THE SURFACTANT SYSTEM AND PULMONARY DISEASE

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"And a thousand thousand slimy things
Lived on; and so did I"

Samuel Taylor Coleridge
The Ancient Mariner

Introduction

For a few days in the summer of 1963 the nation was transfixed and ultimately saddened by the struggle for life of Patrick Bouvier Kennedy. Born 5 weeks premature and weighing 4 pounds, 1 ounce the infant son of President and Mrs. John F. Kennedy suffered from hyaline membrane disease, an entity characterized by respiratory distress in premature infants. Only a few years earlier it had been noted that infants suffering from this disease had pulmonary surfactant which functioned abnormally. Lacking effective treatment the child of the worlds most glamorous and powerful couple died on the third day after delivery.

The development of neonatal intensive care units and the utilization of surfactant replacement in the intervening three decades have greatly changed the management of newborn respiratory distress syndrome (RDS). In 1995 a child of similar weight and maturity with RDS would have an overwhelming chance of survival. The knowledge derived from study of surfactant, a complex mixture of lipid and proteins produced by type II pneumocytes which is unique to the pulmonary environment, has also impacted on the understanding of many lung diseases in the adult. However for most internists the surfactant system and its role in pulmonary function remain a distant memory. This review will therefore focus on the structure and function of pulmonary surfactant and the diverse role that components of surfactant play in promoting gas exchange, participating in host defense against microorganisms, and modulating the function of immune and inflammatory cells in the lung.

Physiologic Role of Surfactant

Optimal pulmonary gas exchange depends on a precise matching of ventilation (V) and perfusion (Q). Blood supply to an alveolar unit with little or no ventilation (Figure 1) results in the return of oxygen poor blood to the pulmonary vein, left atrium and ventricle and subsequently to the systemic circulation. Many circumstances may lead to V/Q mismatching but one of the most common is atelectasis, a condition where segments of gas exchanging units essentially collapse against themselves.

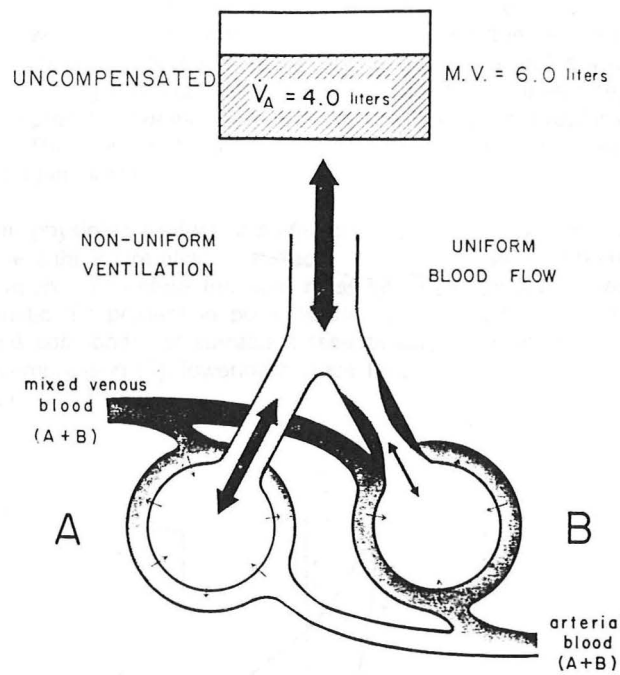

FIGURE 1 Production of ventilation-perfusion mismatch. Lung segment B is relatively hypoventilated. Blood leaving segment B is deoxygenated and mixes with normally oxygenated blood leaving segment A to produce the final O_2 concentration found in the systemic circulation.

The major force promoting the tendency of the lung to collapse inward on itself is elastic recoil. Elastic recoil acts much like a stretched spring, decreasing volume to the point where there is no further tension. The two major components of elastic recoil are the intrinsic tension of the connective tissue structures of the lung and surface tension at the alveolar air-tissue interface.

The major physiologic role of surfactant is to decrease surface tension. This is important in both inspiration and expiration. However it is perhaps easier to understand the importance of this phenomenon at low lung volumes. According to LaPlace's law the amount of pressure (P) necessary to increase the size of a sphere (such as an alveolus at the onset of inspiration) is defined by :

$$P=\frac{2T}{R}$$

where T = surface tension and R = radius of the sphere. Thus at low alveolar volumes (low R) if surface tension remained high the amount of pressure required to "open" the alveolus would be high and the alveolus would likely stay closed, producing atelectasis and a V/Q mismatch. Furthermore, at any lung volume the pressure necessary to produce an increase in alveolar volume would be high. This loss of compliance in turn would require increased work of breathing during inspiration.

The physiologic effect of surfactant is produced by a monolayer of lipid which covers the entire alveolar surface. This monolayer must be recreated with each inflation. Therefore the lipid must be adsorbed and spread rapidly, a characteristic not present in pure diplamitoyl-phosphatidylcholine (DPPC) the major lipid component of surfactant (see below). On deflation the surfactant layer is compressed (1), lowering surface tension to a value approaching zero (Figure 2).

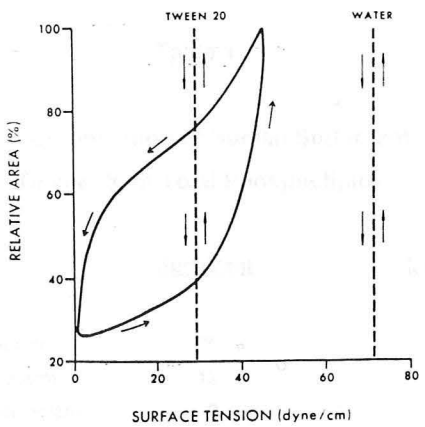

FIGURE 2 Surface area-surface tension relationships for normal surfactant (solid lines), Tween 20 (a detergent) and water. As surfactant is compressed in area surface tension decreases nearly to zero. The hysteresis loop for surfactant closely parallels the compliance curves for intact normal lungs during the respiratory cycle.

The function of surfactant relies on a complex inter-relation of lipids and proteins which ultimately enables a surface tension lowering monolayer to be formed. Disease states may be produced by abnormalities of either lipid or protein.

Constituents of Surfactant

Lipids

Surfactant isolated from the alveolar space by bronchoalveolar lavage (BAL) is composed approximately 90% of lipid. These lipids are predominantly phospholipids with some triacylglycerols and cholesterol. The major phospholipid (70-80%) in surfactant (Table 1) in all species studied to date is phosphatidyl choline (PC) the majority of which is fully saturated with diplalmitoyl species to form DPPC (2,3).

Table 1

Phospholipid Composition of Human Surfactant and Lung Tissue (% of Total Phospholipid)

	Surfactant	Lung Tissue
Phosphatidylcholine	73	50
Phosphatidylglycerol	12	3
Phosphatidylethanolamine	3	19
Phosphatidylinositol	3	3
Phosphatidylserine	3	7
Sphingomyelin	4	12
Others	2	6

Phosphatidyl glycerol (PG), which is an uncommon lipid in mammalian cell membranes, accounts for ~ 10% of lipid. Common membrane phospholipids such as phosphatidylserine, sphingomyelin, and phosphatidylethanolamine are minor components of surfactant.

Thus although surfactant lipids are found in other substances, the relative composition of surfactant lipids is unique. Indeed the ratio of phosphatidylcholine (lecithin) to sphingomyelin (L/S ratio) is commonly used to assess fetal lung maturity. It should be remembered however that studies examining the regulation of phospholipid synthesis in the lung can not distinguish between surfactant lipids and those associated with cell membranes. Nevertheless, significant data exists concerning the regulation of phospholipid synthesis in developing fetal lung or isolated type II pneumocytes (4).

Regulation of Phosphatidylcholine Synthesis and Secretion

The rate limiting enzyme in PC synthesis is choline-phosphate cytidyltransferase (CYT). The activity of CYT is regulated by glucocorticoids, thyroid hormone and estrogen (5-10). These hormones appear to act indirectly on CYT activity. Several studies suggest that the quantity of CYT is not increased by glucocorticoids but that activity is enhanced through up-regulation of another enzyme, fatty acid synthase (FAS). The up-regulation of FAS is accompanied by increased expression of the gene and synthesis of the enzyme. Fatty acids then serve as a co-factor to directly activate CYT through a variety of mechanisms including translocation of the enzyme to the cell membrane.

The time course of PC appearance in the developing neonate is relatively linear from midgestation to term. Significant secretion as reflected by accumulation in amniotic fluid does not occur until 35 weeks in normal pregnancies. In adults, PC synthesis can also be regulated by hormonal manipulation and has been shown to be inhibited by some inflammatory mediators such as tumor necrosis factor (TNF; see below).

Promoters of PC secretion include B-adrenergic agents, other agents that increase cAMP, purine nucleotides, leukotrienes, and active labor (11-21).

Proteins

The majority of protein isolated from surfactant is serum protein. However there are four unique surfactant associated proteins (Table 2) designated SP-A, B, C and D. These proteins are expressed in a lung-specific fashion and their synthesis is subject to numerous regulatory forces. SP-A and SP-D have many structural simularities and are encoded for by genes on chromosome 10. SP-B and SP-C are small hydrophobic proteins which play a prominent role in promoting adsorption of phospholipid to the air-tissue interface and rapidly reduce surface tension. Surfactant proteins have been the subject of intensive investigation and play a major role in the function of surfactant.

Table 2

Surfactant Associated Proteins

	Molecular Weight	Chromosome	Hydrophobic	Protein Class
SP-A	36KD	10	No	Collectins
SP-B	7KD	2	Yes	Saposins
SP-C	5KD	8	Yes	?
SP-D	43KD	10	No	Collectins

SP-A and SP-D

Structure

SP-A is a glycoprotein with a molecular weight of ~ 36 KD which exists in the alveolus as a multimer of six separate triple helical structures (18 polypeptide chains) with a molecular weight of ~ 700 KD (22,23). SP-A contains two distinct domains, a collagen like amino terminal end and a carboxy terminal lectin-like structure. SP-A is thus capable of binding lipids and carbohydrates as well as interacting with specific cell surface receptors.

SP-D is a glycoprotein with MW ~ 43 kd. SP-D exists in the alveolus as multimers of trimers and has collagen and lectin-like ends similar to SP-A (24,25). However the collagen-like domain of SP-D is considerably longer than SP-A. Both SP-A and SP-D have been demonstrated to be present only in type II cells and nonciliated bronchiolar epithelial (Clara) cells.

Function

SP-A has a number of functions related to the assembly of surfactant (26-28). First, SP-A binds strongly to phospholipids and in concert with calcium, SP-B and SP-C promotes the structural transformation of lamellar bodies to tubular myelin. Secondly, SP-A in concert with SP-B and SP-C promotes the formation of phospholipid surface films. Third, SP-A may augment the uptake and re-utilization of surfactant components by endocytosis on the apical surface of type II cells. Finally SP-A can inhibit PC synthesis by isolated type II cells suggesting that SP-A may provide "feedback inhibition" on surfactant secretion. The role of SP-D in the function of surfactant is largely unknown. Knockout mice lacking SP-A or SP-D may further clarify the role of these proteins in surfactant function.

Both SP-A and SP-D may have important roles in host defense and modulation of immune and inflammatory cell function (see below).

Regulation of SP-A and SP-D Gene Expression

The major regulators of SP-A gene expression are glucocorticoids, arachidonic acid metabolites and cAMP (29-33). Prostaglandin E2 and cAMP both produce an up-regulation of SP-A gene transcription, mRNA levels and protein production. In contrast glucocorticoids up-regulate gene transcription but actually decrease SP-A mRNA by rendering the mRNA less stable and decreasing its half-life. Thus the overall effect of glucocorticoids on SP-A production may vary.

SP-A is first detectable in human amniotic fluid at 30 weeks gestation. In contrast SP-D gene transcription is initiated late in gestation after transcription of other surfactant proteins and phospholipid synthesis has begun. The regulation of SP-D gene expression is largely undetermined.

SP-B and SP-C

Structure

Both SP-B and SP-C are small hydrophobic molecules (22). SP-B is initially synthesized as a precursor polypeptide with MW ~ 40 KD. The proteolipid derived from this precursor has a reduced MW of ~ 7KD. SP-B contains seven cysteine residues which participate in the formation of three intrachain disulfide bonds and one inter-chain bond with another SP-B to form a dimer. This structure is similar to a functionally diverse group of proteins designated saposins, which bind a variety of lipids. SP-B shows a particular affinity for binding phosphatidyl glycerol (34-36).

SP-C is produced as a 22kd precursor which is processed to a 5 KD proteolipid in its reduced form. SP-C contains an alpha helix capable of spanning a lipid bilayer (37-39).

Function

SP-B and SP-C markedly enhance the surface tension lowering properties of surfactant phospholipid. Both SP-B and SP-C promote rapid adsorption of the lipid though SP-B appears to be more potent in this role. It has been suggested that SP-B functions to remove unsaturated phospholipids from the lipid monolayer. Recent data in children with congenital alveolar proteinosis also suggests that SP-B may play a role in the polar nature of surfactant secretion from the type II cell (see below).

Regulation of SP-B and SP-C Gene Expression

The gene for SP-B is located on chromosome 2 and expression has been detected in both type II and Clara cells (40). The gene for SP-C is located on chromosome 8 and expression is found only in type II cells (41,42). Gene expression for both SP-B and SP-C occurs early in gestation with mRNA being detected by 13 weeks. In contrast to SP-A gene regulation, cAMP analogs have only mild stimulatory effects on SP-B or SP-C transcription. Glucocorticoids stimulate transcription of mRNA for both SP-B and SP-C and actually enhance SP-B mRNA stability (43-47).

Secretion, Processing, Degradation and Re-Utilization of Surfactant

Phospholipids are synthesized in the endoplasmic reticulum of the type II cell (Figure 3) (48), modified in Golgi complexes and then transported to immature lamellar bodies (composite bodies). Surfactant proteins are transported from the Golgi in small intracellular carriers called multi-vesicular bodies (mvb). The multivesicular bodies fuse with composite bodies to form mature lamellar bodies. Lamellar bodies containing protein and lipid are then secreted by exocytosis in a polarized manner at the apical epithelial surface. There is evidence that some surfactant proteins, particularly SP-A, may also be directly secreted independent of lamellar bodies.

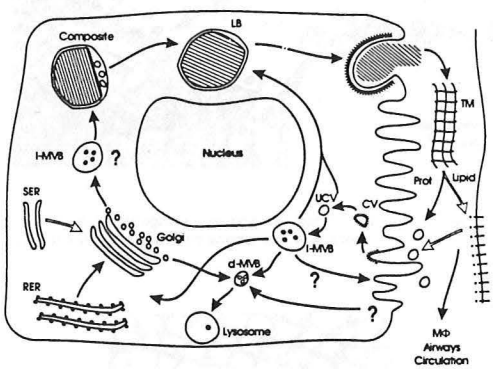

FIGURE 3 Schema of surfactant secretion. Multivesicular bodies (MVB) containing surfactant protein fuse with composite bodies to form lamellar bodies (LB). LB are secreted from the type II cell, ultimately forming tubular myelin (TM) (48).

After leaving the type II cells (Figure 4) the lamellar bodies undergo a series of modifications in the hypophase (i.e. the layer beneath the surfactant monolayer). Ultimately the lamellar body contents give rise to an extended lattice called tubular myelin, which is likely the precursor of the phospholipid monolayer at the air/tissue interface (Figure 5).

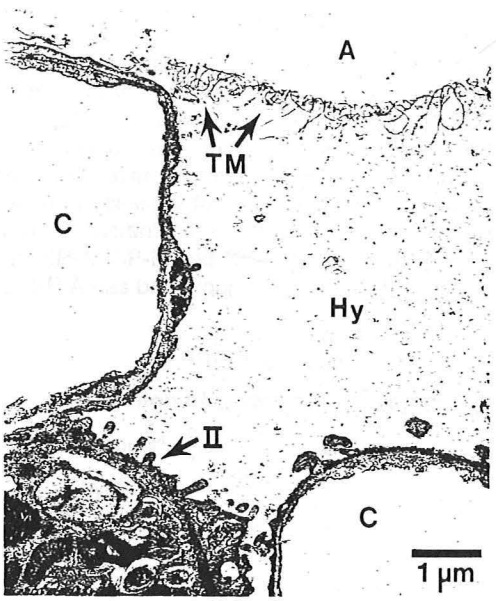

FIGURE 4 Electron micrograph showing type II cell, capillaries (C), hypophase fluid (Hy), tubular myelin (TM), and the alveolar space (A) (48).

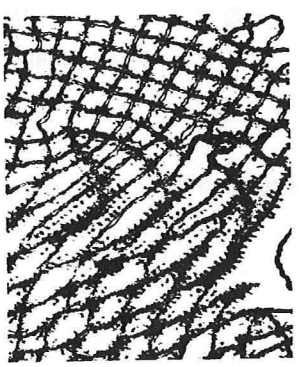

FIGURE 5 Electron micrograph of tubular myelin. Regularly spaced globular profiles on short stalks are found on the lipid bilayers.

Surfactant secretion is controlled (49) (Table 3) by a number of regulatory influences (cAMP, B-agonists, purine nucleotides, protein kinase cascades) but some component of surfactant secretion is constitutive. The constant production of surfactant in combination with the need to remove some proteins during expiration as the monolayer contracts and the need to re-generate the protein/lipid interaction necessary for adsorption and spreading during the next breath requires that surfactant be constantly recycled (Figure 6) (83). Data suggests that lipid, SP-A, SP-B and SP-C all re-enter the type II cell. A high affinity receptor for SP-A has been found on type II cells (27).

Table 3 (50)

Regulation of Surfactant Secretion

Glucocorticoids	∅
Thyroxine	+
Adrenergic agents	+
B-blockade	-
Estrogens	+
Androgens	∅
Prostaglandins	+
cAMP	∅
Ventilation	+
SP-A	-

∅ = none or variable

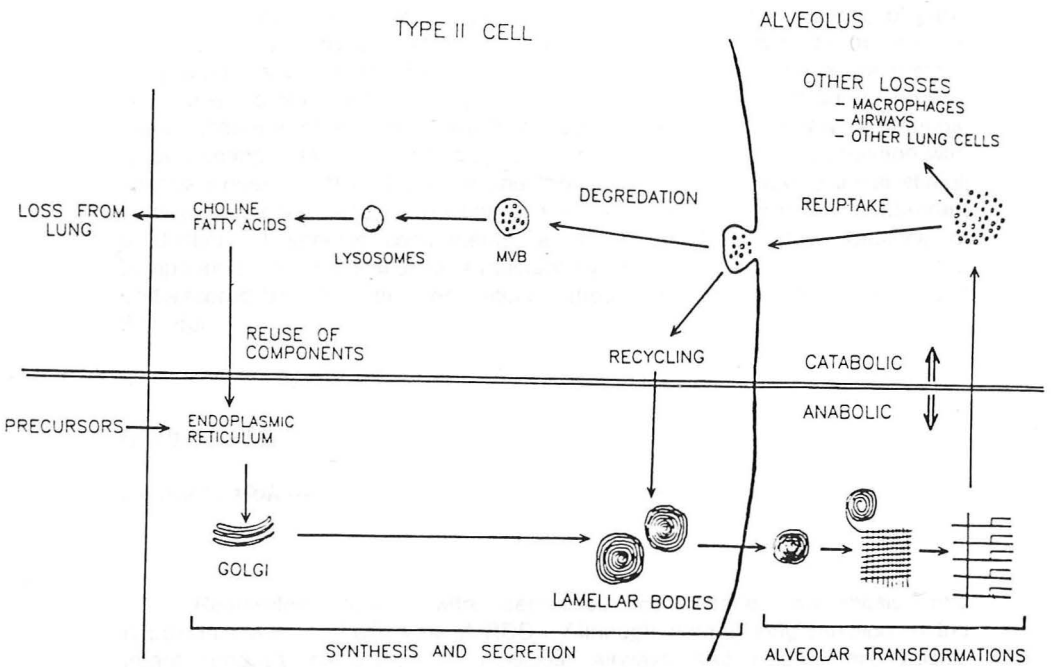

FIGURE 6 Secretion and breakdown of surfactant phosphatidylcholine (51).

Phosphaditylcholine is primarily recycled directly into lamellar bodies while only a minority is degraded and re-utilized for synthesis (50). One of the functions of SP-A intracellularly may be the targeting of lipid directly into lamellar bodies rather than shunting it to degradation. SP-A and lipid are also phagocytosed by alveolar macrophages. This compartment may represent ~ 10% of total "alveolar" SP-A (51). Overall the alveolar surfactant pool remains relatively constant. The administration of large quantities of exogenous surfactant to neonates or experimental animals has suggested the turnover time for DPPC is ~ 14 hours and that only 20-30% of administered radiolabeled surfactant is still present in the alveolus 24 hours after administration (52).

Thus the constituents of alveolar surfactant reflect the balance of gene transcription, stability of mRNA, production and secretion, catabolism, removal by type II cells and macrophages, recycling and degradation. All of these steps are subject to multiple regulatory influences. It is therefore not surprising that the components of alveolar surfactant may be altered in a variety of settings. Indeed changes in surfactant composition have been noted in association with serious illness, such as bacterial pneumonia, and after relatively trivial stimuli such as exercise. Altered function of surfactant may relate to either aberrant production of specific components, a change in the relative balance of components, or modification of surfactant by an inflammatory milieu. This is underscored by contrasting respiratory distress syndromes in the neonate and the adult.

Neonatal RDS

Pathophysiology

Respiratory failure with decreased compliance, atelectasis and hypoxemia are the hallmarks of RDS. Although normal lung function in the infant requires maturation of muscles, airways and neural mechanisms substantial evidence suggests that a reduction in surfactant lipid, specifically DPPC, is the central mechanism in most cases of RDS (53).

In 1959 Avery and Mead reported that saline extracts from the lungs of infants who died of RDS had poor surface tension lowering properties (54). Subsequently, much of the data concerning surfactant pools in the neonate has been derived from studies in pre-term lambs who develop an RDS like syndrome when delivered at 90% of gestational age. Lambs with little alveolar surfactant have severe hypoxemia and require high pressures during mechanical ventilation (Figure 7) (55). The average surfactant pool size in pre-term lambs with RDS was 20% of spontaneously delivered lambs at term.

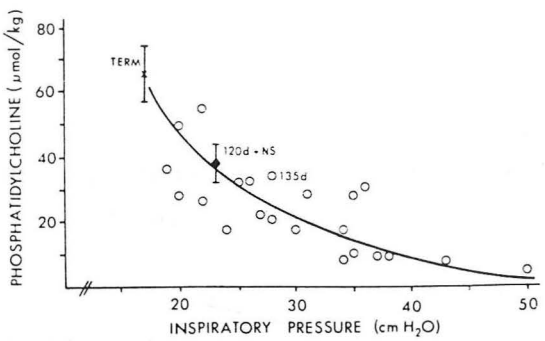

FIGURE 7 Correlation of surfactant pool size with peak inspiratory pressures in premature lambs delivered at 135 days gestation (O), 120 days and replaced with natural surfactant (NS), and term pregnancies (55).

In humans exact data on DPPC pool size of the full term neonate is not available. Most data suggests that neonates with RDS have a similar DPPC pool size compared to adults, but less than a term newborn (56). Complicating factors impacting on surfactant function in neonates with RDS include loss of surfactant into hyaline membranes, as the phospholipids can interact with plasma proteins to initiate a coagulation cascade. In addition plasma proteins may directly alter the surface tension lowering properties of surfactant. This appears most likely to occur in the setting of decreased endogenous surfactant protein content. Epithelial damage leading to pulmonary edema may also result in altered function (57,58).

Hormonal Manipulation of the Surfactant System

Many critical components of surfactant are up-regulated by glucocorticoids and thyroid hormone. A number of trials have examined the use of these agents in neonates. Because these hormones produce their effect through increased gene transcription, there is general agreement that these agents have maximal effect when given at least 24 hours prior to delivery.

The majority of studies support the use of glucocorticoids (Table 4) (59,60)in reducing the incidence of RDS in infants born 1-7 days after administration. It appears that steroids do not work particularly well after 34 weeks of gestation. Some studies have reported little or no benefit and there is data suggesting an increased risk of maternal infection if prolonged rupture of membranes is present. An NIH consensus report (61) in 1994 however strongly endorsed the use of glucocorticoids antenatally in mothers at risk for premature delivery. There is considerable data to suggest that steroids may synergize with surfactant replacement therapy in infants under 30 weeks gestation (62).

Table 4

Incidence of RDS In Infants Born 1-7 days After Maternal Corticosteroid Administration

	Study 1 (59)		**Study 2** (60)	
Gestational Age	**Control**	**Steroid**	**Control**	**Steroid**
<30 wk	58%	28%*	42%	60%
30-32	56%	9%*	-	-
32-34	13%	0	-	-
34+ wk	5%	6%	6%	5%
Total	24%	9%*	18%	9%*

*p<.05 compared to controls

T3 and T4 do not cross the placental membrane, while TRH does. Although thyroid releasing hormone (TRH) has been beneficial in some trials, more recent data suggests it is largely ineffective in altering the risk of RDS (63). Overall, however, the role of TRH when given in optimal doses has not been completely defined (64).

Surfactant Replacement Therapy

Early attempts at surfactant replacement involved the aerosol delivery of DPPC and were unsuccessful (65). In 1980, Fujiwara and colleagues used a surfactant prepared from an organic solvent extract of bovine lung to treat 10 pre-term infants with severe disease (66). The results were sufficiently encouraging that multiple trials soon followed (67-73).

There are currently several commercially available surfactant preparations in use around the world (Table 5). They can be divided into surfactants prepared from mammalian lungs and synthetic surfactant.

Table 5

Exogenous Surfactants

Synthetic	Source	Contents
ALEC (Pumactant)	Synthetic	DPPC, PG
Exosurf (Colfosceril palmitate)	Synthetic	DPPC, hexadecanol, tyloxapol
Human		
Human	Amniotic fluid	All lipids, SP-A, SP-B, SP-C
Animal		
Survanta (Beractant)	Minced cow lung & synthetic lipids	Lipids, SP-B, SP-C
Surfactant-TA (Surfacten)	Minced cow lung & synthetic lipids	Lipids, SP-B, SP-C
Curosurf	Pig lung	Lipids, SP-B, SP-C
Alveofact	Lipid extract of cow lung lavage	Lipids, SP-B, SP-C
CLSE	Lipid extract of calf lung lavage	Lipids, SP-B, SP-C
Infasurf	Lipid extract of calf lung lavage	Lipids, SP-B, SP-C

Survanta is prepared from minced bovine lungs by extracting lipids, SP-B and SP-C with organic solvents. DPPC, palmitic acid, and triglyceride are then added to improve the surface properties of the mixture. Survanta is widely utilized in the United States. It does not contain either SP-A or SP-D. Similar surfactant preparations include Curosurf, Alveofact, CLSE, and Infasurf.

Exosurf is a synthetic surfactant approved for use in the United States. It contains a mixture of DPPC with a synthetic polymer, tyloxapol, and hexadeconol which facilitate the spreading of DPPC. Exosurf contains no surfactant protein. ALEC is a 7:3 mixture of DPPC and phospatidyl glycerol used is England. One of the potential advantages of the synthetic surfactants is the concern about producing an immune response to foreign proteins contained in natural surfactants. However most studies have failed to detect antibodies against bovine proteins in infants who have received repetitive doses of Survanta (74,75).

Administration of Surfactant

From animal data an empiric dose of 100 mg of phospholipid/kg of body weight has been utilized in most series. The drug is suspended in 3-5 cc of saline/kg and delivered directly through an endotracheal tube. Because administration of such a large volume of liquid immediately upon delivery may hamper the recusitation of the infant there is debate as to the proper timing of administration. The majority of literature suggests that immediate instillation (prophylaxis) in high risk infants or waiting until symptoms develop prior to administration (rescue therapy) produce equivalent outcomes (Figure 8) (76).

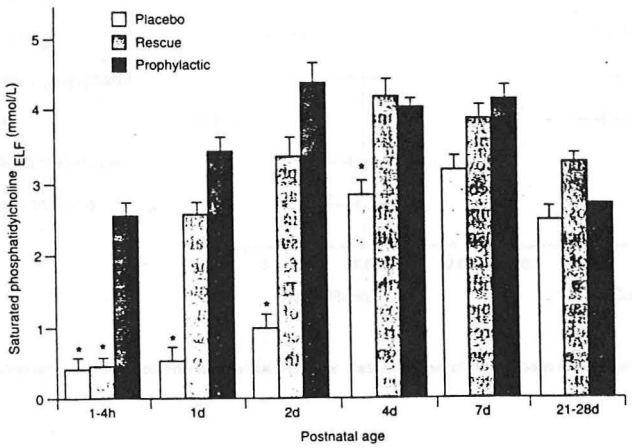

FIGURE 8 Saturated phosphatidylcholine in lavage from neonates with RDS receiving either rescue or prophylactic surfactant replacement (76).

Results of Surfactant Administration

There is overwhelming evidence that administration of surfactant to infants with RDS reduces mortality. In addition the incidence of pneumothorax, pneumomediastium, and pneumopericardium are decreased (Figure 9) (52). Most studies suggest that multiple doses of surfactant are superior to a single dose, but therapy is usually individualized. Beneficial effects on lung compliance and oxygenation can be seen within minutes in some infants. The only serious side effect reported with surfactant is a somewhat increased incidence of pulmonary hemorrhage. There is no eveidence that surfactant administration depresses endogenous surfactant production.

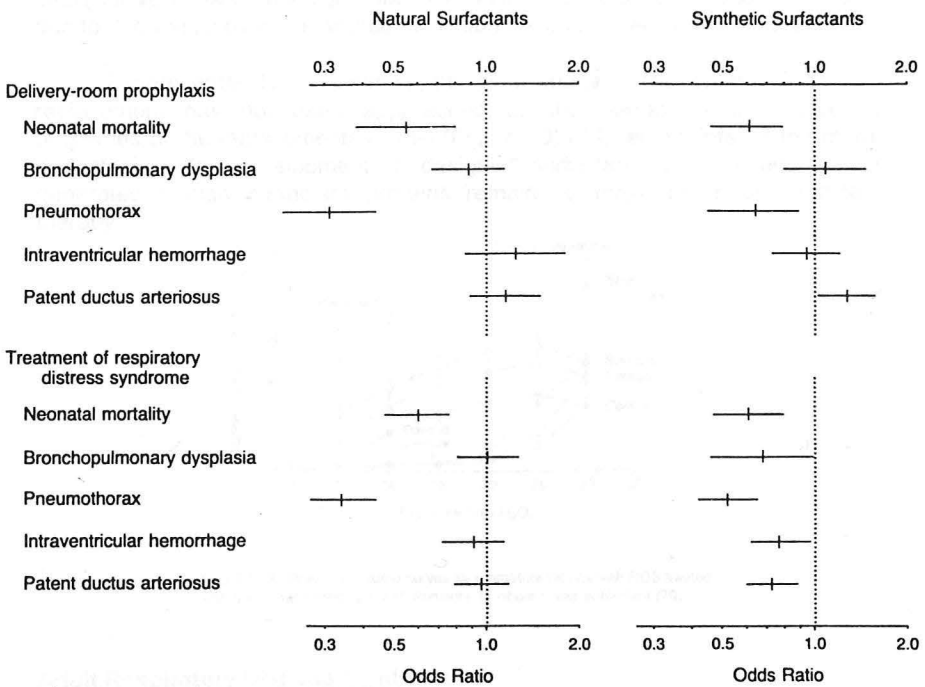

FIGURE 9 Odd ratios and 95% confidence intervals from four meta-analyses of clinical trials of the effects of surfactant in RDS (52).

In a recent multicenter study (77) Survanta and Exosurf were compared in neonates weighing 500-1500 gm with RDS. In this high risk group of patients death occurred in 19% of Survanta and 23% of Exosurf treated patients. Bronchopulmonary dysplasia was present in 43% of both groups of survivors. Survanta appeared to work faster than Exosurf resulting in significantly lower mean airway pressures and FiO2 requirements during the first 72 hours of therapy.

Overall the impact of Surfactant administration on morbidity and mortality of RDS has been great (78). Following the general introduction of surfactant replacement therapy in 1989 the overall infant mortality rate dropped 8.5% in 1989 and another 6% in 1990. Recent studies have demonstrated that the majority of this drop was accounted for by the use of surfactant. The rate of death for very low birth weight infants was reduced by 30%. Economic benefits due to decreased overall resource utilization have also been demonstrated.

Experimental data in animals, however, suggests that optimal surfactant replacement has not been approached as the surface tension lowering properties of the replacements *in vivo* (Figure 10) (79) remain inferior to natural surfactants. The development of "designer" surfactants utilizing recombinant generated human surfactant proteins remains a major focus of surfactant therapy.

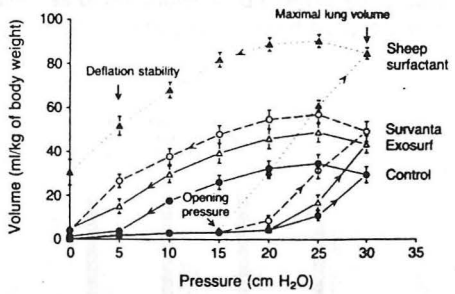

FIGURE 10 Pressure-volume curves for premature rabbits with RDS treated with placebo (control), Exosurf, Survanta, or whole sheep surfactant (79).

Adult Respiratory Distress Syndrome

ARDS is a syndrome with multiple etiologies which is characterized by an acute lung injury leading to impaired oxygenation, decreased compliance, bilateral pulmonary infiltrates and in its early stages noncardiogenic pulmonary edema (80). In many patients multiple organs in addition to the lung are involved. Mortality from ARDS without other organ system failure is 40-50%; patients with multiorgan failure have mortalities of 80-90% (81,82).

Similar to early studies of neonatal RDS, abnormal surfactant function has been demonstrated in material obtained from ARDS patients (83,84). Even when protocols to isolate surfactant were utilized where serum proteins were removed, purified surfactant from ARDS patients had impairment of surface tension lowering properties. Indeed, analysis of surfactant from patients hospitalized with known risk factors for ARDS but without clinical evidence of ARDS disclosed reduced function. This data as well as studies in animal models of sepsis (85) suggest that alveolar surfactant is abnormal early in the disease process and correlates with severity of pulmonary dysfunction.

Surfactant Composition in ARDS

Conflicting data exists about the size of the surfactant pool in patients with ARDS. Much of the difficulty in measuring the pool relates to the use of bronchoalveolar lavage (BAL) where the harvested surfactant is diluted in a non-standardized fashion by saline. However other variables include the nature of the injury itself. In animal models injury produced by oleic acid or 100% oxygen result in surfactant pools which are either normal or slightly reduced (86,87). In contrast injury produced by 85% oxygen or bleomycin are actually associated with increased surfactant pool size (88,89). In humans with ARDS both normal and decreased total phospholipid pools have been reported. In general severe injury of type II cells has been shown to decrease surfactant pool size while less severe injuries and catechol responses would be expected to increase the pool size (Figure 11).

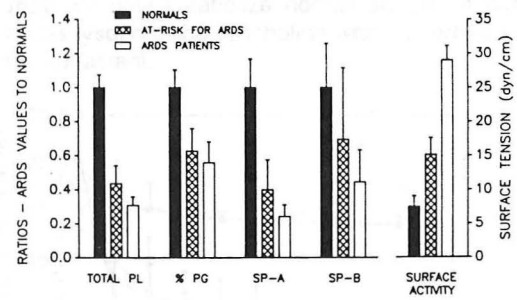

FIGURE 11 Alterations in surfactant recovered from BAL of patients at risk for ARDS, or with ARDS. In this study total phospholipid, % phosphatidylglycerol, SP-A, SP-B were reduced in ARDS. Surface tension of surfactant was increased in ARDS (84).

A more uniform finding in ARDS patients is the altered phospholipid composition of surfactant (Table 6) (84). Multiple studies (90-92)have demonstrated decreased relative concentrations of DPPC and PG with concomitant increases in phosphatidylinositol (PI), phosphatidyl ethanolamine (PE), sphingomyelin, and other minor lipid components. These changes occur

early in the course of the disease. In addition decreased levels of SP-A and SP-B have been demonstrated in ARDS. The relation of these changes to diminished physiologic function of surfactant has not been established.

Table 6 (84)

Phospholipid Composition of Surfactant In ARDS (% Total)

	PC	PG	PI	PE	Sph	LPC
Control	76.3	11.6	3.9	3.3	1.5	0.2
At-risk for ARDS	73.3	7.3	4.7	4.9	1.6	1.3
ARDS	62.6	6.5	7.0	5.9	5.4	2.3

Inactivation of Surfactant

The transudation of serum proteins associated with a capillary leak syndrome may lead to altered surfactant function, particularly if surfactant proteins are concomitantly reduced (93). More importantly, however, surfactant lipids peroxidated by oxygen radicals produced by inflammatory cells have markedly reduced function (Figure 12) (94,95). In addition, phospholipases capable of degrading phospholipid are found in BAL from ARDS patients (96). These enzymes may not only catabolize normal surfactant but also produce intermediaries such as lysophosphatidylcholine which interfere with the surface properties of normal surfactant.

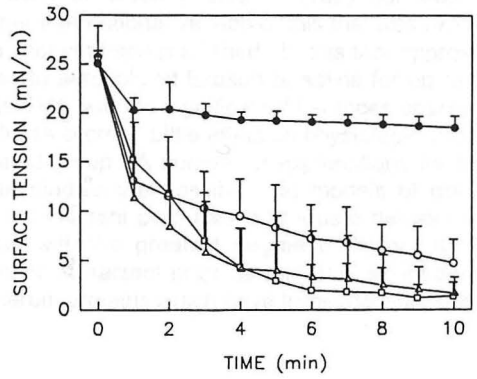

FIGURE 12 Surface tension of surfactant exposed to oxidants ($CuCl_2$) for 24 hours was higher (closed circles) than surfactant exposed to oxidants for 5 minutes (open circles) or controls (open triangles and squares). Thus oxidants altered the surface properties of surfactant (94).

The conversion of surfactant to its optimal form may also be impeded in ARDS. Surfactant exists in different aggregate forms in the alveolus (97-99). Large aggregates are tubular myelin-rich fractions with marked surface tension lowering properties. These are converted to small aggregates with poorer functional properties by alveolar movement and a serine protease. This conversion is inhibited by SP-A. There is significant evidence that the ratio of small: large aggregates is increased in ARDS, perhaps owing to both an increased protease burden in the alveolar space and a reduction in SP-A (100).

Surfactant Replacement Therapy in ARDS

As opposed to neonatal RDS the major cause of death in ARDS is failure of organs other than the lungs. Indeed respiratory insufficiency is thought to be the cause of death in only 25% of ARDS patients (82,101). Therefore surfactant therapy should not be expected to impact overall mortality of ARDS in a fashion similar to neonatal RDS. A more pertinent test would be the ability of surfactant to improve gas exchange.

Several anecdotal reports have cited success with surfactant replacement. However, larger controlled trials have yet to demonstrate significant results using surfactant. In one recently published study 51 patients were randomized into either 12 or 24 hours of aerosolized Exosurf or saline. No difference in physiologic parameters such as compliance, need for PEEP or shunt fraction were observed (Table 7) (102) nor was there a change in mortality. A larger international version of this trial was reported in abstract form last year but has not yet been published. In this trial approximately 500 patients were randomized to aerosolized Exosurf or saline for up to 5 days. Mortality in both groups was 41%, with no significant differences observed when accounting for severity of illness scores. Little effect on physiologic impairment was noted in the Exosurf treated group. A number of explanations for the poor response to Exosurf in these studies are possible. In models of non-uniform lung injury aerosolization of surfactant produces inadequate delivery to some lung areas, particularly those with the greatest degree of injury (103,104). Additionally, Exosurf contains no surfactant proteins and thus would be more susceptible to inactivation by serum proteins which have transuded into the alveolus.

Table 7 (102)

Physiologic Parameters In ARDS Patients After Receiving Placebo, 12 or 24 Hours/Day Aerosolized Exosurf (Expressed As Change From Baseline)

Group	Shunt Fraction (%)	P_aO_2/F_IO_2 ratio	Compliance (cc/cmH_20)
Placebo	-3.9	+44.0	-0.2
Exosurf-12	-8.1	+42.2	+1.3
Exosurf-24	-3.7	+22.8	+0.2

These potential limitations would not be present utilizing instilled Survanta. Small studies have been published or reported in abstract form which suggest that Survanta may improve physiologic function in some patients (105). Overall, however, the changes reported with Survanta have not been impressive.

It is possible that in the future better surfactant replacements may prove useful in managing severe hypoxemia in ARDS. It would appear that instillation is superior to utilization of aerosols. At present there is no data to support routine use of surfactant replacement in ARDS. Given the extensive data suggesting that surfactant lipids and proteins are altered by the inflammatory milieu in ARDS there is little reason to suspect that exogenous surfactant would be resistant to inactivation. Furthermore given the relatively short half life of exogenous surfactant in the alveolus, large quantities over a prolonged period would likely be necessary.

Alveolar Proteinosis

Although the composition of surfactant is altered in a number of diseases there are few disease entities where abnormalities of surfactant production and metabolism are believed to play a primary role in producing respiratory distress. One group of diseases where surfactant abnormalities clearly produce abnormal gas exchange is pulmonary alveolar proteinosis (PAP). PAP may either be congenital or acquired and is characterized by the accumulation of amorphous, lipid-rich, periodic acid-schiff positive proteinaceous material in the alveolar space. Although the congenital and acquired forms of PAP have similar gross histology, the mechanisms responsible for the diseases are vastly different.

Congenital PAP

Congenital PAP is an uncommon cause of respiratory failure in full term newborns. Recently it has been determined that PAP in this setting is due to an absence of SP-B; SP-A and SP-C are present (Figure 13) (106). The disease is characterized by intractable respiratory failure and death within weeks to months of birth. It should be noted that deficiency of SP-B may cause respiratory failure without accumulation of PAP-like material in the alveolar space. Therefore, SP-B deficiency may produce a heterogenous clinical picture (107).

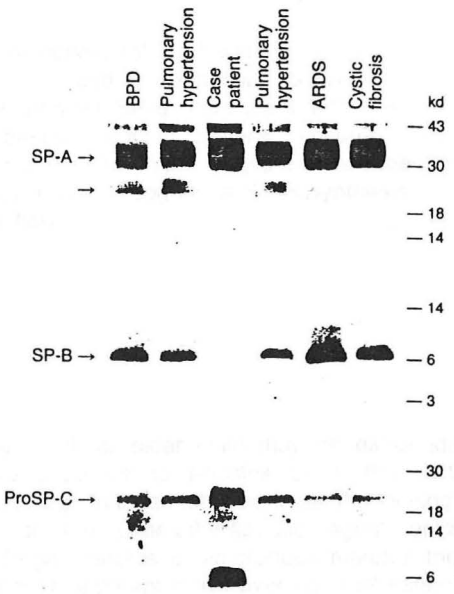

FIGURE 13 Protein blotting for SP-A, SP-B, and SP-C pre-protein in children with various diseases. The case patient had congenital PAP and had no SP-B (106).

In patients with congenital PAP the most common defect is a frameshift mutation substituting GAA for a single C nucleotide at codon 127 of the DNA sequence for SP-B (108). This results in a premature signal for termination of translation after codon 214. This mutation has been noted in multiple families.

The histologic picture of SP-B deficiency in these patients is striking (109). Large numbers of membranous vesicles and only a few lamellar bodies are noted in the alveolus. Typical tubular myelin is not observed. More importantly, membranous vesicles from type II cells, SP-A and SP-C are found below the basement membrane of type II cells despite intact inter-epithelial cell junctions. These latter findings suggest that SP-B plays an important role in the directional orientation of surfactant secretion from the type II cell into the alveolar lumen.

Treatment of congenital PAP with Survanta, which contains SP-B, has been ineffective (110). Infants treated with Survanta developed evidence of an immune response against SP-B, consistent with the absence of this protein during thymic development. Several children have undergone lung transplantation for congenital PAP. Together, the experiences with Survanta in this disease suggest that endogenous SP-B synthesis is crucial for surfactant secretion and function.

Acquired PAP

PAP in the adult or older child may be either idiopathic or acquired following massive exposure to silicates or in the setting of hematologic malignancies (111,112). In these latter patients, the inciting agent may be either the malignancy or the chemotherapeutic agent utilized. Despite the accumulation of large amounts of amorphous material there is essentially no inflammatory response apparent in the alveolus or interstitium.

Histologic and electron microscopic examination of the alveoli of PAP patients discloses numerous lamellar body-like structures in the alveolus and in alveolar macrophages (Figure 14). Lipid analysis discloses a high concentration of DPPC. Both SP-A and SP-D have been found in large quantities in harvested alveolar material (113,114).

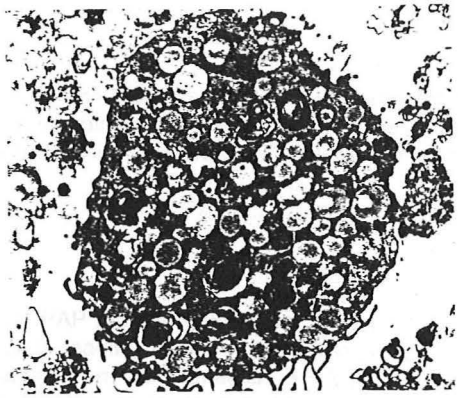

FIGURE 14 Electron micrograph of an alveolar macrophage from a patient with PAP. Lamellar bodies are easily identified in the cytoplasm.

Clinical Presentation

PAP is characterized by insidious onset of dyspnea on exertion. Some patients may have productive cough but overall this is unusual. Radiographic evaluation reveals bilateral infiltrates similar to pulmonary edema but with a normal heart size. In some institutions, high resolution CT scanning of the chest yields a "geographic" pattern of infiltrate and thickened lobular septa suggestive of the diagnosis. Pulmonary function testing characteristically discloses a marked reduction of diffusion capacity with only mild decreases in vital capacity. Arterial desaturation with exercise is common.

Of particular interest, PAP is one of the few pulmonary diseases characterized by diffuse infiltrates and an increase in serum LDH. This is particularly striking because the amorphous material in PAP is similar to that observed in patients with pneumocystis carinii pneumonia (PCP), which also produces an increase in LDH. Extensive efforts to link PCP to the development of PAP have failed to demonstrate any relationship between the diseases (115,116). However, it should be noted that PCP also results in an increase in alveolar SP-A (see below). Material obtained from patients with PAP is usually sterile; however an increased incidence of infection with Nocardia is noted in patients with PAP.

Diagnosis of PAP is usually established on bronchoscopy. BAL yields milky fluid which rapidly sediments in a collection vesicle. Transbronchial biopsy discloses the characteristic lipid material in alveoli without inflammation.

Etiology

The etiology of PAP is largely unknown. The most attractive hypothesis is injury to type II cells leading to either an overproduction of surfactant or a failure to clear surfactant from the airspaces. SP-A can be found in the serum of PAP patients suggesting that some degree of type II cell dysfunction with loss of directional secretion of surfactant has occurred (117).

Therapy

The therapy for symptomatic PAP is whole lung lavage. This is done in an operating room using a double lumen endotracheal tube and usually involves several hours of lavage. Up to 15L of normal saline may be instilled and retrieved in some cases. Lavage of the other lung is usually performed at a separate setting. The beneficial effect of this therapy is rapid (Figure 15) (118). Some patients may require repeat lavage at later time intervals; many patients resolve their disease over a several year period (Figure 16) (118). Overall the prognosis for idiopathic PAP is excellent.

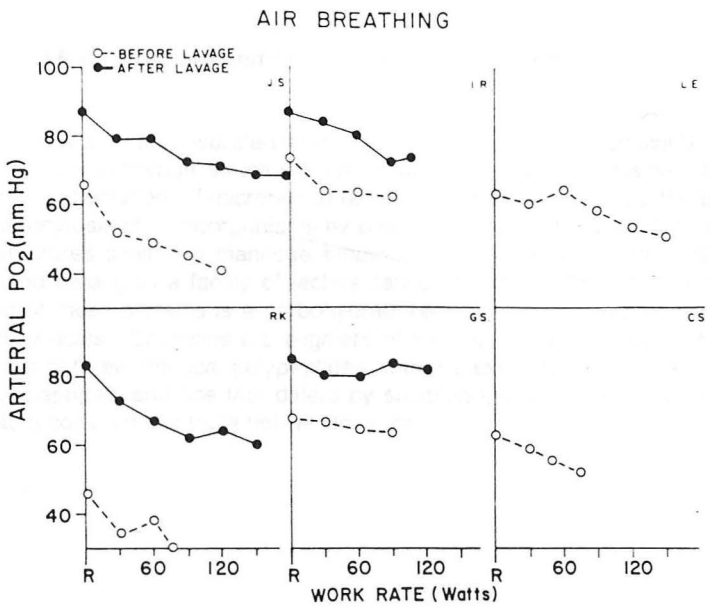

FIGURE 15 Response of arterial pO_2 to exercise in patients with PAP before and 2 days after therapeutic lung lavage (118).

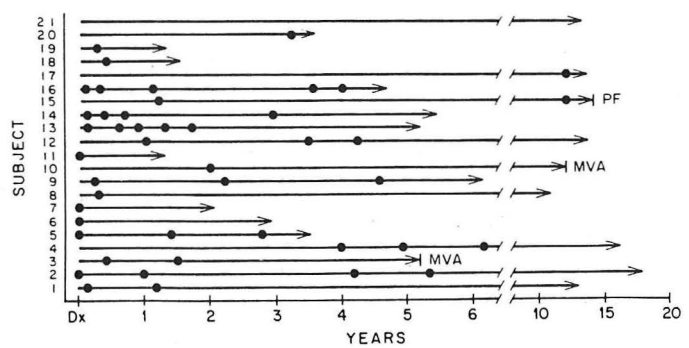

FIGURE 16 Follow-up of 21 patients with PAP. Need for lavage is identified by closed circles. Two patients died of other causes, one patient developed pulmonary fibrosis (PF) (118).

The Surfactant System and Microbial Defense

SP-A and SP-D: Structure and Function of the Collectins

Lectins are carbohydrate-binding proteins capable of recognizing a large range of oligosaccharide structures synthesized by living organisms. Lectins mediate the attachment of microbes to target cells and also facilitate the binding and phagocytosis of microorganisms by phagocytic cells. Both SP-A and SP-D have structures similar to mannose binding protein (MBP) and other "C-type" lectins and belong to a family of lectins called collectins (Figure17) (119). At one end of these proteins is a carbohydrate-recognition domain (CRD) of 115-130 amino acids. Collectins are oligmers of trimeric subunits. Each subunit is composed of three identical polypeptides, with the exception that SP-A has two identical peptides, and one that differs by six amino acids. The three peptides twist into a collagen-like triple helical structure.

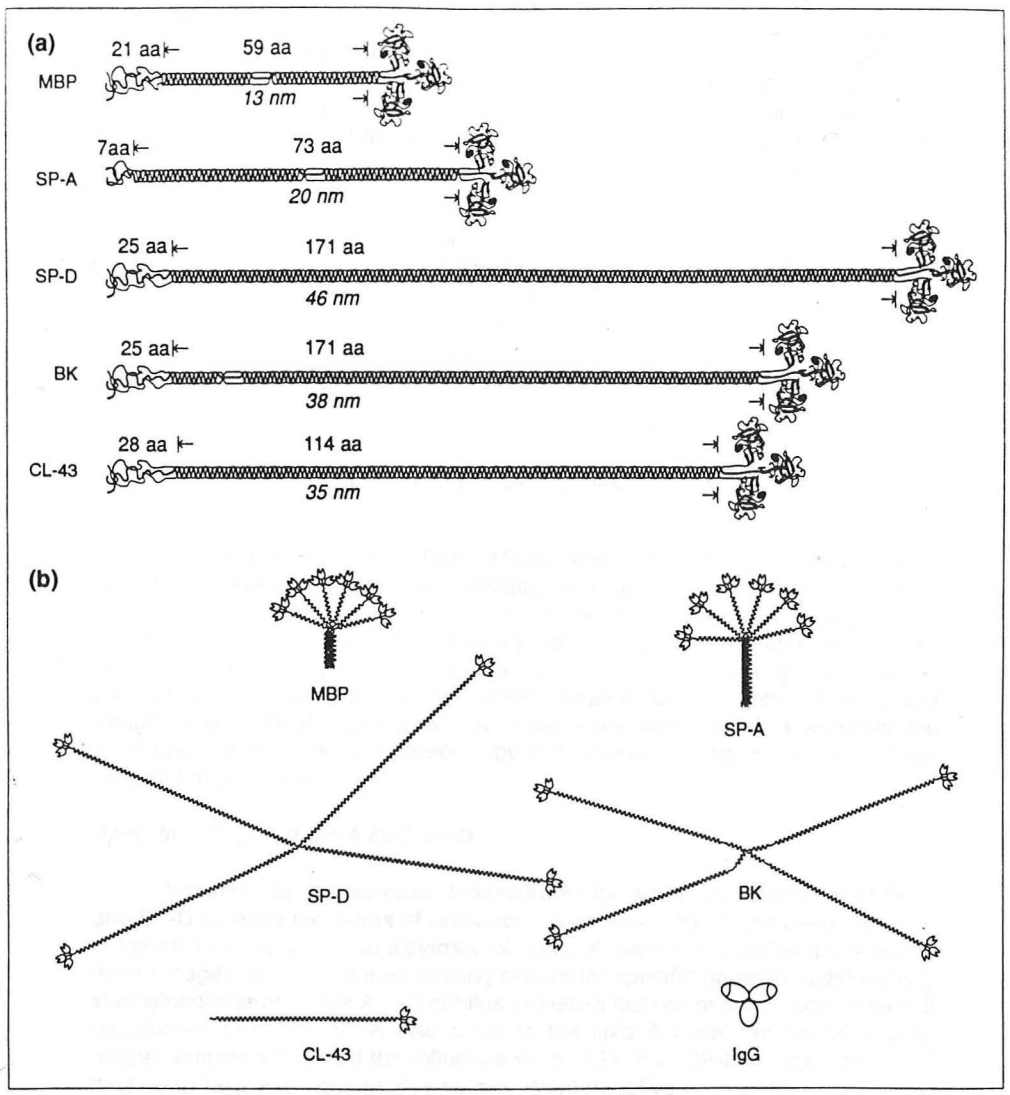

FIGURE 17 The structure of collectins including SP-A and SP-D is remarkable for CRD's (on the right) at the end of a long collagen-like triple helix. The quaternary structure of the collectins is shown in (b), with IgG shown for a size comparison (119).

The collectins bear a striking similarity to the first component of complement, designated C1q (Figure 18) (120). SP-A and C1q have an ultrastructure referred to as a "bouquet of tulips". The receptor for collectins is the C1q receptor and is present on many cells including leukocytes, fibroblasts and epithelial cells. SP-A binds to the C1q receptor on alveolar macrophages. A receptor for SP-D has also been described on alveolar macrophages. However SP-D binding may involve additional receptors beside C1q.

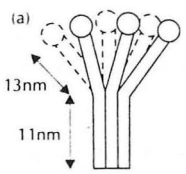

FIGURE 18 Ultrastructure of both C1q and SP-A (120).

The role of collectins in host defense likely involves several mechanisms (121-129). First, owing to their similarity to C1q, the collectins can mediate activation of the complement system. Secondly, collectins may act as opsonins for micro-organisms by either directly facilitating phagocytosis or by up-regulating other receptors that mediate attachment and phagocytosis. This is particularly true for immune complexes cleared by Fc receptor mediated phagocytosis. Finally collectins may under some circumstances augment the production of toxic oxygen species by mononuclear phagocytes which have ingested micro-organisms.

Specific Effects of SP-A and SP-D

A number of studies have demonstrated the potential importance of SP-A and SP-D as early mediators of anti-bacterial defense. SP-A has been shown to enhance binding and phagocytosis of several bacterial species by alveolar macrophages. SP-A mediated binding occurs for specific bacteria, most notably staphylococcus and type A hemophilus influenza but not pneumococci or type B hemophilus (130-32). SP-A also binds to the lipid A moiety of bacterial LPS, herpes simplex virus, and the influenza virus (133-35). SP-D is also capable of binding to LPS and agglutinates several gram-negative organisms (136). SP-D also may play an important role in defense against influenza A virus (Figure 19) (137) as it significantly decreases viral hemagglutination activity.

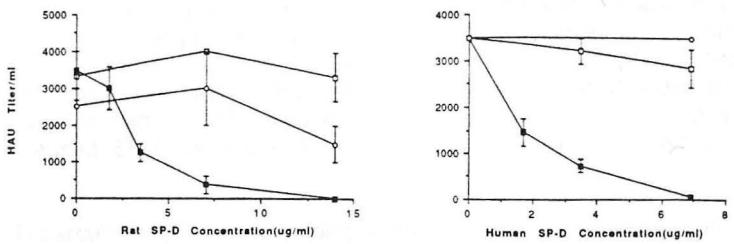

FIGURE 19 Rat and human SP-D inhibit the hemagglutination activity of influenza A virus (solid squares) compared to control (open circle) or SP-D exposed to maltose (open squares), a sugar which binds to the CRD of SP-D (137).

The importance of SP-A and SP-D to overall immune defense in an immunocompetent host is unclear. It is likely that these collectins serve as a first line of defense in sub-clinical infections. However in hosts incapable of mounting an effective immune response the surfactant system may be an important variable in mediating defense.

Patients with AIDS have evidence of altered surfactant metabolism. This is particularly true of patients with PCP (138,139). The surfactant profile observed in AIDS would be consistent with injury to type II cells. Relative DPPC concentration is generally reduced and isolated type II cells from rats with PCP have been shown to respond abnormally to secretagogues which enhance DPPC secretion in control animals (140). In addition the content of SP-A in the alveolus is markedly increased and correlates with the severity of respiratory disease (Figure 20) (141). This contrasts with the reduced SP-A content observed in bacterial pneumonias (142).

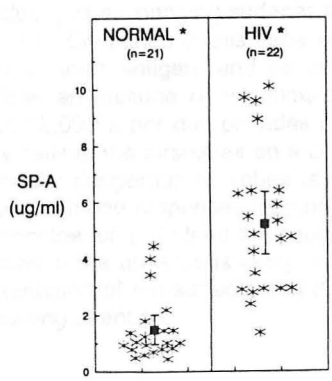

FIGURE 20 SP-A content in BAL of HIV+ patients with pneumonia and normal volunteers. SP-A content was significantly higher in HIV+ individuals with any type of pneumonia ($p<0.0001$) (141).

Pneumocystis organisms must attach to a surface for replication to take place (143). Data suggests that a 120 KD surface glycoprotein on pneumocystis binds to SP-A (144). SP-A may then act as an "anchor" in mediating adherence to alveolar epithelium. Although it is clearly established that CD4 deficiency is the primary mechanism responsible for the development of PCP (145), it is likely that increased SP-A facilitates a rapid increase in organism load in some patients.

Tuberculosis (TB) is a leading cause of pulmonary disease in AIDS patients. Mycobacterium tuberculosis is an obligate intracellular pathogen. In order for infection to become established TB must gain access to an alveolar macrophage and be phagocytosed. A number of recent studies have demonstrated that SP-A enhances attachment of TB to AM. A recent study (146) demonstrated that BAL from HIV+ patients increased the attachment of TB to AM three fold. When SP-A was removed from BAL by immunoprecipitation the binding of TB organisms returned to levels seen using control BAL. Whether this "opsonic" effect is related to use of SP-A as a classic opsonin is unclear. Prior studies have suggested that TB binds to C1q and mannose receptors on macrophages and that these receptors are up-regulated by SP-A (147,148). Attachment in these studies is also accompanied by increased phagocytosis. The increase in alveolar SP-A in AIDS patients may therefore constitute an additional risk factor for primary TB infection. Of note patients with PAP, also an "SP-A rich" disease, may have some increased incidence of mycobacterial infection (149).

Surfactant, Inflammation, and Immune Responses.

In the average adult, gas exchanging surfaces of the lung are exposed to 12,000 litres of air per day. Contained in this large volume of inspired gas are numerous microorganisms, inert antigen, and noxious substances potentially capable of initiating either an immune or inflammatory response. Moreover, alveolar blood supply of 12,000 L per day provides close access of circulating immune or inflammatory cells to the airspaces on a continual basis. Although a response against potentially dangerous microbes is beneficial, many airborne stimuli capable of eliciting immune responses may not require such a response in order to be cleared from the lung. Indeed the suppression of routine immune or inflammatory responses in the alveolus is likely crucial to preserving optimal gas exchange. The interaction of the surfactant system with immune cells has therefore received increasing attention.

Effect of Inflammation on Surfactant

In addition to the direct inactivation of surfactant lipids by oxidants a number of effects on lipid and protein synthesis have been described. TNF alpha reduced phosphatidylcholine production by isolated human type II cells by 68% in one study (150). A smaller reduction was observed for phosphatidylinositol and phosphatidyl-ethanolamine; other lipids were unaffected. In other studies TNF has been shown to markedly downregulate both SP-A and SP-B synthesis. The effect of TNF was rapid with nearly complete loss of SP-A mRNA by 24 hours (Figure 21,22) (151). The effect of TNF requires protein synthesis and is blocked by actinomycin D, an inhibitor of protein synthesis. The mechanism of reduced SP-B mRNA has been shown to relate to decreased mRNA stability and is mediated by a cis-active element located in the 3' untranslated region of SP-B mRNA (152). The effect of TNF can be blocked by steroids or pentoxifylline (153).

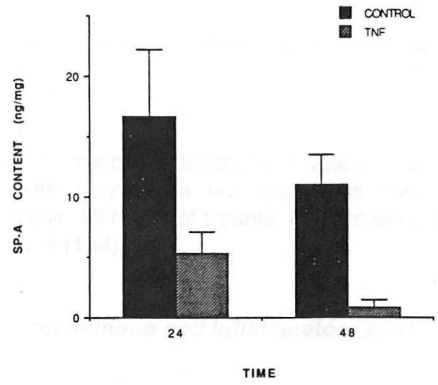

FIGURE 21 Effect of TNF alpha on SP-A content of a type II cell line after 24 or 48 hours (151).

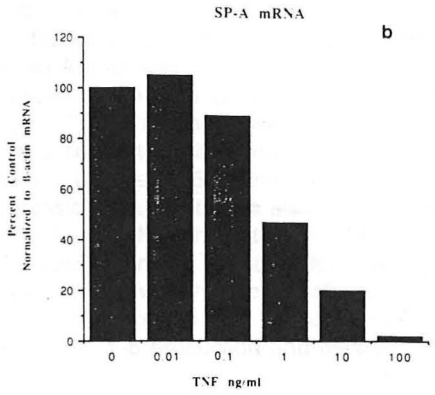

FIGURE 22 The inhibition of SP-A mRNA by TNF is dose related (151).

Decreased SP-A has been described in many inflammatory states. In a group of patients with polytrauma but not direct chest trauma SP-A was decreased by 50% within 18 hours of trauma, despite what must be presumed to be a high catechol state (154).

Effect of Surfactant on Immune and Inflammatory cells

The effect of whole surfactant and isolated components on immune and inflammatory cells may differ. Purified SP-A has been reported to directly stimulate lymphocyte proliferation, immunoglobulin production, release of growth factors from type II cells, and synthesis of IL-1, IL-6 and TNF by peripheral blood mononuclear cells (155-157). In contrast surfactant lipids, particularly in combination with SP-B and SP-C are markedly inhibitory of immune and inflammatory cell function.

Surfactant significantly inhibited T cell proliferation in response to mitogen and alloantigen, as well as activation of lymphokine activated killer cells by IL-2 (158-164). It is noteworthy that the reduced function of surfactant treated peripheral blood lymphocytes is identical to the properties of lymphocytes obtained directly from the lung (165). Exosurf inhibited LPS induced production of TNF-alpha by human AM as well as production of IL-1 beta and IL-6 (166). This inhibition was greater using the SP-B and SP-C containing preparation Survanta (Figure 23) (167). At optimal doses Survanta inhibited 70% of TNF alpha secretion and this correlated with reduced TNF mRNA. Similar findings have been reported using Curosurf (168), and the phospholipid fraction appears to be mediating suppression. Survanta has also been shown to inhibit 80-90% of gamma interferon-induced TNF production in monocytes(169). TNF is a proximal mediator of inflammation and important for inducing IL-8 production (IL-8 is a potent chemotaxin for neutrophils) by a variety of cells in the alveolus. The inhibition of alveolar macrophage TNF production by surfactant is likely an important down-regulator of IL-8 production and neutrophil recruitment into the lung.

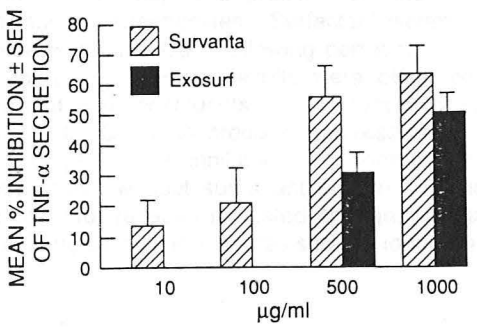

FIGURE 23 Comparison of Survanta and Exosurf-mediated inhibition of LPS induced TNF alpha production by human alveolar macrophages. Exosurf had no effect at 10 or 100 ug/ml (167).

The mechanisms responsible for altering immune and inflammatory cell function remain to be fully elucidated. However, several studies suggest that one mechanism by which surfactant exerts its effect is the disruption of second messenger pathways. Surfactant isolated from BAL altered the function of protein kinase C (PKC), an important generator of second messengers. In response to activating stimuli PKC translocates from the cytoplasm of phagocytic cells to the cell membrane. Following a 30 minute incubation with surfactant PKC translocation in response to the activating stimulus PMA was significantly inhibited in monocytes (Figure 24) (170).

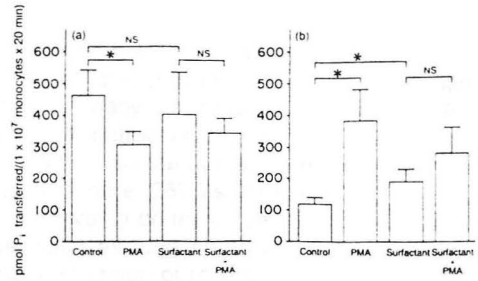

FIGURE 24 Effect of surfactant on translocation of protein kinase c (PKC) in monocytes activated with PMA. Cytosolic PKC is shown in (a); membrane PKC activity in (b). Normally PMA causes an increase in the membrane bound fraction of PKC with corresponding decrease in cytosolic PKC. This translocation was muted in surfactant exposed cells (170).

Other studies have demonstrated that surfactant may also interrupt receptor-mediated increases in intracellular calcium concentration [Ca^{2+}i] in lymphocytes. This calcium flux is a proximal second messenger believed integral in the activation of lymphocytes. Surfactant isolated from human BAL significantly inhibited increased Ca^{2+}i following pertubation of the T cell (CD3) receptor (Figure 25) (171). Similar results were observed using Survanta. Analysis of protein and lipid constituents of surfactant disclosed that SP-B and SP-C were necessary for DPPC to produce suppression of calcium fluxes as neither pure DPPC or SP-A were inhibitory. In contrast phosphatidylglycerol was markedly suppressive without surfactant protein. Of note, the effect of surfactant was specific for receptor-mediated changes in Ca^{2+}i; the inhibition could be overcome using calcium ionophores such as ionomycin.

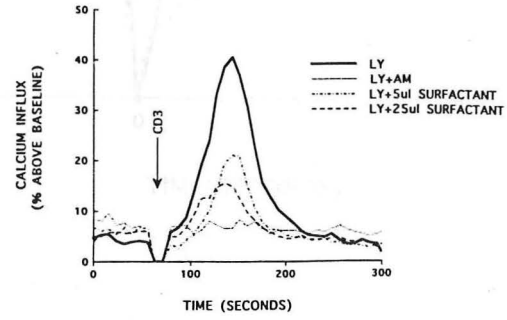

FIGURE 25 Effect of human alveolar macrophages and surfactant on CD3 mediated increases in [Ca^{2+}i] in lymphocytes. Both constituents of the alveolar space inhibited calcium fluxes in T cells (171).

The mechanisms responsible for inhibiting second messenger generation are unclear. However one possible explanation is an alteration of the fluidity of cell membranes. Many membrane bound receptors generate second messengers through pathways requiring movement of the receptor in the membrane lipid bilayer. Survanta produced a significant reduction in membrane fluidity in T cells (Figure 26) as measured by fluorescent photobleaching. Similar effects of Survanta on membrane fluidity have also been observed using structural tissue such as lung fibroblasts. A more rigid cell membrane may contribute to the interruption of receptor-mediated second messenger pathways in response to some activating stimuli.

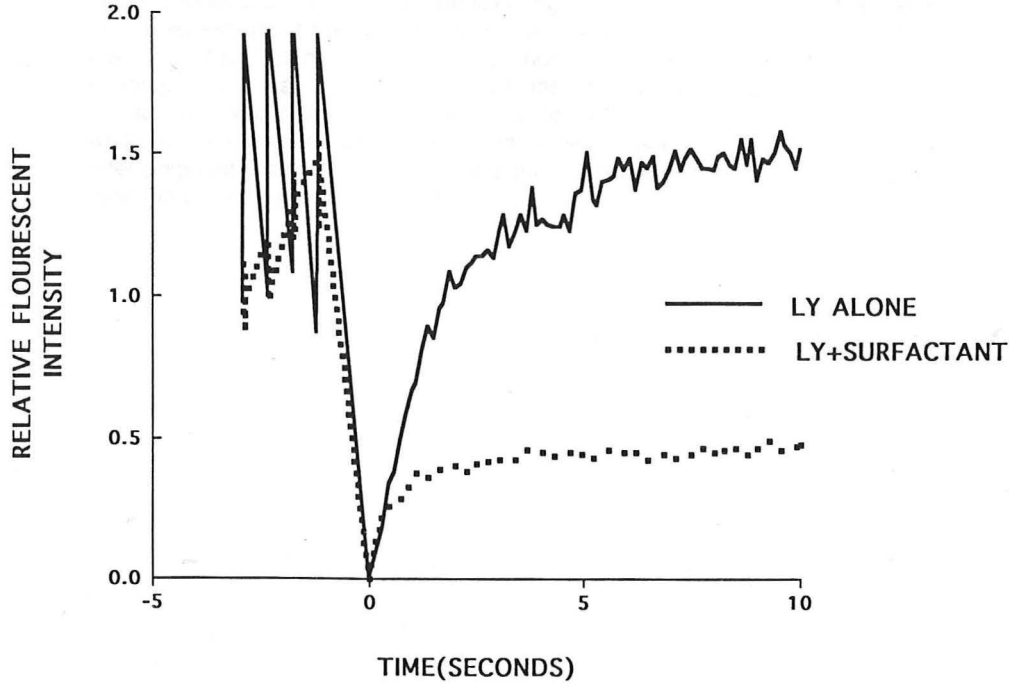

FIGURE 26 Effect of Survanta on membrane fluidity of lymphocytes. A fluorescent marker is bleached by a laser at time 0; the rate at which fluorescence returns is a reflection of the ease with which new fluorescent material migrates through the membrane lipid bilayer. Surfactant markedly reduced fluidity.

The majority of available data would suggest that normal surfactant inhibits proliferation of immune cells and release of pro-inflammatory mediators, including prostanoids and cytokines by alveolar macrophages. It is therefore tempting to speculate that changes in surfactant during an inflammatory response, most notably decreases in SP-B, DPPC and PG contribute to ongoing inflammation and proliferation of immune cells and perhaps structural tissue. Conversely the restoration of normal surfactant composition may be crucial to terminating an inflammatory response.

Summary

The study of the surfactant system has revealed that surfactant performs multiple functions in addition to the lowering of surface tension. Much remains to be learned about the role of specific components of surfactant in mediating the effect of surfactant on gas exchange, host defense and immune cell function. Great progress has been made in the last decade in applying the fruits of surfactant research towards clinical medicine. The tens of thousands of children who are alive today because of the study of surfactant over the past 4 decades offer poignant testimony to the benefits that accrue to a society wise enough to invest in basic biomedical research.

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