Surfactant proteins A and D

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Pulmonary surfactant is a complex lipid–protein mixture that lowers the surface tension at the air–fluid interface in the lung. It consists of about 90% lipid and 5–10% surfactant-specific protein. The components of surfactant are synthesized by alveolar epithelial type II cells [1]. These cells secrete the surfactant into the fluid layer that covers the alveolar epithelium. The surfactant lipids can subsequently spread as a monolayer at the air–fluid interface and, by decreasing surface tension, protect the alveoli against collapse at end-expiration and against alveolar oedema. Although the major physiological function of pulmonary surfactant is to confer mechanical stability to the alveoli, components of surfactant may also play a role in pulmonary defence [2]. Four surfactant proteins have been described: surfactant proteins A, B, C and D. The structure and possible functions of surfactant protein A (SP-A) and D (SP-D) are discussed.

Structure and localization of SP-A

The primary structure of SP-A has been determined for several species including the human protein [3, 4] and is highly conserved. SP-A is a large complex molecule assembled from 18 polypeptide chains [5, 6] (Figure 1). The primary translation product of human SP-A is 248 amino acids long and has distinct structural domains. The N-terminal part of the primary translation product is a signal peptide of 20 amino acids. Secreted SP-A has an N-terminal end of seven amino acids, containing a cysteine residue that forms an intermolecular disulphide bond with another polypeptide chain of SP-A. The short N-terminal domain is flanked by a collagen-like structure extending from 10 to 14 triplets that forms a monolayer at the air–fluid interface and, by decreasing surface tension, protect the alveoli against collapse at end-expiration and against alveolar oedema. Although the major physiological function of pulmonary surfactant is to confer mechanical stability to the alveoli, components of surfactant may also play a role in pulmonary defence [2]. Four surfactant proteins have been described: surfactant proteins A, B, C and D. The structure and possible functions of surfactant protein A (SP-A) and D (SP-D) are discussed.

Abbreviations used: CRD, carbohydrate recognition domain; LPS, lipopolysaccharide; SP, surfactant protein.

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Figure 1
Structure of SP-A

Properties of SP-A

SP-A binds lipids and causes aggregation of phospholipid vesicles in the presence of Ca\(^{2+}\) [11]. SP-A-induced aggregation is dependent on an intact collagenous domain [12, 13]. At physiological extracellular Ca\(^{2+}\) concentrations, SP-A shows self-aggregation. There is an excellent linear relationship between the extent of vesicle aggregation induced by SP-A at each Ca\(^{2+}\) concentration and the extent of SP-A self-aggregation at the same Ca\(^{2+}\) concentration [12]. Aggregation of phospholipid vesicles by SP-A is mediated by Ca\(^{2+}\)-induced protein–protein interactions between carbohydrate-binding domains and oligosaccharide moieties [14].

SP-A binds to immobilized monosaccharides in a Ca\(^{2+}\)-dependent fashion [15]. An important subgroup of the C-type lectins is the collectin family (group III lectins) [16]. The proteins of this group are multimeric proteins characterized by CRDs bound to collagen stalks. SP-A, mannan-binding protein and also SP-D and conglutinin are members of this group. SP-A binds to agarose-bound \(\alpha\)-mannose, \(\beta\)-fucose and also to \(\alpha\)-galactose and \(\beta\)-glucose, but does not interact very well with \(N\)-acetyl-\(\beta\)-galactosamine and \(N\)-acetyl-\(\beta\)-glucosamine.

number of proteins. These proteins bind carbohydrates in a calcium-dependent fashion and are termed C-type lectins [8]. The CRDs of these proteins are characterized by 14 invariant residues and 18 that are conserved in character in fixed positions within a 120–130 amino acid domain. Four of these residues are cysteines, which form two intrachain disulphide bonds that are necessary for carbohydrate binding. The cysteines in SP-A form bonds between residues 135–226 and 204–218 [6]. Another post-translational modification of this part of SP-A is the N-linked glycosylation of asparagine at position 187.

Using in situ hybridization with autoradiography as the detection method, it was shown that, while the human SP-A gene is strongly expressed in alveolar type II cells, the gene is also focally expressed in bronchiolar and bronchial epithelial cells in 40% of the examined airways [9]. This would suggest that not only bronchiolar Clara cells, but also cells of the larger conducting airways, express SP-A. The fact that secretory granules of the Clara cell contain SP-A, but are not part of the endocytic pathway, also indicates that these cells express SP-A [10].
Similar binding characteristics were observed if the binding of human or canine SP-A to bovine serum albumin linked monosaccharides was measured [17]. Binding of partially purified SP-A, from bronchoalveolar lavage fluid obtained from healthy volunteers, to solid-phase mannan using an enzyme-linked lectin-binding assay was found to be optimal between 0.1 mM and 1 mM CaCl₂ [18]. It was found that SP-A has a high affinity for N-acetylmannosamine. The binding selectivity of SP-A is comparable to that of mannose binding protein [18]. SP-A binds to a variety of glycolipids including its own carbohydrate moiety [14] and several glycolipids [17, 19]. Its lectin activity can be used to purify SP-A [15].

**Functions of SP-A**

The localization, structure and properties of SP-A have led to speculations on the possible physiological functions of this protein. Tubular myelin is thought to be the structure from which the surface film forms in vitro. From in vitro reconstitutions [20] it is clear that tubular myelin formation is dependent on the presence of SP-A. In the tubular myelin lattice, SP-A has a preferential localization, suggesting that the protein provides the framework of this lattice [21]. The SP-A-SP-A interactions may be mediated in part by CRD-oligosaccharide interactions [14].

A second physiological function of SP-A may be the regulation of surfactant homeostasis [22]. This putative function of SP-A has been suggested because the protein modulates uptake and secretion of phospholipids by isolated alveolar type II cells in vitro. However, in a recent study it was demonstrated by several techniques that SP-A does not enhance the gross rate of lipid endocytosis by type II cells [23]. Instead, SP-A only increases lipid uptake by the few type II cells that are in the immediate vicinity of SP-A liposome complexes. Thus, SP-A may not stimulate lipid uptake by type II cells by receptor-mediated endocytosis but may induce a local concentration-dependent uptake. SP-A has also been shown to inhibit secretion of phosphatidylcholines by type II cells that were labelled overnight with labelled choline (reviewed in [22]). These in vitro studies suggest that SP-A may play a role in regulating alveolar pool size, by balancing surfactant release and clearance.

When its carbohydrate-binding properties were described it was recognized that SP-A may play a role in the pulmonary defence system [15]. It was proposed that SP-A recognizes polysaccharides on the bacterial surface and initiates coating and phagocytosis of bacteria by alveolar macrophages. Since then, many results have indicated that SP-A may play an important role in the innate first-line defence mechanisms of the lung:

**Binding to alveolar macrophages**

Binding and receptor-mediated endocytosis of SP-A to rat alveolar macrophages [24] was reported to occur via its CRD. Other investigators came to the conclusion that SP-A binds alveolar macrophages via its collagen domain because both Clq and soluble collagen compete for the binding of SP-A to macrophages [25].

**Effects on phagocytic functions**

SP-A enhances phagocytosis of pathogens by alveolar macrophages. Van Iwaarden and co-workers demonstrated that SP-A from human alveolar proteinosis patients stimulates phagocytosis of radiolabelled, serum-opsonized, *Staphylococcus aureus* by rat alveolar macrophages in a surface phagocytosis assay [26]. In line with these observations it was reported that phagocytosis of complement and IgG coated erythrocytes is enhanced if macrophages are cultured on SP-A-coated plates [27]. These seminal studies indicated that SP-A may facilitate complement- and immunoglobulin-mediated phagocytosis by alveolar macrophages. Recently SP-A-enhanced phagocytosis of non-opsonized bacteria by alveolar macrophages was also reported [28]. Human SP-A stimulates serum-independent phagocytosis of *Eschericia coli, Pseudomomas aeruginosa* and *Staphylococcus aureus* by rat alveolar macrophages, depending on the growth phase of the bacteria. SP-A binds *Staph. aureus* and *Streptococcus pneumoniae* (type 25) in a calcium-dependent manner and induces association of *Staph. aureus*, but not of *Strep. pneumoniae*, with rabbit alveolar macrophages [29]. It is not clear if the CRD of SP-A is involved in these interactions. The CRD was implied in the binding of SP-A to other organisms: The interaction of SP-A with *Pneumocystis carinii* involves the interaction of the CRD with the mannose-rich surface membrane glycoprotein gp120 [30]. These observations suggest that SP-A may act as an opsonin in the phagocytosis of bacteria and perhaps other microorganisms as well.

SP-A has also been reported to act as an opsonin in the phagocytosis of herpes simplex virus type 1 by rat alveolar macrophages [31]. The opsonic capacity of SP-A was found to be twice as potent as that of serum. Clq could not substitute for this activity of SP-A. SP-A binds herpes simplex
virus, as was shown indirectly by the increased binding to virus-infected cells expressing viral proteins at the cell surface [32]. Binding of SP-A to infected cells is inhibited by heparin, but not by yeast mannan. Interestingly, deglycosylated SP-A, obtained by digestion with N-glycosidase F, did not bind to infected cells. These observations indicate that the carbohydrate moiety, but not the CRD, of SP-A is involved in recognition of viruses [32]. In a recent study it was found that the carbohydrate moiety of SP-A is also involved in virus neutralization [33]. Infection of LL-C MK2 cells with influenza A (H3N2) virus was prevented by preincubation of the virus with SP-A. Viral infectivity was measured by the appearance of the viral proteins on the cell surface. After removal of the carbohydrate moiety of SP-A, by enzymic digestion with N-glycosidase F, SP-A no longer prevented viral infection of the cells.

**Induction of oxygen radical production by alveolar macrophages**

Human SP-A, purified from the lavage of alveolar proteinosis patients, enhances the lucigenin-dependent chemiluminescence response by rat alveolar macrophages [26]. In addition, the chemiluminescence response induced by rat surfactant can be abolished by antibodies against SP-A. These observations indicate that SP-A may also induce killing of microorganisms. The effect of SP-A is depressed by lipids. The SP-A-induced stimulation of superoxide radical production is not observed with peritoneal macrophages, polymorphonuclear leukocytes or monocytes [26]. SP-A itself is very susceptible to oxidation [34]. Oxidized SP-A was not able to stimulate alveolar macrophages and act as an opsonin of viruses [35].

**Lipopolysaccharide (LPS) scavenging**

SP-A binds LPS (endotoxin) from Gram negative bacteria, and prevents binding to granulocytes and activation of these cells [36]. Recent work from our laboratory indicates that SP-A binds the Lipid A region of LPS, the region implicated in the pathogenesis of septic shock. Interestingly, SP-D also binds LPS but to the core region and not to the core-oligosaccharide-deficient Re chains or Lipid A. It can be speculated that SP-A and SP-D act in concert to scavenge free endotoxin.

**Macrophage chemotaxis**

Another possible function of SP-A was suggested recently by Wright and Youmans [37]. These investigators observed that SP-A (human, rat) stimulates rat alveolar macrophage chemotaxis.

SP-A is a multifunctional protein. It has several functions in surfactant metabolism. In addition SP-A has many functions in the first line defence against inhaled pathogens. Although there are indications that SP-A levels change in toxic and diseased states [38], it should be emphasized that most of the described functions are properties of SP-A in vitro. All these putative functions await experimental verification in vivo. It will be interesting to investigate regulation and site of SP-A expression under conditions of altered surfactant metabolism and in infection models in vivo.

**Structure and localization of SP-D**

SP-D is a 43 kDa glycoprotein with a collagenous and non-collagenous domain [39, 40]. The primary in vitro translation products of rat and human SP-D have an apparent molecular mass of 39 kDa [41]. The only consensus sequence for asparagine-linked oligosaccharides (Asn-Gly-Ser) is within the collagen domain [41]. The primary structure of SP-D has been determined for the human [41-43], rat [44] and bovine [45] protein.

The protein comprises four regions (Figure 2): a short N-terminal, non-collagen sequence; a collagen domain of 59 Gly-Xaa-Yaa repeats; a short linking domain (‘neck’ region) that connects the collagen domain to the fourth region; and the carboxy-terminal CRD. This latter domain contains all of the invariant residues, including four conserved cysteine residues, characteristic of the family of Ca++-dependent C-type lectins. Rotary shadowing electron microscopy shows that SP-D has a similar cruciform structure to that of conglutinin [46], although the majority of the bovine preparation consisted of single stalks with globular heads. The collagen domain of SP-D is much longer than that of SP-A (59 versus 24 Gly-Xaa-Yaa repeats). Furthermore, the collagen domain of SP-D has no kink in the triple helix. This results in a length of the collagen triple helix of about 46 nm (bovine SP-D) [46].

By immunoelectron microscopy, SP-D was found in Clara cells, alveolar type II cells and alveolar macrophages [10, 47]. In Clara cells, labelling was found in the endoplasmic reticulum and the Golgi complex, and was most prominent in granules present in the apical domain of the cell [10]. Like SP-A, SP-D is present in the endoplasmic reticulum and the Golgi complex of type II cells. In contrast to SP-A, which is present in lamellar bodies and tubular myelin [21], SP-D is absent from these forms of stored and secreted surfactant [10]. Double labelling experiments showed that the
secretory granules of Clara cells, which were heavily labelled for SP-D, also contain SP-A. Interestingly, SP-A was distributed throughout the granule contents, whereas SP-D was confined to the periphery of the granule. The largest numbers of SP-D immunoreactive cells were observed in the distal membranous bronchioles. Scattered immunoreactive cells were found in the largest noncartilagenous airways but no labelling was observed in the major bronchi or trachea [47]. SP-A is focally expressed in airway cells [9]. In situ hybridization studies of SP-D mRNA are required to assess whether SP-D is also focally expressed in airway cells. Production of both SP-A and SP-D in bronchi would indicate that these proteins may also be important in the host defence in the airways and not only in bronchioli and alveoli.

Properties of SP-D

SP-D is not a real surfactant protein in the true sense since 90–95% of the protein was present in 48 000 g supernatants of lavages from normal rats. Until now there have not been many indications that the protein has an important function in surfactant homeostasis. Persson and coworkers examined the carbohydrate binding properties of rat SP-D [48]. These studies indicated that SP-D has a considerable specificity for α-D-glucosyl sugars. SP-D binding to maltosyl-BSA was most effectively completed with maltose, maltotriose, isomaltose and glucose [48]. The shape of SP-D is ideal for agglutination reactions. The molecule consists of four globular carbohydrate binding heads each connected to 46 nm long rods, which are associated via the free end. Thus, the molecule spans a long distance of 92 nm, a feature that may be important for the agglutination of microorganisms.

Functions of SP-D

Although termed surfactant protein D, it is not immediately clear how this protein would play a role in surfactant homeostasis. There are indications that SP-D is involved in the first line defence against inhaled pathogens rather than in surfactant metabolism. The first indication is in its structure; SP-D is a member of the collectins, a group of proteins that play a role in the immediate response to infectious challenge (recently reviewed in [16]). Secondly, its absence from intracellular surfactant and its loose association with extracellular surfactant. Thirdly, its site of production; SP-D is synthesized in surfactant producing alveolar type II cells but also in bronchiolar Clara cells and possibly even in cells in the upper airways. Fourthly, its
effects in vitro on bacteria and alveolar macrophages; SP-D causes agglutination of Gram negative bacteria such as E. coli, E. aerogenes, Salmonella paratyphi and Klebsiella pneumoniae but not of Staph. aureus, the only Gram positive bacteria tested [49]. The aggregation of E. coli Y1088 was inhibited by outer membrane, or purified LPS preparations isolated from this strain. SP-D binds the core region of LPS. If SP-D plays an important role in the nonclonal immunity, it should have a broad binding specificity towards Gram negative bacteria. Since the production of oxygen radicals by rat alveolar macrophages has not been reported. However, recent work of Van Iwaarden and coworkers indirectly suggests binding of SP-D to alveolar macrophages but not to peritoneal macrophages. These investigators showed that rat SP-D enhances the production of oxygen radicals by rat alveolar macrophages but not by peritoneal macrophages [50].

SP-D agglutinates Gram negative bacteria and activates alveolar macrophages. Therefore it may enhance binding, uptake and killing of bacteria by alveolar macrophages. In addition, agglutinated bacteria may be cleared more rapidly from the lung via mucociliary transport. Another exciting function of SP-D (and SP-A) may be to scavenge free LPS (endotoxin). This would prevent LPS from binding to granulocytes and would consequently protect against septic shock. All these putative functions await experimental verification in vivo.

In mammals, two separate nonclonal defence systems have evolved based on collectins: one circulatory and one lung system. The human lung is an organ with a large inner surface that is continuously in contact with the environment. The surfactant proteins SP-A and SP-D may have important roles in the first line defence against inhaled pathogens. Although in vitro structure and properties of the circulatory collectins may be comparable to those of the lung collectins, it should be realized that each of these proteins probably has unique biological functions in vivo.

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Interactions of carbohydrates and lectins with complement

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The complement system is a major component of immune defence against infection. It is a large system, consisting of about 20 soluble (plasma) proteins (Figure 1) and a similar number of cell-surface proteins. The cell-surface proteins are widely distributed, and act as receptors for fragments of soluble complement proteins, or as regulatory proteins, which control the activities of soluble complement proteins. The complement system in humans is well-characterized at the biochemical level: primary sequences are known for most of the proteins of the system, and secondary and tertiary structure data

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Abbreviations used: CRP, C-reactive protein; MAC, membrane attack complex; MASP, mannose binding protein-associated serine protease; MBP, mannose binding protein.

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