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Pulmonary Surfactant and Its Apoproteins

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Introduction

Six decades have gone by since Kurt von Neergaard published his classical work on the effects of surface tension on the mechanical properties of the lungs (1). For three decades his insights were largely ignored by medical scientists. Interest in his results became widespread after the discovery that the lung regulates its surface tension by lining itself with surfactant (2, 3), and that deficiency of this vital material is the proximate cause of respiratory distress syndrome in premature infants (4). In the last three decades investigators have brought to bear a wide variety of research disciplines on the surfactant system of the lung and have generated a large fund of information about this complex system (5, 6). Currently, the most modern methods of biological research are being applied to lung surfactant with the result that our understanding of its functions, components, and metabolism is increasing with unprecedented speed. Concomitantly, practical application of these fundamental research results to common lung diseases has begun in earnest, especially in newborn and adult respiratory distress syndromes where treatment with exogenous surfactants is being evaluated (7).

This reduction to practice does not mean, however, that the research on pulmonary surfactant is completed. Many questions about its functions remain unanswered, particularly those mediated by its apoproteins, and these are the main subject of this essay. But first, the complex nature of the surfactant deserves comment.

Heterogeneity of pulmonary surfactant

It is well known that lung surfactant as commonly isolated contains many kinds of molecules: phospholipids, cholesterol, other neutral lipids, and many proteins (8). It is not so often pointed out that the surfactant is also morphologically and functionally heterogeneous (9) (see Fig. 1) and that differences in form and activity correlate with apoprotein content (9, 10). When stored in the lamellar bodies of the alveolar type II cells, the surfactant is tightly packed in multilayer arrays. Upon secretion and hydration in the alveolar lining liquid, it expands into a complicated, latticelike structure called tubular myelin, from which the interfacial monolayer appears to spread (11).

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This is the structure that lowers surface tension and helps stabilize the lung. The lining liquid also contains small vesicles (12) whose function is not certain. Sequential centrifugation of surfactant obtained by lavage of the lung gives subfractions that are enriched in multilayer vesicles and tubular myelin or in small vesicles (9). Interestingly, the former material lowers surface tension rapidly, is preferentially taken up from the alveoli, and contains surfactant apoproteins, whereas the latter lacks apoproteins and functions poorly (9, 10). Experiments with radioactive labeling of surfactant components suggest that they cycle through these forms and are taken back into the type II cells, reassembled, and resecreted (9, 10, 13-15). Thus, the apoproteins appear to determine important characteristics of the surfactant. The structure and properties of the surfactant apoproteins are the focus of the remainder of this perspective.

Apoprotein A

Surfactant protein A (SP-A)¹ is the most abundant and best characterized of the surfactant proteins. Approximately 3 mg of SP-A is associated with each 100 mg of phospholipid in the surfactant recovered from bronchoalveolar lavage. The protein, first identified in 1973 (16), is a large lung-specific glycoprotein synthesized in both the alveolar type II cell and the bronchiolar Clara cell (17, 18). Although SP-A can be isolated from the surfactant lipids *in vitro* in a water-soluble form, no significant pool of SP-A free of lipid has been identified *in vivo*.

The synthesis of SP-A is developmentally regulated. The protein is detectable in the lung only during the last third of human fetal gestation and is secreted into the alveolar space and therefore detectable in amniotic fluid from ~ 34 wk gestation (19). The concentration of SP-A correlates well with the concentration of surfactant phospholipid in amniotic fluid during gestation and inversely with the incidence of respiratory distress syndrome (19). The mechanisms involved in this developmental regulation of SP-A gene expression are clearly complex. Transcription of the SP-A gene in cultured lung preparations can be regulated by a number of hormones and growth factors including glucocorticoids, cAMP, insulin, interferon gamma, epidermal growth factor, and transforming growth factor beta (20), but to date only glucocorticoids have been shown to effect the synthesis of SP-A *in vivo* (21).

The human SP-A primary translation product is 248 amino acids long with a molecular mass of 28,000 D (22, 23). A number of modifications are made to the SP-A amino acid chain during cellular processing. The amino-terminal one-third of the protein is rich in glycine and proline residues.

1. Abbreviations used in this paper: SP-A, SP-B, and SP-C, surfactant proteins, A, B, and C, respectively.

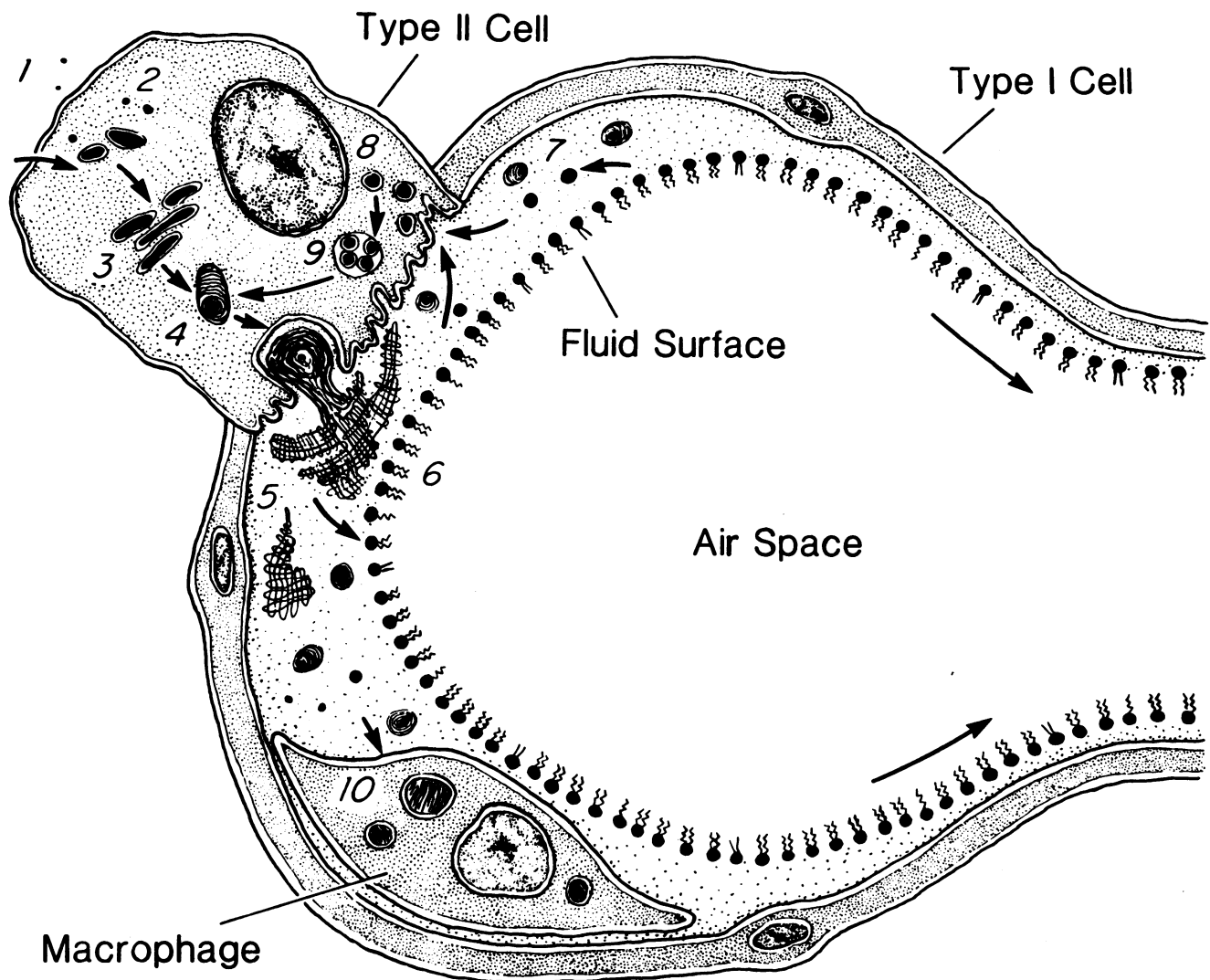


Figure 1. Schematic diagram of the surfactant system. A single alveolus is shown with the location and movement of surfactant components depicted. Surfactant components are synthesized from precursors (1) in the endoplasmic reticulum (2) and transported via the Golgi apparatus (3) to lamellar bodies (4), which are the intracellular storage granules for surfactant. After secretion into the liquid lining the alveolus, the surfactant forms tubular myelin (5), which is thought to generate the surface monolayer (6) which lowers surface tension. Subsequently, surfactant components are taken back into type II cells, possibly in the form of small vesicles (7) apparently by a specific pathway involving endosomes (8) and multivesicular bodies (9) and culminating again in storage of surfactant in lamellar bodies. Some surfactant in the liquid layer is also taken up by alveolar macrophages (10). A single transit of the phospholipid components of surfactant through the alveolar lumen normally takes a few hours. The phospholipids in the lumen are taken back into the type II cell and reutilized ~ 10 times before being degraded.

Many of the proline residues in this region are modified by hydroxylation in the 4 position. This modification, presumably important for stabilizing the collagenlike triple helix into which the amino-terminal one-third of SP-A is assembled (24) appears to be a prerequisite for passage of SP-A through the secretory apparatus of the cell (25). Human SP-A is also glycosylated close to the carboxy terminus with a sialylated oligosaccharide chain, but the role of this modification is not known.

The secreted form of SP-A is assembled into a large oligomer composed of 18 similar subunits with a molecular mass of ~ 700,000 D (24). The active protein is structurally asymmetric with a rodlike amino-terminal stem consisting of six closely associated triple helices, each connected to a globular head made up of the carboxy-terminal 150 amino acids. As assessed

by electron microscopy, the collagenlike region of SP-A is ~ 20 nm long. The six globular heads of the protein appear to be held in a roughly circular array, ~ 20 nm in diameter (26). The overall plan of SP-A resembles closely the "bunch of tulips" organization of complement component C1q. The steric relationship of the globular SP-A heads dictated by the collagenlike stems may be important in the interaction between SP-A and a number of the ligands, including carbohydrates (27), phospholipids (28), and cell-surface receptors (29-31) to which it binds.

Apoprotein B

Although a small hydrophobic protein was observed in lung surfactant in early experiments (16), it was not until much later that two distinct proteolipids, SP-B and SP-C, were char-

acterized in human surfactant (32, 33). These proteolipids (proteins that preferentially extract into organic solvents) are associated with the surfactant phospholipids in roughly equal abundance. Approximately 1 mg of each proteolipid can be isolated from 100 mg of surfactant phospholipid.

SP-B is synthesized in the type II cell and bronchiolar Clara cell (18). In human fetal lung the SP-B gene is transcribed during the second trimester, well before the appearance of morphologically recognizable lamellar bodies. SP-B mRNA is also detectable in human fetal lung earlier than either the mRNA for SP-A or the increase in phospholipid content (34). Whether the appearance of SP-B mRNA during normal lung maturation is matched by a parallel increase in SP-B protein content is not well established. Similar to other components of the surfactant system, SP-B gene expression and protein accumulation are markedly enhanced by glucocorticoids (35).

The primary translation product of the SP-B mRNA is a 42,000-D proprotein (33, 36), which is glycosylated in the regions flanking the active form of SP-B (37). The active proteolipid is a 79-amino acid protein (38) consisting of residues 201–279 in the precursor. Isolated type II cells process the SP-B precursor to the smaller active proteolipid, but the compartments in which the processing occurs have not been established (37). The SP-B precursor has not been identified in bronchoalveolar lavage material and only the fully processed form of SP-B has been identified in lamellar bodies (37).

There is currently very little information available beyond the amino acid sequence on which to base a model of SP-B proteolipid structure. Mature SP-B contains 7 cysteines in only 79 amino acids. These cysteines probably contribute to both inter- and intrachain disulfide bonds. In the oxidized state the SP-B proteolipid but not the precursor protein migrates during electrophoresis as a disulfide-dependent dimer and less abundant higher order oligomers. The oxidized protein is very resistant to chemical or enzymatic cleavage, suggesting a very compact folded conformation. Both positively charged and hydrophobic residues are distributed throughout the SP-B proteolipid sequence. The periodicity of charged and hydrophobic residues suggests that much of the SP-B molecule may have an amphipathic character (39) similar to many other peptides that interact with lipid membranes. The abundance of positively charged residues may also be important for interactions with negatively charged phospholipid head groups. Many critical issues such as the size and structure of the native SP-B complex in the lipid environment have yet to be addressed.

Apoprotein C

Apoprotein C (SP-C), like each of the other surfactant apoproteins, is synthesized in the type II cell (40). While there is evidence in different species that SP-A and SP-B are also synthesized in bronchiolar Clara cells, no such information is yet available for SP-C. The mRNA for SP-C is readily detectable in human fetal lung late in the second trimester of gestation. The content of message for SP-C in fetal lung explant culture rises dramatically after exposure to glucocorticoids (34). Specific antibodies for SP-C are not readily available as native SP-C has proven to be a very poor antigen. The content of the protein, as distinct from the mRNA, in fetal and adult lung therefore remains to be clearly defined. Although the transcription rates of all three surfactant apoprotein genes are increased by glucocorticoids, several details of the time course

and dose response to glucocorticoids suggest that the three genes may not be regulated in a tightly coordinated fashion under all conditions (20).

The primary translation product of SP-C mRNA is 197 amino acids long (32). Although SP-C is generally assumed to be a secreted protein, there is no classical amino-terminal leader sequence to direct the precursor into the lumen of the endoplasmic reticulum. The form of SP-C found in association with extracellular surfactant lipids is a 36-amino acid fragment of the larger precursor (41). This form of SP-C is active in *in vitro* assays of phospholipid adsorption (32, 42), and while it is generally considered to be the native biologically active form of the SP-C protein, nonspecific proteolysis of an active larger form of SP-C during the isolation of surfactant cannot be entirely excluded at this time. Specific antibodies will be necessary to determine the cellular or extracellular compartments in which the SP-C precursor is processed.

The only significantly hydrophobic region in the SP-C precursor sequence is a stretch of 24 amino acids from residues 35 through 58 of the precursor, which includes six contiguous valines and is located in the region of the protein that forms the active proteolipid. The sequence of this region is highly conserved across species and is strongly suggestive of a membrane-associated domain. Immediately preceding the hydrophobic region in the native peptide is a conserved lysine-arginine pair. These positively charged residues may have important interactions with the negatively charged headgroups of certain surfactant lipids. Complicating interpretation of SP-C structure is recently published evidence that the peptide is palmitoylated on the cysteine or cysteine pair located near the amino terminus (43), adding a strongly hydrophobic substituent to an otherwise relatively hydrophilic region. How this modification may affect SP-C folding and interaction with lipids remains to be seen.

Physiological Roles of the Apoproteins

Many lines of evidence suggest that the surfactant apoproteins function at several points in the surfactant metabolic pathway. The major features of this pathway are depicted in Fig. 1. At all stages in the movement of surfactant lipids through this "alveolar cycle," with the possible exception of the interfacial monolayer itself, the surfactant lipids are associated with proteins but the specific apoprotein content and composition of surfactant appears to vary considerably at different points in the cycle (10).

The site of the assembly of the surfactant complex remains uncertain. Although it is generally assumed that the lipids and apoproteins of surfactant are assembled intracellularly and secreted together, as a complex, more than a decade of work has not precisely defined the protein composition of the surfactant secretory organelle, the lamellar body. Analysis of the protein content of lamellar bodies obtained by subcellular fractionation indicates that SP-A is a major protein in these organelles (25). The fully processed SP-B proteolipid has also been identified in a lamellar body fraction (37). Although it therefore seems that both proteins are present in lamellar bodies in significant amounts, their possible role in organizing the structure of this organelle has not been addressed. The intracellular localization of SP-C has not been reported.

The apoproteins SP-A and SP-B have a major role in the remarkable transformation of the multilayered membranes of lamellar bodies into the complex three-dimensional lattice of

the tubular myelin structure that develops from secreted surfactant. In a recent series of experiments Suzuki and his colleagues have been able to assemble this structure *in vitro* from purified surfactant components (44). These studies convincingly show that both SP-A and SP-B are required to generate tubular myelin from surfactant phospholipids. They also confirm the critical role of calcium in tubular myelin formation (45). Lamellar bodies may have a high total calcium content (46), suggesting that all the components required for tubular myelin may be assembled in the cell before secretion. The rapid hydration of lamellar body contents after secretion into fluid containing millimolar calcium ions may therefore be the essential step for their dramatic structural transformation.

Reconstitution studies with the purified apoproteins and phospholipids have shed some light on the interactions between individual components of surfactant. Although several surfactant components including the phospholipids may bind calcium, the high-affinity interaction between calcium and SP-A seems to be of particular importance in surfactant function. Calcium mediates the specific self association of SP-A through interactions dependent on the rigid collagenlike stem of the protein (47). As SP-A also avidly binds phospholipid (28), the result of the interaction between calcium, phospholipid, and SP-A is extensive lipoprotein aggregation (48). The surfactant proteolipids also contribute to the transformations in surfactant structure and, most significantly, to the surface activity of the resulting lipoprotein structures, but probably by quite different mechanisms. The surfactant proteolipids, like several other small amphipathic cationic proteins, cause fusion of negatively charged phospholipid membranes (49) and probably increase short-range disorder in the lipid layers. Interestingly, the lipid mixing induced by the surfactant proteolipids is also somewhat calcium dependent (49), again suggesting a regulatory role for this ion in surfactant function.

A role in the movement of phospholipid from lamellar structures in the alveolar liquid layer into the interfacial surface film appears to be an extremely important function of the surfactant apoproteins. Both surfactant proteolipids SP-B and SP-C markedly accelerate the rate of formation of phospholipid films at the air-water interface (32, 36, 42, 50, 51). Both proteolipids are found in exogenous surfactant preparations currently being evaluated in clinical trials (52). SP-A by itself has a lesser effect on phospholipid adsorption than the proteolipids but acts in a cooperative manner with SP-B in forming an extremely fast-acting lipoprotein complex (36). These reconstitution studies support the hypothesis, generated from studies of native surfactant fractions (9), that tubular myelin is the structure from which the surface film spreads. The relationships of the apoproteins to the film itself have received very little attention from investigators to date, but are interesting subjects for further research.

The pool of surfactant in the alveolar lumen is adjusted by changing the rates of surfactant secretion and removal (53). Surfactant is cleared from the alveolar space by a number of routes, including uptake into both type II cells and alveolar macrophages (5). Treatment of isolated type II cells with the surfactant apoprotein SP-A decreases the rate of surfactant secretion in response to a variety of stimuli (54, 55). Also, addition of SP-A to phospholipids increases their uptake into the cells (56). Although these observations have yet to be supported by definitive studies in whole animals, they suggest that apoproteins, particularly SP-A, may regulate the turnover of

alveolar surfactant by mechanisms that may involve the interaction between the apoproteins and specific receptors on the surface of type II cells (29–31). Isolation and characterization of such receptors would help elucidate these mechanisms.

The interesting possibility that the surfactant apoproteins may have additional physiological roles distinct from those involved in the maintenance of alveolar stability has been raised by recent observations suggesting that SP-A may have immunomodulatory effects on alveolar macrophages. These experiments stemmed at least in part from the realization that SP-A, C1q, and the acute phase serum mannose-binding protein are similar in sequence and organization. It is of interest in this context that transcription of the SP-A gene, but not the genes for the other surfactant apoproteins, is enhanced by the immunoregulatory protein interferon gamma (20). Purified SP-A enhances the uptake into macrophages of phospholipids (56), opsonized red cells (57), and bacteria (58). Potentially related observations of the effect of the serum mannose-binding protein on the uptake of the human immunodeficiency virus into lymphocytes (59) suggest that carbohydrate recognition properties of SP-A may be at work in its effect on macrophages.

Clinical applications

The growing knowledge of the surfactant system has not escaped the attention of physicians responsible for the care of patients with serious pulmonary diseases. In particular, the recognition that surfactant deficiency accompanies respiratory distress syndrome in the premature infant raised the hope that repletion therapy might be lifesaving. After some false starts this now appears to be true in some cases. Currently, many clinical trials are underway aimed at defining more precisely the risks and benefits of substitution therapy and identifying the patients most likely to be helped by surfactant administration (60). At present, several thousand infants have been treated with various available preparations. Adverse effects have been rare and impressive improvements have been noted in gas exchange, ventilatory requirements, chronic lung disease, and mortality from respiratory failure. Evidence that similar benefits might accrue in adult lung diseases characterized by atelectasis remains to be accumulated.

Prospectus

It is obvious, even from this brief essay, that the lore of pulmonary surfactant has far transcended its humble beginnings and is leading us now into fascinating pathways of research and towards useful applications. It is probable that proteins, as the smart molecules in the surfactant system, will continue to occupy center stage. Fortunately, methods for studying proteins are constantly improving in efficiency, sensitivity, and precision. We can expect, therefore, that we will learn more about the functions and disorders of the surfactant system in the next decade than was discovered in the previous six decades.

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