	Guinea pigs	Bovine modified natural	Instilled	170
Bilateral vagotomy	Rabbits	Sheep natural	Instilled	105
N-Nitroso-*N*-methylurethane	Rats	Bovine modified natural (Survanta)	Instilled	171
	Rabbits	Bovine modified natural (Survanta)	Instilled and aerosolized	172
Prolonged hyperoxia	Rabbits	Calf lung surfactant extract (CLSE)	Instilled	173–175
	Baboons	Artificial surfactant (Exosurf)	Aerosolized	176,177
Hydrochloric acid	Rabbits	Bovine (Survanta, 178) or porcine (179) modified natural	Instilled	178,179

to explore the benefit of surfactant replacement in ARDS using a saline lavage model in guinea pigs [161]. Intratracheal administration of 50 mg of natural surfactant immediately and 30 minutes after lavage resulted in a rapid and dramatic improvement in gas exchange and lung function, maintained over the 5 hour period of the experiment. In subsequent studies they showed that improvement was obtained even if the treatment was given up to 2 hours after the lavage [162,163].

Histologically, saline lavage results in extensive atelectasis, marked necrosis and desquamation of type I alveolar epithelial cells but lesser damage to type II cells, and hyaline membrane formation [160]. The histological changes in surfactant treated animals are minimal by comparison [162]. The benefit of tracheal instillation of exogenous natural surfactant has also been demonstrated in lavage models using adult rabbits [164,165], and more recently in a lavage model using adult sheep in which the efficacy of tracheal instilled was compared with aerosolized exogenous surfactant given by nebulizer [166]. Both preparations improved oxygenation and lung compliance but the levels of improvement were greater for instilled delivery [166]. However, in a study using a different model nebulized delivery was superior [172]. The authors concluded that different delivery approaches may be required dependent upon the nature of the underlying injury.

Saline lung lavage models of ARDS have been criticized because the injury is homogeneous rather than inhomogeneous, as in the clinical syndrome; and because the marked deficiency of surfactant produced is more similar to the primary surfactant deficiency of IRDS than the mechanisms causing surfactant functional deficiency in ARDS. Despite these criticisms such studies have been important in providing a standard model for the testing and comparison of different preparations of therapeutic surfactants, which is essential for pharmacological development.

Other models

There are a number of other experimental models of ARDS which induce surfactant deficiency as a secondary consequence of lung injury, thereby resembling the mechanisms in ARDS, although the insults used are not necessarily comparable to the clinical risk factors. Surfactant replacement therapy has been shown to be of benefit in several of these models.

Infections

Various models mimicking the development of ARDS in patients with pneumonias or systemic infections are available. In 1987, acute lung injury in mice with influenza A pneumonia was shown to be improved by instillation of 200 mg/kg natural bovine surfactant [180]. The infection is normally lethal within 6 days, but both thoracic lung compliance and survival were improved by therapy. Therapy with natural surfactant (200 mg/kg) has also been reported to improve arterial oxygenation in rats with pneumonia induced by nebulized live Sendai virus [168]. In this model, abnormalities in surfactant function are associated with increased total protein in BAL. Instillation of natural bovine surfactant (200 mg/kg) has also improved gas exchange in experimental models of respiratory failure induced by *Pneumocystis carinii* pneumonia in rats [169] and *Streptococcus pneumoniae* infection in mice (111). Experimental models mimicking ARDS associated with Gram-negative septicemia are also available. These models have been induced, for example, by intravenous or intraperitoneal injection of live *Escherichia coli* or *E. coli* endotoxin in a variety of species [181–183]. The features are similar to those of the clinical syndrome and include alterations in surfactant function associated with high permeability edema and reduced synthesis of DPPC. Although sepsis is one of the most common and severe risks associated with the clinical development of ARDS, the benefit of surfactant replacement therapy in these models has yet to be investigated.

Antilung serum

A different model of acute lung injury can be induced by injection of antilung serum containing antibodies to surfactant associated proteins and to antigens of alveolar-capillary basement membrane [170]. Inactivation of surfactant function occurs due to edema and decreased lung phospholipid content. Evidence was obtained using this model suggesting that some alveolar phospholipid is lost by leakage into the blood across the damaged capillaries [111,170]. This identifies another mechanism which may contribute to alterations in surfactant composition after acute lung injury. Tracheal instillation of natural surfactant (350 mg/kg) soon after exposure to antilung serum was able to induce a significant improvement in gas exchange [170].

Bilateral vagotomy

Bilateral vagotomy in rabbits induces severe hypoxemia and reduced lung compliance within 4 hours [105]. The histological features show interstitial and intra-alveolar edema and hyaline membrane formation; and impaired surfactant function develops due mainly to protein inhibition. Tracheal instillation of natural surfactant (50 mg/kg) has also been reported to improve gas exchange and lung compliance in this experimental model of acute lung injury [105].

N-nitroso-N-methylurethane:

Changes very similar to those in ARDS can be induced in animals by subcutaneous injection of *N*-nitroso-*N*-methylurethane (NNNMU). The changes develop within a few days, and include alterations in BAL phospholipid composition resembling those in ARDS, and increased BAL protein [184]. A beneficial effect of instilled natural surfactant (100 mg/

kg) has been reported using this model in rats [171] and in rabbits using either instilled or aerosolized surfactant [172]. More recently the metabolism of exogenously administered surfactant has been investigated in NNNMU injured rabbits [185]. The clearance of ^{3}H-labeled surfactant from the alveolar wash and lung tissue was the same in both the normal and NNNMU injured groups, suggesting similar catabolic pathways [184,185]. However, the levels of DPPC were lower in the alveoli and lung tissue of the injured compared with the control animals 24 hours after treatment suggesting decreased endogenous surfactant synthesis and/or secretion [185]. This and other [173] studies indicate that a single dose of instilled surfactant is unlikely to result in prolonged supplementation of the intra-alveolar pool of DPPC because of its rapid clearance and catabolism. It is important to bear this in mind when devising optimal approaches to surfactant therapy in ARDS. Even in IRDS, rescue therapy after the development of lung injury often requires several instilled doses given over the first 24 hours (1).

Hyperoxia

Reactive oxidants (e.g. superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-), singlet oxygen and other free radicals) released from activated inflammatory cells are thought to play a role in the pathogenesis of ARDS [81–83]. They may contribute to tissue damage by oxidation of thiol groups on proteins, peroxidation of cell membrane associated fatty acids, and by generating reactive products that can cause further damage. Prolonged exposure to hyperoxia (100% O_2) can be used to produce experimental models of oxidant induced ARDS [186–188]. Injury develops after 24–48 hours of exposure, when the PaO_2 drops acutely shortly before death [189–191]. There is a progressive increase in alveolar permeability, a fall in BAL phospholipid content, an increase in BAL proteins and impaired surfactant function [186]. Matalon *et al.* [173] reported that intratracheal instillation of a saline suspension of 125 mg of calf lung surfactant extract (CLSE) (an organic extract of alveolar lavage containing 99% lipid and 1% hydrophobic proteins) after 64 hours of O_2 exposure could improve hyperoxia induced lung injury in rabbits. A second dose was given 12 hours after the first to maintain supplementation. Prolonged survival in rabbits with oxygen induced lung injury has also been achieved by prophylactic instillation of 125 mg of CLSE after 24 hours of 100% O_2 exposure before evidence of injury, followed by repeated doses at 24 hourly intervals [174]. Others have also reported a beneficial effect of early treatment with instilled surfactant in the rabbit model [175]. More recently, it has been demonstrated that administering exogenous surfactant by aerosol at the start of exposure to hyperoxia can improve oxygenation and reduce alveolar epithelial cell damage in adult baboons [176,177]. However, late stage treatment of oxygen induced lung injury does not necessarily achieve response [192], and the timing of surfactant therapy after injury is likely to be of considerable importance in relation to the benefit achieved. Moreover, the optimal timing may differ for different risk factors where the mechanisms and rate of development of lung injury are variable.

Oleic acid induced lung injury

ARDS can develop in patients who suffer fat embolism due to the release of fatty acids from fractured long bones after trauma. An experimental model of this type of injury has been developed using intravenously injected oleic acid [193,194]. Surfactant dysfunction appears to be mainly due to increased contamination of the alveolar lining fluid with protein and unsaturated fatty acid [195–197]. An attempt has been made to treat oleic acid induced lung injury in sheep with an aero-

solized preparation of the artificial surfactant Exosurf (mainly DPPC), but no response was achieved [198]. There is much controversy over the comparative advantages and disadvantages of aerosolized versus instilled delivery of therapeutic surfactants. It is also possible that protein-free artificial surfactants may not be able to counteract the complex disturbances of the surfactant system which occur in ARDS. These difficulties are discussed below in relation to the few clinical trials of surfactant replacement therapy in ARDS.

Installation of hydrochloric acid

ARDS can be induced by aspiration of gastric contents; and an experimental model mimicking this situation has been produced by intrabronchial instillation of hydrochloric acid [178,179]. Interestingly, the efficacy of surfactant therapy (modified natural porcine surfactant 75 mg/kg) was improved in this model by prelavaging the damaged lung with saline to remove inhibitory proteins before instillation of the therapeutic surfactant [179]. This suggests that whole lung lavage immediately before therapy could be considered in clinical situations where there is protein inhibition, if no response is achieved using the conventional approaches.

These experimental models mimicking different risk factors associated with clinical ARDS help to define better the rationale for surfactant replacement therapy in a variety of circumstances leading to surfactant deficiency. The findings justify the continuation of efforts to develop further this approach for the treatment of patients with ARDS.

CLINICAL EXPERIENCE OF SURFACTANT REPLACEMENT IN ARDS

Despite encouraging results in experimental models of ARDS, there have been only a few clinical trials of surfactant replacement therapy in patients with ARDS to date (Table 16.4). The major difficulty in designing clin-

Table 16.4 Clinical trials of surfactant replacement therapy in ARDS

Number of patients	*Risk factor*	*Surfactant*	*Delivery*	*Reference*
Case reports				
1 child	Viral and bacterial pneumonia	Bovine modified natural, 300 mg/kg	Instilled	199
3 adults	Sepsis Pancreatitis Aspiration pneumonia	Porcine modified natural (Curosurf) approx. 60 mg/kg within 72 h of onset	Instilled	200
2 adults	Burn injury Cardiopulmonary bypass	Bovine modified natural (Surfactant-TA) 240 mg/day	Instilled repeated doses	201
7 children	?	Bovine modified natural, 100 mg lipid/kg of a lipid extract from alveolar lavage at 6–8 h intervals to max. 4 doses	Instilled repeated doses	202
Controlled trials				
49 adults	Sepsis	Artifical (Exosurf), 40.5 or 81 mg/ml for up to 5 days after onset	Nebulized	203
498 adults	Sepsis	Artificial (Exosurf), 13.5 mg DPPC/ml for up to 5 days after onset	Nebulized	204
59 adults	Trauma Multiple blood transfusions Sepsis Aspiration pneumonia	Bovine modified natural (Survanta) 50 mg PL/kg at minimum 6 h intervals to max. 8 doses; or 100 mg PL/kg to max. 4 doses; or 100 mg PL/kg to max. 8 doses	Instilled repeated doses	205,206

PL = total phospholipid

ical trials is due to the lack of knowledge of the precise nature of surfactant alterations at different stages during the course of ARDS, and the influence of the different risk factors. The first clinical trials reported in the literature were mainly uncontrolled case reports of empirical treatment early after the onset of ARDS [199–201]. At this early stage, protein contamination often makes the major contribution to surfactant dysfunction [97,98]. The first report was of a terminally ill child with viral and bacterial pneumonia treated with instilled natural bovine surfactant, 300 mg/kg [199]. There was a dramatic improvement in arterial oxygen tension (Pa_{O_2}) which rose from 19 to 240 mmHg (2.5 to 32 kPa) within 4 hours of treatment. The chest radiograph also showed a marked improvement. The second report was of three patients with severe ARDS related to sepsis, pancreatitis or aspiration pneumonia, treated by tracheal instillation with 4 g (approximately 60 mg/kg) of the modified natural porcine surfactant Curosurf in 50 ml volume within 72 hours of onset [200]. Treatment was well tolerated and produced a transient improvement in gas exchange. Nosaka *et al.* then reported that repeated instilled doses (240 mg/day) of the modified bovine natural surfactant Surfactant-TA approximately 15 times over 38 days in a 66 year old woman who developed ARDS after severe burn injury, and three times in a 51 year old man who developed ARDS after cardiopulmonary bypass, achieved more persistent improvement in gas exchange [201].

In 1992, the preliminary results of the first multicenter, randomized, placebo controlled trial of exogeneous surfactant therapy in ARDS were presented [203]. A nebulized preparation of the artificial surfactant Exosurf was used in this trial, aiming to achieve more prolonged delivery and even distribution. Nebulized doses (40.5 or 81 mg/ml) were given over the first 5 days after onset to patients with sepsis induced ARDS. The initial results on an intake of 49 cases indicated a trend towards reduced mortality and improved physiology at 14 days. However, results recently reported for a larger intake of 498 patients revealed no significant improvement in any parameter compared with the matched controls [204]. Patients were stratified by risk of mortality (APACHE-III score) and randomized to receive either Exosurf (13.5 mg DPPC/ml) or placebo (0.45% saline) aerosolized for up to 5 days; 249 of the patients received surfactant (138 males and 111 females, mean age 49 years ± 17) and 249 received placebo (141 males and 108 females, mean age 53 years ± 18). At entry to the study, both groups had similar histories and APACHE-III score distribution. Twenty-eight percent of patients did not complete the full 5 days of therapy due to early discontinuation of ventilation or death, but the surfactant was well tolerated and safety assessments showed no consistent differences between the groups. Improvement was judged by the assessment of mortality at 30 days for the group overall, for the patients stratified according to APACHE-III distribution, and for the subset of patients with pneumonia. Treatment had no significant effect on 30 day mortality, or on physiological measurements over the 5 days of treatment in any of these groups.

By contrast with the Exosurf trial, more encouraging results have been obtained in another multicenter, randomized controlled trial in progress using the modified natural bovine surfactant Survanta, employing multiple instilled doses, and comparing several dosing strategies [205,206]. Patients with ARDS associated with trauma, multiple blood transfusions, sepsis sydrome and/or witnessed aspiration of gastric contents are being studied and the preliminary results have recently been presented [205,206]. Patients were included in the trial within 48 hours of onset if the Pa_{O_2}/F_{IO_2} ratio was < 200 and positive end expiratory pressure PEEP $> 10\ cmH_2O$, and four groups were compared. One ($n = 8$) received doses of 50 mg phospholipid/kg, repeating this dose at a

minimum dosing interval of 6 hours to a maximum of eight doses according to prospectively determined criteria (PaO_2/FIO_2 falling to below 250). A second group ($n = 16$) were given 100 mg phospholipid/kg, employing a maximum of four doses, while a third group ($n = 19$) were given 100 mg phospholipid/kg to a maximum of eight doses. The fourth ($n = 16$) was treated in the standard way without surfactant supplementation. Response was determined from arterial blood gas tensions and ventilator settings recorded at baseline and 120 hours after the initial dose of surfactant. Patient outcome at 28 days was also recorded. Evaluation of the changes from baseline to 120 hours indicated that a significant improvement in ventilatory requirements was achieved using 100 mg phospholipid/kg to a maximum of four doses ($P < 0.05$ compared with standard therapy). Twenty-eight day mortality was significantly decreased, and this trend was also observed using 100 mg phospholipid/kg to a maximum of eight doses. BAL samples were obtained at baseline and 120 hours after the initial dose of surfactant to evaluate the surface active function and chemical composition of surfactant before and after Survanta supplementation. The highest supplementation was obtained using 100 mg phospholipid/kg to a maximum of eight doses [206]. In another recent study, preliminary results showing improvement have also been obtained using repeated instilled doses of bovine natural surfactant (100 mg lipid/kg of a lipid extract from alveolar lavage) in an uncontrolled trial in seven pediatric patients with ARDS [202]. Up to four doses were given at 6–8-hourly intervals depending on oxygen criteria. Four of the seven patients had significant improvements in oxygenation ($PaO_2/FIO_2 > 200$ in three, and > 150 in one) peaking within 2 hours after the first dose. These patients also had improvements in ventilation efficiency and peak inspiratory pressure values. On the basis of these results, it was proposed that a randomized double blind clinical trial of exogenous surfactant therapy should be performed in pediatric patients with ARDS.

There are many possible explanations for the contradictory results obtained in the first two multicenter, randomized controlled trials of surfactant therapy in ARDS. The formulations of the two surfactants used differed in major respects, as did the modes of delivery and doses. The patient selection criteria also differed; the Exosurf trial included only patients with sepsis associated ARDS, while the Survanta trial included patients with ARDS associated with a range of risk factors. Regarding the influence of different formulations, many clinical trials in IRDS have indicated that 'natural' or 'modified natural' surfactants are more efficacious for rescue therapy than the protein-free artifical surfactants [1]. The results of the first two controlled trials suggest that this may also be the case in ARDS. Regarding methods of delivery, the proposal that nebulized delivery might have significant advantages was not validated by the Exosurf trial [166,204], but the efficacy of natural surfactant preparations using this method of delivery is unknown. Nebulization has the advantage that surfactant can be delivered continuously during ventilation (linked with cycles of inspiration) to maintain supplementation over prolonged periods but it has the disadvantage that $< 30\%$ of the administered dose is deposited within the lungs [207]. There is also evidence that the process of nebulization may cause foaming, which reduces the surface tension lowering ability of surfactant preparations *in vitro* [208]. Preferential delivery to more normal areas of the lungs is also a potential problem [209]. If instilled multiple doses prove inadequate to maintain supplementation at optimal levels, it is possible that a combined approach to delivery, commencing with high dose instilled surfactant to achieve maximal initial supplementation followed by nebulization to maintain supplementation, may be an appro-

priate way to achieve optimal delivery of therapeutic surfactants to adult lungs.

The timing of treatment after encountering the initiating risk factor is likely to be of great importance in relation to the response achieved. Prophylactic therapy is more efficacious than rescue therapy in IRDS [1], and this raises the question of whether prophylactic use of surfactant replacement therapy can reduce the development of acute lung injury in patients at risk of ARDS. To address this question, a clinical trial has been conducted recently to determine the effect of exogenous surfactant therapy (3.2 g of ALEC instilled 60 minutes after bypass) on lung function after cardiopulmonary bypass [210]. No improvement was detected in eight treated compared with eight untreated control patients monitored at regular intervals up to 180 minutes after bypass. Moreover, the carbon monoxide transfer factor (TLCO) was significantly lower in the treated group at 120 minutes after therapy, raising the question whether treatment may be disadvantageous in individuals who may not require it. This emphasizes the need for early prognostic indicators to identify and select those patients especially at risk.

There is very little information on surfactant replacement therapy in patients at a late stage of severe ARDS, when there is deficient surfactant production as well as excessive protein contamination of the system [97,98]. We investigated the effect of single dose intrabronchial instillation of the artificial surfactant ALEC (75 mg/kg) in four such patients [211]. There was no sustained clinical improvement, but BAL samples before and at 24 hours after treatment showed that measurable supplementation of *in vivo* PC and PG levels and proportions had been achieved. ALEC is composed of 70% DPPC and 30% PG, but BAL levels of PC at 24 hours were increased up to 4.4-fold and PG levels up to 34.7-fold. PC proportions (percent of total phospholipid) remained below the normal range, but PG increased to above the normal range in three patients. These findings are consistent with experimental evidence that PC is cleared from the air spaces of the lungs more rapidly than PG [1,212–214]. This suggests that doses and formulations of therapeutic surfactant to produce greater and more sustained supplementation of PC are required in patients with late stage ARDS. These findings demonstrate the value of BAL in monitoring the levels of supplementation and other *in vivo* effects of surfactant therapy, to guide therapeutic modifications that may be required to optimize response.

Our study in patients with late stage ARDS [211] also indicated that surfactant therapy does not reduce neutrophil counts in BAL. This suggests that combined treatment with anti-inflammatory drugs may be advisable to prevent further damage to intrinsic or instilled surfactant by oxidants and proteases from such cells. Many clinical trials of corticosteroids have been conducted in ARDS with the aim of suppressing neutrophils [215]. Most were trials of prophylactic treatment of patients with sepsis at risk of ARDS, or early stage treatment after onset of the syndrome. They concluded that early treatment with corticosteroids is of no therapeutic benefit. The only exception to this was a trial in patients with ARDS associated with fat embolism [216]. However, recent evidence suggests that corticosteroids can be of value in patients at a later stage of ARDS who develop the complication of pulmonary fibrosis [217]. We have had the opportunity to study one such case and observed that BAL neutrophil counts, phospholipid levels and proportions of PC and PG normalized after commencement of treatment in association with a striking clinical response [218]. This suggests that the benefit of corticosteroids may be due not only to their anti-inflammatory effects but also to their ability to stimulate surfactant phospholipid production by type II alveolar epithelial cells, either directly or by stimulating fibroblasts to produce a 'fibroblast pneumocyte

factor' which can enhance surfactant synthesis [219].

Suppression of neutrophils in ARDS may also reduce damage to surfactant-specific proteins which have been shown to be susceptible to degradation by neutrophil derived proteases *in vitro* [85]. There is little information on *in vivo* levels of surfactant-specific proteins in ARDS, but a recent report indicates that BAL levels of Sp-A and Sp-B are significantly decreased, and Sp-A levels are also decreased in patients at risk [99]. These reductions are likely to contribute to surfactant dysfunction because of the importance of these molecules in surfactant biophysical function and turnover. Sp-A and Sp-D also have antimicrobial functions (see below) which could be of relevance to the increased susceptibility of patients with ARDS to nosocomial infections. In the light of these observations, it is probable that more complete formulations of natural or synthetic therapeutic surfactants containing surfactant associated proteins and phospholipids will prove to be of greatest benefit for the treatment of ARDS.

ROLE IN HOST DEFENSE

The pulmonary surfactant system plays an important role in pulmonary immune defense mechanisms, although less is known about these functions than its biophysical properties. Normal surfactant enhances the innate 'nonspecific' immune defense mechanisms which clear inhaled particles and microbes from the lungs, and suppresses the development of specific T lymphocyte mediated immune responses to inhaled antigens, preventing a constant state of immune hyperreactivity detrimental to life. Lymphocytes from the alveolar spaces of normal lungs are less responsive to mitogenic and antigenic stimulation than their counterparts in blood [220]. In 1979, Ansfield *et al.* first reported that normal surfactant from canine lungs suppresses the proliferation of canine blood lymphocytes [221]. Work from our group has shown subsequently that normal pulmonary surfactant from humans, pigs and rabbits also suppresses human blood lymphocyte proliferation to mitogens and alloantigens [222]. The major phospholipid components, PC and PG, are highly immunosuppressive, but the minor components, SM and PE (which increase during tissue damage), are highly immunostimulatory [223]. This suggests that the alterations in surfactant composition which develop during acute lung injury could have an influence on the regulation of pulmonary inflammatory responses.

By contrast with its immunosuppressive effects on T lymphocytes, normal pulmonary surfactant enhances 'nonspecific' pulmonary immune defense mechanisms. It aids mechanical clearance of cells and particles from the air spaces by its biophysical properties which promote movement towards the mucociliary escalator at end expiration, due to the increasing film pressure exerted by the surface monolayer of phospholipids. It is also thought to play a role in recruiting alveolar macrophages to the air spaces of the lungs, as both natural surfactant and Sp-A are chemotactic for alveolar macrophages *in vitro* [224–226]. Alveolar macrophages are the main phagocytes responsible for the immune defense of normal lungs, and it has been demonstrated that Sp-A and Sp-D can enhance the phagocytosis and killing of micro-organisms by these cells [44,45, 227–230]. Neutrophils recruited during inflammatory responses also phagocytose and kill bacteria, and assist wound healing through their scavenger functions by degrading and removing products of tissue damage. However, an excessive neutrophil response, as occurs in ARDS, can have adverse effects which outweigh the advantages [231]. It is not known whether pulmonary surfactant plays a role in regulating neutrophil responses, but a study using the porcine modified natural surfactant Curosurf indicates that this has no

effect on neutrophil adherence or migration and may slightly reduce bacterial phagocytosis and killing by these cells [232]. Surfactant has also been reported to enhance CD11b/CD18 adhesion molecule expression on neutrophils *in vitro* [233] and we have observed that there is no reduction in BAL neutrophil counts after ALEC therapy in patients with ARDS [211], suggesting that surfactant may not inhibit neutrophil activation nor recruitment to the lungs. Elucidation of the effect of surfactant on neutrophils is an important area for future research, especially in relation to ARDS. On the other hand, the synthetic surfactant Exosurf has been reported to inhibit secretion of the cytokines tumor necrosis factor (TNF)α, interleukin (IL)-1 and IL-6 by activated human alveolar macrophages *in vitro* in a dose dependent manner [234]. Decreased secretion of TNFα after preincubation of human blood monocytes with the natural porcine surfactant Curosurf has also been reported [235]. These and other cytokines are thought to play an important role in the inflammatory mechanisms involved in the pathogenesis of ARDS [90,91,231,236–238]. The mechanisms are described in detail elsewhere.

Apart from providing information on surfactant composition and function, BAL investigations have the potential to clarify the relationship between changes in levels of inflammatory cells and their products, and the development of surfactant dysfunction in ARDS. New therapeutic agents are being developed to modulate the effects of inflammatory mediators, including antibodies to endotoxin or cytokines [239–241], low molecular weight antagonists of cytokines or cytokine receptors [242] and agents to modulate expression or block interaction with adhesion molecules [243–245]. These developments, together with the research in progress to produce more efficient synthetic therapeutic surfactants, appear to offer better prospects for the future.

SUMMARY

Alterations in pulmonary surfactant are an important contributory factor in the pathogenesis of ARDS. Their consequences on rate of progression of the syndrome, prognosis and increased susceptibility to nosocomial infection need to be better elucidated. Continuing work on surfactant therapy, including efforts to develop synthetic surfactants which more closely emulate natural surfactant, is important, as this has the potential to improve survival and/or speed recovery by reducing ventilatory requirements.

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