Ligands and receptors of lung surfactant proteins SP-A and SP-D

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1. ABSTRACT

Surfactant Protein A (SP-A) and D (SP-D) are calcium-dependent collagen-containing lectins, also called *collectins*, which play a significant role in surfactant homeostasis and pulmonary immunity. The role of SP-A and SP-D in immune defence is well- established. They are known to bind to a range of microbial pathogens that invade the lungs and target them for phagocytic clearance by resident alveolar macrophages. They are also involved in the clearance of apoptotic and necrotic cells and subsequent resolution of pulmonary inflammation. To date, the molecular mechanisms by which SP-A and SP-D interact with various immune cells are poorly understood. In spite of overall structural similarity, SP-A and SP-D show a number of functional differences in their interaction with surface molecules of microorganisms and host cells. The aim of this review is to provide an overview of the current knowledge of ligands and receptors that are known to interact with SP-A and SP-D.

2. INTRODUCTION

Upon inspiration, the airway epithelium is challenged by an array of airborne substances and potentially pathogenic microorganisms entering the lung. A system of defense mechanisms exists, which effectively neutralizes invading microorganisms and ensures that occurrence of pulmonary infections is relatively uncommon. These mechanisms include both innate as well as adaptive immune responses within the lungs.

Recognition of potential targets for phagocytosis is accomplished by a wide range of mechanisms. Among these, major contributors are soluble proteins of the innate immune system, present in blood plasma and in other body fluids, which bind to targets of various types, including microorganisms, and altered or damaged self molecules and host cells, then mediate interaction with phagocytes. The innate immune system recognition molecules include complement, the collectins, and the ficolins. These molecules express either direct antimicrobial activity, or
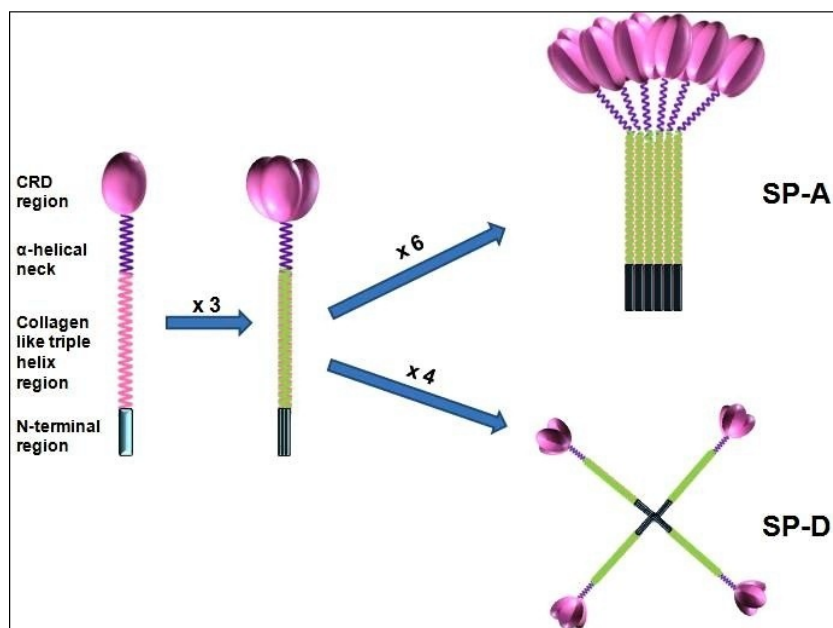
Figure 1. Structures of SP-A and SP-D. The basic structural unit of collectins is the trimer, and in mature fully-assembled SP-A, three polypeptides form 1 trimeric subunit, and six trimers form an octadecamer, which resembles a bunch of tulips, the collagen-like domains making up the stems and the CRDs the flowers (92).

may facilitate the elimination of infectious agents by phagocytic cells by acting as opsonins. The cellular and molecular constituents of the innate immune defence play a critical role in balancing the inflammatory response in such a way that containment of infection is sufficient while damage to the delicate respiratory epithelium is kept to a minimum. They also are important for rapid killing and clearance of pathogens.

SP-A and SP-D constitute important molecular components of the pulmonary innate immune defense system, which are members of a group of collagenous host defence lectins, first designated as 'collectins' by Malhotra *et al* (1).

2.1. The collectins

Collectins belong to a group of proteins, which are characterized by the presence of multiple copies of a polypeptide made up of a collagen-like domain and a C-type lectin domain, also referred to as a calcium-dependent carbohydrate recognition domain (CRD) (2-5). These multimeric glycoproteins, which belong to the C-type lectin superfamily (6), can bind to specific patterns of carbohydrates (neutral sugars), found on the surface of a wide variety of microorganisms. This binding is mediated by interactions of the multiple CRDs with terminal monosaccharide residues that are distributed spatially in a pattern characteristic of microbial surfaces, and which, therefore, enable discrimination between self and non-self (6, 7). Binding to collectins can lead to direct agglutination or neutralization of microorganisms, opsonisation in order to present bound microbes directly to phagocytes (8), or complement activation via the lectin pathway [in the case of mannose-binding lectin (MBL) only] (9). Consequently,

collectins are considered important sugar pattern recognition molecules of the innate immune system that can interact directly with live pathogens, and therefore play important roles in the first line of defence against microbes.

At present, five members of the collectin family are well-characterized which include three proteins present in serum, MBL (10) and two collectins only found in cattle: conglutinin and collectin-43 (CL-43) (11). The other two members, SP-A and SP-D, are both synthesized and secreted by airway type II and Clara cells, and therefore are also referred to as 'lung collectins'.

2.2. Structural organization of the collectins

The collectins are characterized by polypeptide chains that are composed of four distinct regions: (i) a short N-terminal region that contains cysteine residues which are involved in the assembly, via disulphide bridges, of the monomers into higher order oligomers; (ii) a collagen-like region characterized by repetitive triplet Gly-Xaa-Yaa sequences which is capable of trimerizing into a collagen triple helix (resulting in formation of a trimeric subunit); (iii) a short α-helical coiled-coil domain, termed the 'neck' region, which initiates trimerization of three monomers due to a heptad repeat of hydrophobic residues, resulting in strong hydrophobic interactions between the polypeptide chains in this domain; and (iv) a C-type CRD that can recognize glycan structures in a calcium-dependent manner (7, 12). The polypeptides thus assemble into trimeric subunits, and these bind together, covalently and non-covalently via the N-terminal regions to form multimers. For SP-A, a hexamer of subunits (with 6 x 3 = 18 CRDs) is a common form (Figure 1) but smaller oligomers (dimers, trimers, tetramers of subunits) are also found. (13)

The hexamer has a "bunch-of-tulips" shape, found also in complement C1q, the ficolins and MBL. SP-D similarly is assembled from trimeric subunits. A large cross-shaped tetramer of subunits is a common form (14). The affinity of a single CRD interaction with carbohydrate structures is low but the trimeric arrangement of these CRDs and the polymerisation of the subunits allows simultaneous and multivalent interactions of higher avidity with multiple surface carbohydrate structures. This enables not only biologically relevant target recognition, but also requires matching arrangements of glycan structures present on the surface of a target before efficient binding can take place, thus contributing to distinguishing self from non-self.

Although the collectins share a number of structural features, there are also many variations present. The collectin trimeric subunits can associate into various forms of higher order oligomers, stabilized via interchain disulphide bonds between the N-terminal domains. SP-D is organized as a tetramer of these collagenous trimers, generating dodecameric cruciform structures (15) while SP-A is found mainly as octadecamers (hexamers of trimers). SP-D can also form higher order oligomeric 'fuzzy ball' complexes (16). In addition to differing in oligomerization, the collectins also differ in the length of their collagen domains, number and distribution of cysteine residues located in the N-terminal domain and collagen domain, and distribution of N-linked oligosaccharides. Furthermore, differences occur in hydroxylation of proline residues, the degree of O-linked carbohydrate modification of the collagen domain, and carbohydrate binding selectivity of the CRDs. All these factors have an impact on the functional properties of the collectins and their interaction with targets and cells of the immune system.

2.3. The lung collectins SP-A and SP-D

SP-A and SP-D were first identified as components present in the phospholipid-rich material designated 'pulmonary surfactant', which is synthesized by alveolar epithelial type-II cells and secreted into the alveolar space. Pulmonary surfactant consists of lipids (90–95%) and four surfactant proteins (5-10%), the hydrophilic SP-A and SP-D and the small hydrophobic SP-B and SP-C.

2.3.1. Surfactant protein SP-A

SP-A is a large glycoprotein made up of multiple copies of (in humans) two polypeptides, SP-A1 and SP-A2 (each of about 30 kDa).These are products of separate genes, but are 97% identical in amino acid sequence In other mammals, only one SP-A polypeptide is expressed. These polypeptides assemble into subunits of ~90 kDa, which then form larger assemblies of up to ~540 kDa (17). SP-A has functions in surfactant metabolism and in pulmonary host defense, which have been studied both *in vitro* and *in vivo*.

The role of SP-A in surfactant metabolism *in vitro* has been extensively investigated (18). It takes part in surfactant pool size regulation by inhibiting surfactant secretion from type II cells (19, 20). SP-A associates rapidly with the secreted lamellar bodies (21) and assists to

form and maintain the tubular myelin structure (22, 23, 24). SP-A can bind to surfactant phospholipids (25, 26), and there is evidence that it improves surface activity by facilitating the adsorption of surface-active material to the air-fluid interface (27). SP-B is presumed to be the primary surfactant protein needed for phospholipid adsorption, and the role of SP-A in surfactant surface activity may be secondary, i.e. synergistic or regulatory (18). SP-A also takes part in the recycling of surfactant and facilitates the uptake of phospholipids into type II cells (28) and alveolar macrophages (29).

2.3.2. Surfactant protein SP-D

SP-D is a hydrophilic glycoprotein made up of twelve identical 43 kDa polypeptides with a total molecular weight of ~520 kDa (30, 31). SP-D has a single type of polypeptide chain, with a much longer collagenous region than SP-A. The polypeptides form trimeric subunits (130kDa), which then form a tetramer of subunits, in a cross (cruciform) shape, which is very large (8-9nm diameter). The dodecamer of 520 kDa is the dominant form, but natural human and bovine SP-D can include a high proportion of trimers and dimers of the 130kDa subunit (15). SP-D dodecamers may also self-associate at their N-termini to form much more highly ordered stellate multimers ("fuzzy ball" structures) with peripheral arrays of trimeric CRDs (16, 14). These multimers are not dissociated by EDTA or competing sugars, and are cross-linked by disulphide and non-disulphide bonds. They show higher apparent binding to a variety of ligands and are more efficient in mediating microbial aggregation (14) .

The main role of SP-D is in the pulmonary host defence. SP-D can bind to the surface of alveolar type II cells (32) as well as alveolar macrophages (33). There is no direct evidence of SP-D taking part in surfactant metabolism, e.g. phospholipid uptake. SP-D does not substantially bind to surfactant aggregates. However, it binds to phosphatidylinositol (PI) (34, 35) and promotes the formation of tubular structures of phospholipids in the presence of PI, SP-B, and calcium (36). Studies using SP-D gene deficient (-/-) mice also suggest a role for SP-D in surfactant homeostasis. The lungs of SP-D -/- mice contain enlarged alveoli, accumulation of surfactant phospholipids, increased numbers of large foamy alveolar macrophages, as well as abnormal type II cells and surfactant structure (37, 38).

Although SP-A and SP-D are referred to as 'lung collectins', these proteins and/or their mRNAs are also expressed, albeit at lower level, in many other tissues including gastric and intestinal mucosae, mesothelial tissues, synovial cells, middle ear and in the peritoneal cavity (39). SP-A and SP-D are also detectable in human blood plasma (40).

3. LIGANDS AND RECEPTORS

SP-A and SP-D are able to bind to a wide range of microorganisms and apoptotic cells, facilitating their clearance through various mechanisms. These collectins also participate in the clearance of other complex organic
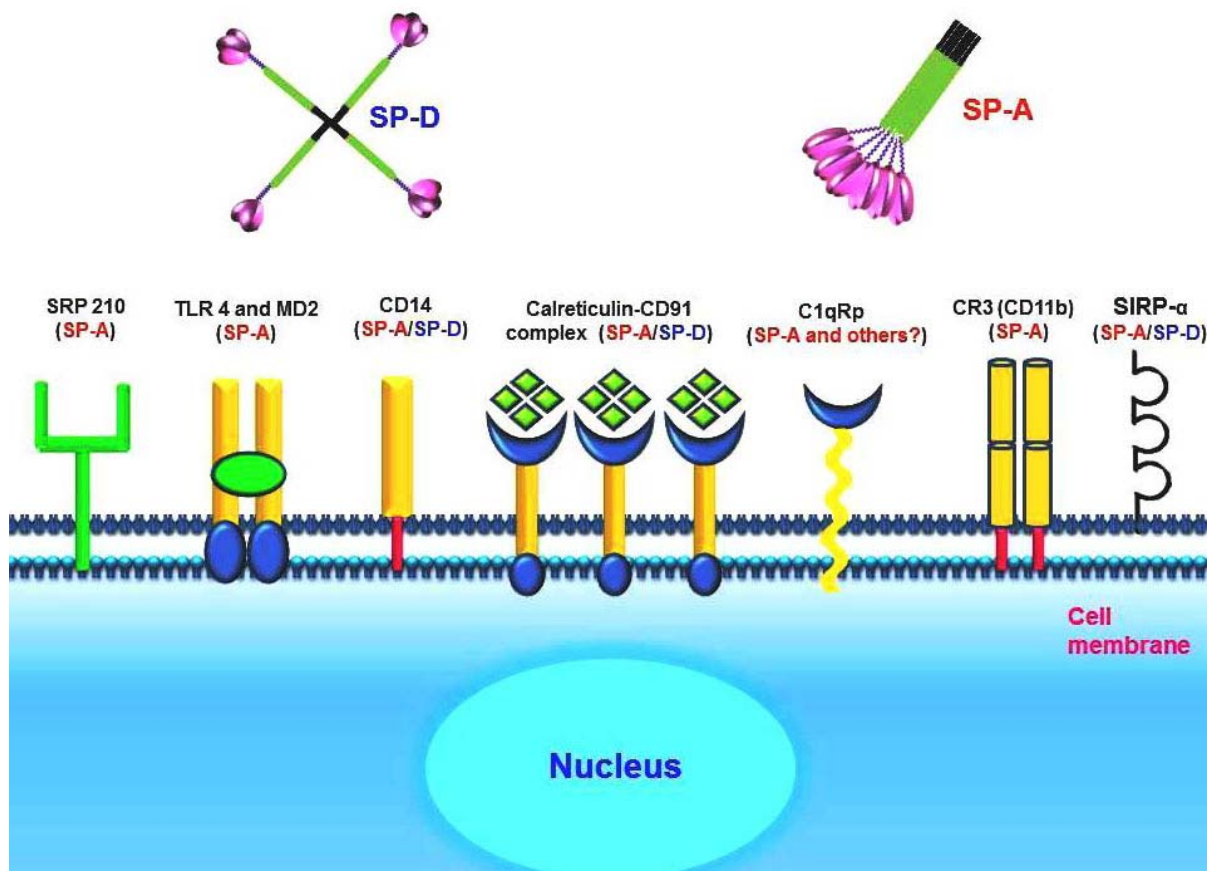
Figure 2. SP-A and SP-D receptors. SP-A and SP-D are able to bind to a range of microorganisms and apoptotic cells, facilitating their clearance through various mechanisms. These collectins also participate in the clearance of other complex organic materials, such as pollens (41) and house dust mite allergens (42). SP-A and SP-D also have the capacity to modulate leukocyte function, further enhancing the clearance of pathogens (43, 17). An overview of the major receptors for both SP-A and SP-D is shown above.

materials, such as pollens (41) and house dust mite allergens (42). SP-A and SP-D also have the capacity to modulate leukocyte function, further enhancing the clearance of pathogens (43, 17). An overview of the major ligands and receptors of SP-A and SP-D is presented below (Figure 2).

3.1. Interactions with carbohydrates

SP-A and SP-D recognise bacteria, fungi and viruses by binding mainly to surface mannose, fucose and N-acetylglucosamine residues, and lipids. The majority of these interactions are mediated through the CRDs and are calcium-dependent. A notable exception is the Herpes Simplex Virus, which appears to bind to the N-linked oligosaccharides of SP-A (44). SP-A and SP-D bind to a broad and overlapping range of microbial targets, but their precise modes of interaction, and the effects of the interaction can differ markedly.

The carbohydrate binding specificity of the C-type lectins is determined by a network of coordination and hydrogen bonds that stabilizes the ternary complex of protein, calcium ion and carbohydrate. SP-A and SP-D show differences in relative saccharide selectivity. Whereas the order of binding preference of SP-A for mono- or disaccharides follows: mannose, fucose > glucose, galactose >> N-acetylglucosamine (GlcNac) (45), human SP-D prefers interaction with maltose> glucose, mannose, fucose> galactose, lactose, glucosamine> N-acetylglucosamine (46). These differences in ligand specificity are likely due to the distribution of non-conserved residues that are located near the ligand-binding pocket of the CRD. Other structural factors are involved that have an impact on the carbohydrate-binding properties of the collectins. The clustering of three CRDs results in the generation of a trimeric high-avidity ligand binding site (47) and makes it possible for two or three CRDs to interact simultaneously with closely-spaced carbohydrate structures.

This multivalent binding depends on a matching arrangement between the three CRDs of the collectin, and the pattern of sugars present on the surface of a target. In addition, asymmetric orientation of the CRDs and charge distributions of the trimeric CRD surface might also affect ligand affinity and specificity. The differences in the oligomeric organization of SP-A (hexatrimeric) and SP-D

Ligands and receptors interacting with SP-A and SP-D

Table 1. Summary of binding of lung collectins to lipids

Lipid	Collectin	Mechanism	Reference
Dipalmitoylphosphatidylcholine (DPPC)	SP-A	Ca^{2+}-dependent, sugar-independent	50
Lipid A (LPS)	SP-A	Ca^{2+}-dependent, sugar-independent	44
Glycolipids (galactosylceramide, lactosylceramide)	SP-A	Ca^{2+}-dependent, CRD involved	51, 45
Dipalmitoylphosphatidylcholine (DPPC), Phosphatidylglycerol (PG)	SP-A	Ca^{2+}-dependent	52
Phosphatidylserine (PS)	SP-A	Ca^{2+} dependent, sugar independent	55
Phosphatidylinositol (PI)	SP-D	Ca^{2+}-dependent, CRD involved	34, 35, 48
Glycosylceramide: Glucosylceramide (GlcCer), Galactosylceramide (GalCer)	SP-D	Ca^{2+}-dependent, CRD involved	47, 56

(dodecameric) result in variations of both number and spatial distribution of their trimeric CRDs, influencing the binding specificity and avidity for carbohydrate ligand patterns present on biologically relevant particles.

3.2. Interactions with lipids

The lung collectins also display interactions with lipids which are mediated by the CRD but can also require the neck domain (47, 48, 49). SP-A binds to DPPC (50), the lipid A moiety of Gram-negative lipopolysaccharides (LPS) (44) and several glycolipids, including galactosyl-ceramide and lactosyl-ceramide, which are abundant in the plasma membranes of eukaryotic cells, although the functional consequences of these interactions are not known. These interactions appear to involve recognition of both the ceramide and saccharide moieties (45, 51).

SP-A also aggregates surfactant phospholipids in the presence of calcium ions (52, 53).With regard to SP-A, it was demonstrated that calcium ions can induce structural changes of the octadecamer which may exist in an "open-bouquet" or a "closed-bouquet" conformation in the absence or presence, respectively, of calcium ions (49). As a consequence, these differences in conformation may result in different interaction of the SP-A head groups (trimeric CRDs) with the lipid membrane surface. SP-A is also tightly associated with lipids *in vivo*, and is involved in the formation of ordered arrays of lipid known as tubular myelin (22). As expected, mice genetically deficient in SP-A (SP-A-/- mice) have very little tubular myelin, but they do not show significant aberration of pulmonary surfactant functions (54).

Jakel and co-workers (55) recently showed that Phosphatidylserine (PS), which becomes exposed on the outside of apoptotic cells, is a relevant binding molecule for human SP-A, but not for SP-D. The binding of SP-A to PS is Ca^{2+}-dependent and is not inhibited by mannose, suggesting that the sugar-binding site of the CRDs of SP-A is not involved. Flow cytometry studies on apoptotic Jurkat cells and apoptotic neutrophils showed inhibition of the binding of annexin V by increasing concentrations of SP-A. Supporting these data, confocal microscopy data showed a co-localisation of annexin V and SP-A in late apoptotic but not early apoptotic cells.

Further studies are needed to exploit the relevance of the interaction of SP-A with PS in the context of phagocytic uptake of apoptotic cells.

SP-D interacts *in vitro* with phosphatidylinositol (PI) in a calcium ion-dependent manner (34, 35, 48). It does not interact strongly with other phospholipid components of pulmonary surfactant but does have a strong association with

glycosyl-ceramide (51). The interactions of SP-D with PI and glycosyl-ceramide are calcium ion-dependent and inhibited by competing monosaccharides, indicating a CRD-mediated interaction, however there is evidence to suggest that PI also interacts with the α-helical coiled-coil region or other regions in the CRD not involved in sugar-binding (56, 47). An overview of the different lipids interacting with SP-A or SP-D is shown below (Table 1).

3.3. Interaction with Nucleic Acids

DNA is often found on the surface of apoptotic cells (57). SP-A and SP-D bind DNA and RNA from various sources including mice and bacteria (58) and enhance the uptake of DNA by human monocytic cells (59, 60). Hence, binding of DNA may be one mechanism by which SP-A and SP-D mediate phagocytosis of apoptotic cells.

3.4. Interaction with Protein Acceptors (Ligands)

SP-A and SP-D bind to cell-surface proteins which act as receptors (i.e. they transmit a signal to a cell on binding of the collectin to the receptor protein). SP-A and SP-D also bind to proteins which are not receptors and do not transmit any signal to a cell. These are referred to here as acceptors, to distinguish them from receptors.

3.4.1. Glycoprotein-340 (Gp-340)

Gp-340 was first identified as an exclusively SP-D binding molecule purified from bronchoalveolar lavage (BAL) of alveolar proteinosis patients (61). The binding of SP-D to gp-340 is inhibited by EDTA, but not affected by maltose, suggesting the involvement of a calcium ion-independent protein-protein interaction through the CRD. SP-A also interacts with gp-340 in a calcium-dependent, but lectin-independent manner (62). cDNA cloning has revealed that gp-340 is a member of a scavenger receptor cysteine rich superfamily consisting of multiple scavenger receptor type B domains (63). Gp-340 is in fact identical to salivary agglutinin - an immune scavenger component of saliva, which has been shown to bind to *Streptococcus mutans* and *Helicobacter pylori* (64). Gp-340 exists in both a soluble form, and in a form associated with alveolar macrophage membranes (63). It is clear now that gp-340 is mainly a soluble SP-A and SP-D binding protein rather than a surface receptor. The possibility that gp-340 acts as an adaptor for SP-A and SP-D binding to surfaces has not yet been investigated (i.e. gp340 binds to some recognition motif on a particle, allowing SP-A and SP-D to bind the particle via gp340).

3.4.2. Myeloperoxidase (MPO)

MPO is an intracellular defense molecule of neutrophils, which becomes exposed on the outside of the

cell upon apoptosis. MPO was identified as a novel binding molecule for SP-A and SP-D (65). The interaction is independent of Ca^{2+}, suggesting involvement of a non-lectin, non-calcium binding site. Both proteins bind directly to purified, immobilised MPO, and show inhibition of the binding of an anti-MPO monoclonal antibody (mAb) to late apoptotic cells. Fluorescence microscopic studies confirm that Anti-MPO mAb and SP-A/SP-D co-localise on late apoptotic neutrophils in a Ca^{2+} -independent manner.

3.4.3. C1q
SP-A was shown to interact with complement protein C1q (66, 67). The lectin domain is not required for this interaction. Uptake of C1q coated beads by alveolar macrophages was enhanced by preincubation with SP-A (67). Again C1q may act as an adaptor, binding to a particle surface then itself forming a binding site for SP-A

3.4.4. Immunoglobulins (Ig)
Nadesalingam *et al* (68) reported that SP-D binds various classes of immunoglobulins, including IgG, IgM, IgE and secretory but not serum IgA. The interaction appears to be through the globular domains of SP-D, both with the Fab and Fc domains in a Ca2+ dependent manner. IgG coated beads were aggregated by SP-D and enhancement of phagocytosis by murine macrophages was shown. It was also shown that SP-A binds to the Fc, rather than the Fab, region of IgG (69). Binding is calcium dependent but not inhibited by saccharides known to bind to SP-A's carbohydrate recognition domain. The binding of SP-A does not inhibit the formation of immune complexes or the binding of IgG to C1q. Recently, a recombinant fragment of human SP-D, composed of trimeric neck and CRDs, have been shown to bind specific IgE that abrogates IgE interaction with specific allergens (AS Qaseem *et al*, unpublished data).

3.4.5. Defensins
Human neutrophil defensins (HNPs) inhibit infectivity of enveloped viruses, including influenza A viruses. HNPs were found to bind to the neck and/or CRD of SP-D. This binding was specific because no, or minimal, binding to other collectins was found (70). HNPs precipitated SP-D from BAL fluid and reduced the anti-viral activity of BAL fluid. HNP-1 and -2 differed somewhat in their independent anti-viral activity and their binding to SP-D. Doss *et al* (71) used surface plasmon resonance to evaluate binding of defensins to SP-D. Human beta-defensins, HD6, and human neutrophil peptide (HNP)-4 bound minimally to SP-D. However HNP-1-3 bound SP-D with high affinity and inhibited SP-D mediated anti-viral activity.

3.4.6. Decorin
Nadesalingam *et al* (72) describe the co-purification of human decorin, a 130-150 kDa proteoglycan and novel SP-D-binding protein, from amniotic fluid.

The CRD region of SP-D binds in a calcium dependent-manner to the sulfated N-acetyl galactosamine moiety of decorin. Complement subcomponent C1q, a complement protein that is known to interact with decorin core protein via its collagen-like region, partially blocks the interaction between decorin and native SP-D. This protein, however, does not block the interaction between decorin and SP-D, a recombinant fragment that lacks the N-terminal and collagen-like regions. Furthermore, the core protein, obtained by chondroitin ABC lyase treatment of decorin, binds SP-D, but not SP-D neck and CRD region. These findings suggest that decorin core protein binds the collagen-like region of the SP-D. Collectively, CRDs of SP-D interact with the dermatan sulfate moiety of decorin via lectin activity and that the core protein of decorin binds the collagen-like region of SP-D *in vitro*.

3.5. Interaction with Protein Receptors
3.5.1. SPR-210
A 210 kDa SP-A-binding protein was first purified from the macrophage cell line U937 and became known as SPR-210 (surfactant protein receptor 210 kDa) (73). This receptor is also found on type II cells and alveolar macrophages. The interaction between SP-A and SPR-210 takes place through the collagen region of SP-A. Antibodies against SPR-210 inhibit binding of SP-A to alveolar type II cells and alveolar macrophages and inhibit the SP-A-mediated uptake of *Mycobacterium bovis*. SPR-210 may thus be a functional cell-surface receptor on both alveolar type II cells and macrophages. The molecular identity of SPR-210 has later been described as myosin 18A (74). This protein, which is expressed in multiple isoforms, may be a candidate for a genuine SP-A receptor, but this requires further investigation.

3.5.2. CD14
Sano *et al* (75) reported that SP-A and SP-D bind to native as well as recombinant soluble CD14, a known receptor for LPS, which is a component of the outer membrane of Gram-negative bacteria that is responsible for sepsis and induction of inflammation. The neck domain of SP-A binds to the leucine-rich peptide portion of CD14 whereas the lectin domain of SP-D interacts with the carbohydrate moiety of CD14. Both SP-A and SP-D appear to modulate LPS/CD14 interactions (76).

3.5.3. Calreticulin-CD91 complex
A common receptor for C1q, MBL and SP-A was described in 1990 (77) and identified later as calreticulin (78). However, calreticulin had been characterized mainly as an intracellular protein; and it took some further research to demonstrate how calreticulin functioned as part of a cell-surface receptor. It was shown (79, 80) that calreticulin binds to the cell-surface receptor CD91 and acts as an adaptor or co-receptor to bind the collagenous region of C1q, MBL and other proteins with similar collagenous structures (the collectins SP-A and SP-D, and also the ficolins) to the cell surface via CD91.

Phagocytic uptake of apoptotic cells, mediated by MBL or C1q binding to the Calreticulin-CD91 complex was demonstrated (80).

On cells which do not express CD91, HLA class I heavy chain (81), or possibly CD59 (82) may act as calreticulin-binding proteins, allowing particles coated with C1q or MBL to adhere to the cells. The region within calreticulin which binds to the collagenous proteins has been identified (83).

3.5.4. C1q receptor for phagocytosis (C1qRp) or CD93

A ~126kDa glycoprotein designated C1qRp and later shown to be CD93, was reported to play a role in C1q/MBL/SP-A-mediated removal of pathogens and immune complexes via phagocytosis, as determined by the ability of antibodies against this receptor partially to block enhancement of phagocytosis by these three proteins (84). However, this molecule was later shown to be an adhesion receptor (85), which does not interact directly or indirectly with any of the C1q-collectin-ficolin proteins. Thus, C1qRp is not a receptor for C1q, SP-A, SP-D etc, although in many databases it is still described as such.

3.5.5. Signal-inhibitory regulatory protein α (SIRP α)

Gardai and co-workers (86) reported that SP-A and SP-D modulate cellular functions through signal-inhibitory regulatory protein-α (SIRP-α), as well as the CD91–calreticulin complex. Their data show that SP-A differentially engages either CD91–calreticulin or SIRP-α, depending on whether the lectin domain of SP-A is bound to a target. For example, in the absence of a pathogen, SP-A binds through its lectin domain to SIRP-α. In the presence of a foreign organism or cell debris, to which the lectin domain of SP-A binds, the free collagen-like region activates immune cells through CD91–calreticulin. Importantly, engagement of the different receptors elicits different responses. When SP-A binds SIRP-α, inflammatory mediator production is inhibited.

3.5.6. Alveolar Type II Cell Receptors

Both SP-A and SP-D have been shown to interact with and be internalized by alveolar type II cells. The interactions of SP-A and SP-D with type II cells could be related to surfactant homeostasis (18). Several putative type II cell receptors for SP-A have been described. Two different SP-A binding proteins were identified using an anti-idiotypic antibody approach, including a 30 kDa protein (87) and a 55 kDa protein known as BP55 that multimerizes to 170–200 kDa (88). Another SP-A binding protein was described by Kresch and co-workers (89). Unlike SPR-210, none of these receptors appears to be expressed on macrophages. None of these proteins has been identified at the molecular level. Important future studies including cloning and characterizing the mechanism by which these receptors mediate type II cell function are needed.

3.5.7. Toll-like receptors (TLR4 and MD-2)

Yamada *et al* (90) showed that SP-A interacts directly with TLR4 and MD-2, which are critical signalling receptors for LPS. SP-A binds to the recombinant soluble form of extracellular TLR4 domain (sTLR4) and MD-2 in a Ca2+ -dependent manner, with involvement of the CRD region. In addition, SP-A attenuated cell surface binding of smooth LPS and subsequent NF- B activation in TLR4/MD-2 expressing cells. These studies indicate that in studies on ligand specificity of TLRs, it may often be important to consider whether a potential ligand has already encountered one of the soluble innate immune recognition systems (complement, collectins, or ficolins) before it encounters the TLR.

3.5.8. CR3 (CD11b)

SP-A has been shown to modulate cell surface expression of CR3 on alveolar macrophages and enhance CR3-mediated phagocytosis (91). Complement receptor CR3 (CD11b, CD18) was expressed at reduced levels on the surface of alveolar macrophages from SP-A -/- compared with SP-A +/+ wild-type mice. Intratracheal administration of SP-A to SP-A -/- mice induced the translocation of CR3 from alveolar macrophage intracellular pools to the cell surface. Intratracheal challenge with *Haemophilus influenza* enhanced CR3 expression on the surface of alveolar macrophages from SP-A -/- and SP-A +/+ mice, but relative expression remained lower in the SP-A -/- mice at all time points post-inoculation. SP-A augmented CR3-mediated phagocytosis in a manner that was attenuated by N-glycanase or collagenase treatment of SP-A, implicating the N-linked sugar and collagen-like domains in that function. The direct binding of CR3 to SP-A was calcium dependent and mediated by the I-domain of CR3, and to a lesser extent by the CR3 lectin domain.

4. CONCLUSIONS

Despite knowledge of carbohydrate and charge pattern recognition mechanisms by SP-A and SP-D, detailed crystal structures of ligand-bound CRD region are not available. They will provide important insights into the three-dimensional orientation of the homotrimeric CRDs bound to a range of self and non-self ligands. The area of domain-specific receptors for SP-A and SP-D still remain feebly understood. Given that SP-A and SP-D are versatile molecules in terms of distribution and functions, a number of reported biological properties and actions of these molecules cannot be explained by the existing repertoire of receptors or binding proteins (acceptors or adaptors). It is also possible that SP-A and SP-D, bound to self and non-self ligands, also act as modifiers of surface receptors. Binding of SP-D via CRDs to the the extracellular domains of soluble forms of recombinant TLR2 and TLR4 are interesting clues (93). In addition to immune-related receptors, receptors, especially for SP-A, on type II cell are also important to be identified. A candidate receptor for SP-A, designated

Ligands and receptors interacting with SP-A and SP-D

Table 2. Summary of proteins interacting with SP-A or SP-D

Acceptor	Protein	Collectin	Mechanism	Reference
Acceptor	Gp 340	SP-A / SP-D	Ca^{2+}-dependent, sugar-independent	61, 62
	MPO	SP-A / SP-D	Ca^{2+}-independent	65
	C1q	SP-A / SP-D	Ca^{2+}-dependent, sugar-independent	66, 67
	Igs	SP-A	Ca^{2+}-dependent	68
	Defensins	SP-D	Ca^{2+}-dependent, via CRD / neck	70, 71
	Decorin	SP-A	Ca^{2+}-dependent, via CRD and collagen-like region	72
Receptor	SPR-210	SP-A	via collagenous region	73
	CD14	SP-A / SP-D	neck domain of SP-A; lectin domain of SP-D	75
	Calreticulin-CD91 complex	SP-A / SP-D	via collagenous region	77, 78, 79, 80
	C1qRp	SP-A (and others?)	Not clear	84, 85
	SIRP α	SP-A / SP-D	via lectin or collagenous domain	86
	Alveolar Type II Cell Receptors	SP-A / SP-D	Not clear	87, 88, 89
	TLR4 and MD-2	SP-A	Ca^{2+}-dependent, sugar-independent	90
	CR3 (CD11b, CD18)	SP-A	Ca^{2+}-dependent	91

P63/CKAP4, which is a 63 kDa, non-glycosylated, highly coiled, transmembrane protein, has recently been described on the surface of type II cells (94). An overview of proteins interacting with SP-A or SP-D is given below (Table 2).

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