Structures and Functions of Mammalian Collectins

Uday Kishore[1] and Kenneth B.M. Reid[2]

1 Introduction

Protein–carbohydrate interactions serve multiple functions in the immune system. Many animal lectins (sugar binding proteins) mediate both pathogen recognition and cell–pathogen interactions using structurally related calcium-dependent carbohydrate recognition domains (C-type CRDs). The collectins are a group of mammalian lectins containing collagen regions. They include mannose-binding lectin (MBL), lung surfactant protein A (SP-A), lung surfactant protein D (SP-D), bovine conglutinin (BC), and collectin-43 (CL-43). Pathogen recognition by these collectins is mediated by binding of terminal monosaccharide residues characteristic of bacterial and fungal cell surfaces. The broad selectivity of the monosaccharide binding site and the geometrical arrangement of the multiple CRDs in the intact collectins explain the ability of these proteins to bind tightly to arrays of carbohydrate structures normally found on the surfaces of the micro-organisms and thus mediate discrimination between self and non-self.

The primary structure of each of the collectins is organised into four regions: an N-terminal region involved in the formation of inter-chain disulphide bonds, a collagenous region composed of Gly-Xaa-Yaa repeats, an α-helical neck peptide, and a C-terminal C-type CRD (Hoppe and Reid 1994). The collectins are large oligomeric structures, each assembled from multiple copies of a single polypeptide chain (with the exception of human SP-A, which has two closely related types of chains; Fig. 1). The C-type CRDs are spaced, in a trimeric orientation, at the end of triple-helical collagenous stalks. It is becoming increasingly clear that the CRD domains, by binding to carbohydrate ligands on the cell surface of the pathogens, fulfill a recognition function that can bring about effector functions, such as complement activation (by MBL) or phagocytosis (by SP-A and SP-D; Hoppe and Reid 1994; Kishore et al. 1997; Crouch 1998).

[1] Institute of Molecular Medicine, John Radcliffe Hospital, University of Oxford, Headington, Oxford OX3 9DS, UK
[2] Medical Research Council Immunochemistry Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK

Results and Problems in Cell Differentiation, Vol. 33
Paul R. Crocker (Ed.): Mammalian Carbohydrate Recognition Systems

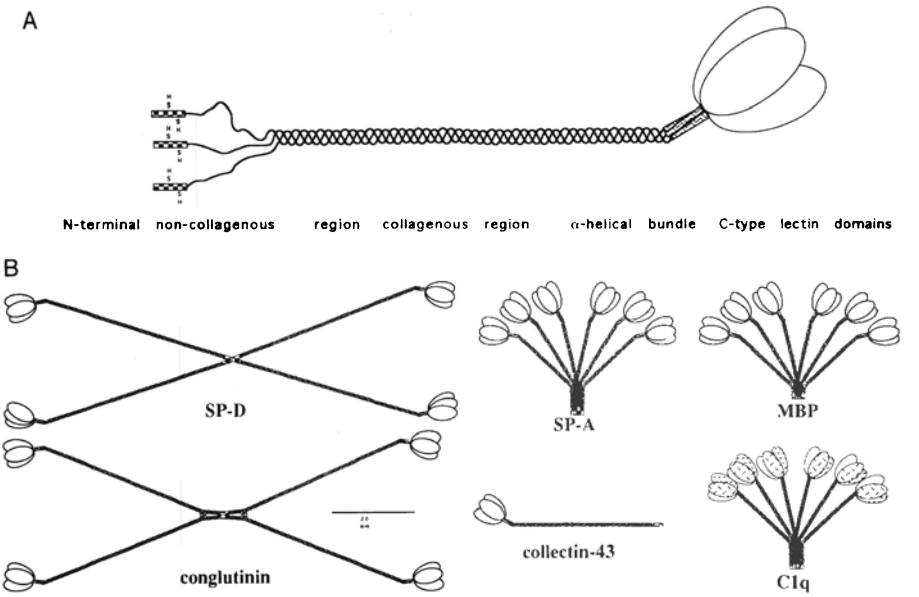

Fig. 1A,B. Domain organisation and assembly of the collectin molecules. **A.** The primary structure of each of the collectins is organised into four regions: an N-terminal, non collagenous region involved in the formation of interchain disulphide bonds, a collagenous region composed of Gly-Xaa-Yaa repeats, an α-helical neck peptide, and a C-terminal C-type CRD domain. The collectins are large oligomeric structures, each assembled from multiple copies of a single polypeptide chain (with the exception of SP-A, where two types of chain are formed). The C-type CRDs are spaced, in a trimeric orientation, at the end of triple helical collagenous stalks. It is becoming increasingly clear that the CRD domains, by binding to carbohydrate ligands on the cell surface of the pathogens, fulfill a recognition function that can bring about effector functions, such as complement activation (by MBL) or phagocytosis (by SP-A and SP-D; Hoppe and Reid 1994). **B.** SP-D and BC appear to be cruciform under the electron microscope, with four arms of equal length ending in globular heads. Oligomers of SP-D are also found. MBL and SP-A seem to resemble serum complement protein, C1q in their overall organisation. The bovine CL-43 exists as monomers (Hoppe and Reid 1994)

2 Mannose-Binding Lectin (MBL)

MBL, a Ca^{2+}-dependent lectin synthesized primarily in the liver, is an acute phase reactant which shows a modest (1.5- to 3-fold) rise in serum levels during stress or infection. The presence of glucocorticoid responsive elements and a cytokine responsive element homologue in the 5′ flanking region of MBL genes (Sastry et al. 1989) suggests that MBL is an acute phase reactant.

MBL binds to carbohydrate structures found on a wide range of pathogenic organisms. On binding to these carbohydrate structures, MBL can bring about activation of the serum complement system, thus allowing recruitment of a variety of inflammatory, killing and clearance mechanisms, in an antibody-independent manner. MBL is, therefore, considered to play an important role

in innate immunity – especially in very young children or immunodeficient individuals. MBL is also found in amniotic fluid, nasal secretions, middle ear fluid, saliva and inflamed sites – such as rheumatic joint fluid (Turner 1996a).

2.1 Molecular Structure and Assembly of MBL

MBL is composed of multimers of identical polypeptide chains of 32 kDa. Three 32-kDa chains combine to make a structural subunit of 92 kDa and oligomeric forms of MBL are composed of two to six of the 92 kDa subunits. Each 32-kDa chain is composed of: an N-terminal region containing cysteine residues involved in inter-chain and inter-subunit disulphide bonding, a region of Gly-Xaa-Yaa repeating triplets which is involved in the formation of collagen-like triple-helical structure, an α-helical neck region of approximately 34 residues, and a C-terminal globular domain which contains the 14 invariant and 18 highly conserved amino acid residues characteristic of the 120-residue C-type carbohydrate recognition domain (CRD). In the electron microscope, the highest oligomeric form of the MBL, the hexamer of the 92-kDa structural unit, appears as a bouquet-like structure with six globular 'heads' each connected by collagen-like strands to a central core (Lu et al. 1990). The largest oligomeric form of serum MBL, which consists of 18 identical 32-kDa polypeptide chains arranged as a hexamer of timers of these chains, has a molecular weight of 576 kDa. MBL isolated from liver appears to be composed of six identical 32-kDa chains and has a molecular weight of 192 kDa. There are seven cysteine residues at positions 5, 12, 18, 135, 202, 216 and 224 (based on numbering of the 32-kDa chain of the mature protein). The disulphide bond arrangement has not been determined, but the residues at positions 5,12, and 18 are probably involved in inter-chain and inter-subunit disulfide bonds. The remaining four residues are expected to form two intra-chain disulphide bonds (135 to 224 and 202 to 216) characteristic of that observed in other C-type lectin domains.

2.2 Biological Functions of MBL

MBL renders innate immunity by (1) recognition of carbohydrate structures on the pathogens via CRDs, and (2) activation of the serum complement system in order to recruit its inflammatory, opsonisation and killing mechanisms. Two possible mechanisms have been proposed for the MBL-mediated activation of the classical complement pathway. Initially it was shown that the higher oligomers (pentamers and hexamers) of MBL could interact with the proenzyme C1r2-C1s2 complex after binding to mannan-coated erythrocytes (Lu et al. 1990). The C1-esterase activity expressed by the bound complex results in C4 and C2 cleavage, leading to assembly of the C3 convertase of the

classical pathway (C4b2a; Ohata et al. 1990). However, it is now considered that the MBL oligomers normally circulate with two serine proteases, designated MBL-associated serine proteases (MASP-1 and MASP-2), each of which show approximately 40% amino acid sequence identity to the complement enzymes C1r and C1s (Matsushita and Fujita 1992; Thiel et al. 1997). The MASP-2 shows functional similarity to C1s since it activates the C4 and C2 components of complement after interaction of MBL with targets, such as mannan. However, at present it is not clear what the stoichiometry is of the MBL-MASP-1/MASP-2 complexes and what the precise role of each of the MASP enzymes within such complexes is.

2.3 Interaction of MBL with Micro-organisms

The MBL CRDs bind glycans terminating with *N*-acetylglucosamine or mannose. Such terminal residues are relatively rare in mammalian tissues (Rademacher et al. 1988) but occur more commonly on microbial surfaces. MBL, via its CRDs, binds to a wide range of pathogens, which include Gram-negative and Gram-positive bacteria, yeasts, viruses, mycobacteria and parasites. Complement activation, brought about by the MBL-MASP-1/MASP-2 complexes interacting with the pathogen surface, results in coating of the target pathogen with large amounts of activated C4 and C3 which leads to opsonisation. One of MBL's main functions is the enhancement of the killing and clearance of pathogens by bringing about antibody-independent activation of the complement system, thus zero, or low, levels of MBL may greatly increase risk to certain infections in young children and immunodeficient individuals (Turner 1996b).

Native and recombinant MBL have been shown to bind wild-type virulent *Salmonella montevideo*, expressing a mannose-rich *O*-polysaccharide (Kuhlman et al. 1989), leading to uptake and killing by phagocytosis. It is likely that MBL-mediated opsonisation involves a complement amplification process and the deposition of C3b / iC3b on microbial surfaces. Non-encapsulated *Listeria monocytogenes*, non-encapsulated *Haemophilus influenzae* and non-encapsulated *Neisseria meningitidis*, *N. cirera* and *N. subflava* show avid binding to MBL. *Streptococci*, *Escherichia coli*, and *N. meningitidis* serogroup show intermediate binding, while encapsulated *N. meningitidis*, *H. influenzae* and *S. agalactive* show weak affinity towards MBL. In binding to different isogenic mutants of *N. meningitidis*, the structure of lipopolysaccharide (LPS) appears to be an important determinant (Jack et al. 1998). The sonicates of *Mycobacteria leprae* and *M. tuberculosis* bind strongly to MBL, probably because of the high D-mannose content found on the surface of these pathogens. MBL also binds to acapsular *Cryptococcus neoformans* and mediates agglutination (Schelenz et al. 1995).

MBL is known to inhibit both haemagglutinin (HA) activity and infectivity of several strains of viruses, in addition to acting as an opsonin and enhanc-

ing neutrophil reactivity against the virus. It has been shown that MBL, as a β inhibitor of influenza A virus (IAV) acts by binding to high mannose structures and masking the cell attachment site of HA (Hartshorn et al. 1993). MBL is also capable of initiating complement mediated neutralisation of IAV (Anders et al. 1990). It has been demonstrated that MBL inhibits HIV infection of $CD4^+$ lymphoblasts and binds to HIV-infected U937 cell lines (Ezekowitz et al. 1989). It can further activate the classical complement pathway by binding to *gp120* from HIV-1 and *gp110* from HIV-2 (Haurum et al. 1993).

2.4 Gene Organisation and Genetics of MBL

The 7 kb gene encoding human MBL is located on the long arm of chromosome 10, within a gene cluster, at 10q11.2-q23, which also includes genes for SP-A, SP-D and a pseudogene of SP-A. Four exons encode the four distinct regions seen in the mature 32-kDa polypeptide chain of MBL: the N-terminal cysteine rich region (exon 1), a collagenous region (exons 1 and 2) and an α-helical neck region (exon 3), followed by a CRD region (exon 4).

There are four allelic forms of the MBL gene which provide structural variants of the MBL polypeptide chain. These allelic forms are designated A, B, C and D. The allele A, being the most common, is taken as the normal, or wild-type, form. In the B and C alleles, one glycine residue is replaced by aspartic acid (allele B) or glutamic acid (allele C) within the collagenous region. In the D allele, an arginine residue in the collagenous region is replaced by a cysteine residue (Madsen et al. 1994). Each of these three substitutions probably affects the formation of a stable collagen-like triple-helix. The homozygotes with respect to the B, C, or D alleles have undetectable or trace amounts of MBL. The heterozygotes (A/B, A/C or A/D) have lower levels of MBL (approximately 15% of that of A/A homozygous individuals). The A/A homozygous individuals can also have low levels of MBL since there are variants within the promotor region of the gene which influence serum levels (Madsen et al. 1995; Turner 1996b). An average value of 1 μg/ml is found in the sera of Caucasians, but there is a wide variation in individual values (0–5 μg/ml), due to the B, C and D alleles and variants in the promoter region.

2.5 Crystal Structure of Trimeric CRDs of MBL

The crystal structure of the trimeric CRDs of MBL, together with the neck region, shows that each CRD is approximately 45 × 25 × 25 Å, and contains two calcium ions, designated sites 1 and 2 (Weis et al. 1991, 1992, 1998; Sheriff et al. 1994). The CRD starts in a β-strand, followed by a ten residue α-helix, an extended stretch of ten residues, and a second α-helix. A short turn after this helix leads into a second β-strand that turns sharply at a conserved glycine (158 of MBL-A). The backbone then enters a region with no regular secondary

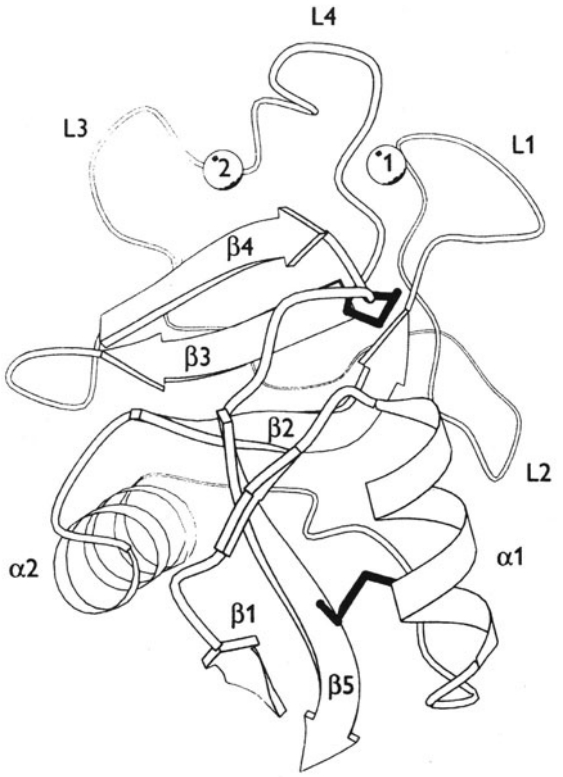

Fig. 2. Ribbon diagram of MBL-A CRD. The α-helices, β-strands, and loops are labelled as *α, β, and L*. The two Ca^{2+} are represented by *spheres*, and the disulphide bonds are shown in *black* (Weis et al. 1998)

structure, containing two loops, an extended stretch, and two more loops, for a total of about 45 residues (Fig. 2). After the fourth loop, the backbone forms two anti-parallel β-strands that end in a tight turn. The structure enters a short loop, and finishes with a β-strand that pairs in an anti-parallel orientation with the first N-terminal strand. The pairing of the first and last β-strands places the beginning and the end of the CRD next to each other. Structure-based sequence alignment of the CRDs reveals the essential sequence determinants of the fold (Weis et al. 1991). There are two disulphide bonds formed by four invariant cysteines: the outer disulphide links the first α-helix to the last β-strand, and the inner disulphide links the beginning of the third β-strand to the loop following the fourth β-strand. A second set of conserved residues form the calcium binding sites.

MBL has a broad carbohydrate specificity well-suited to recognise a variety of pathogenic surfaces. The common feature of these sugars is the presence of equatorial hydroxyl groups in the stereochemistry of the 3- and 4-OH groups of D-mannose. The structural basis of MBL and carbohydrate specificity has

been investigated by high resolution X-ray crystallographic analysis of the two rat proteins, serum MBL-A and liver-associated MBL-C. The structure of MBL-A complexed with a high mannose oligosaccharide at 1.7 Å resolution (Weis et al. 1992), and a series of MBL-C structures complexed with methyl glycosides of mannose, *N*-acetylglucosamine, and fucose at 1.7–1.9 Å, have revealed that the binding occurs through direct co-ordination of the site 2 Ca^{2+} involving covalent bonding of the 3- and 4-OH groups of the ligand (Fig. 3 A). In the interaction of mannose with MBL, there are three non-polar van der Waals contacts: the C4 of mannose contacts the Cβ of residue 189 of MBL-A, the 2-OH contacts a carbon atom, and the exocyclic C6 contacts the terminal methyl group of Ile-207 (Fig. 3 B). Site-directed mutagenesis has revealed that only C4-Cβ contact is energetically significant (Iobst et al. 1994): replacement of His-189 with Ala, and that of Ile-207 with Val, has little effect on ligand binding. However, changing His-189 to Gly, which removes the Cβ contact, reduces the affinity for mannose significantly. Complexes of MBL-C with methyl glycosides of *N*-acetylglucosamine and fucose confirm that the contacts with the hydroxyl groups equivalent to the 3- and 4-OH groups are the principal determinants of the recognition. Di-, tri-, and higher oligomannose oligosaccharides complexed with MBL-A and MBL-C confirm that the site interacts only with a single sugar moiety of the ligand (Weis et al. 1992).

Despite their affinity for a range of monosaccharides, MBLs do not trigger complement-mediated lysis or opsonic reaction to host cells. This can partly be explained by the relative sparsity of terminal mannose, GlcNAc, and fucose residues on vertebrate glycoproteins and glycolipids. The crystal structures of the rat MBL-A (Weis and Drickamer 1994) and human MBL (Sheriff et al. 1994)

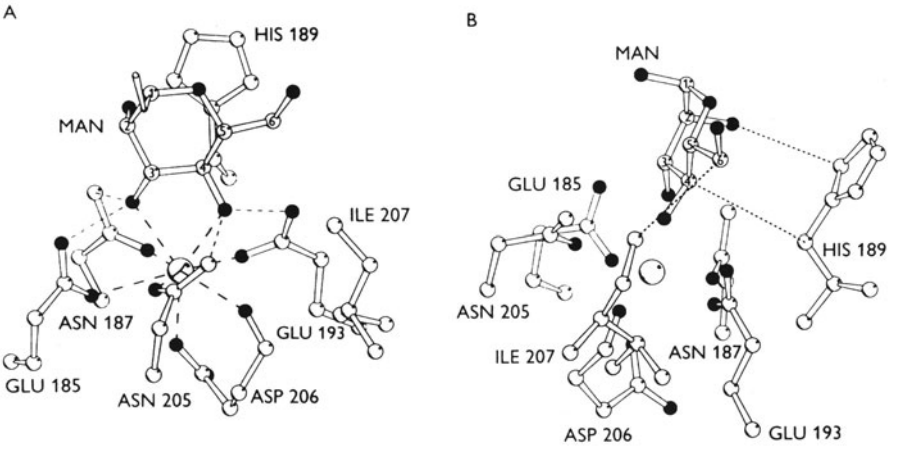

Fig. 3A,B. Binding of mannose at the rat MBP-A calcium site. **A.** Side chain oxygen atoms of residues 185, 187, 193 and 205, and the main chain carbonyl oxygen of residue 206, form the pentagonal, equatorial Ca^{2+} ligands (Weis et al. 1998). **B.** Van der Waals contact (*dashed lines*) between mannose and residues His 189 and Ile 207 (Weis et al. 1998)

trimers provide a likely explanation for the ability of MBL to distinguish non-self from self. A hydrophobic interface between the neck and the CRD maintains a fixed spatial relationship between the two, such that the sugar/calcium binding sites are 53 Å (rat) and 45 Å (human) apart in the trimer. The terminal mannose residues in vertebrate mannose-rich oligosaccharides are about 20–30 Å apart. The binding sites in the MBL trimer are therefore too far apart to interact multivalently with such oligosaccharides. In contrast, pathogenic cell surfaces present dense, repetitive arrays of ligands that can span the distance between binding sites in the MBL CRD trimers, resulting in highly avid multivalent interactions. MBL-A has proven to be an excellent system to probe the determinants of carbohydrate specificity in other C-type lectins. The interactions made by MBP-A and its ligands allow new specificities to be engineered onto the C-type CRD scaffold without affecting the overall structure of the protein. The pair of Glu-185 and Asn-187, when replaced with Gln and Asp, respectively, confers upon MBL-A preferential binding for galactosides, instead of mannose (Drickamer 1992).

3 Surfactant Protein A (SP-A)

SP-A accounts for 5% of the weight of the surface active mixture of phospholipids and proteins which are essential constituents of pulmonary surfactant. Nearly all the SP-A is tightly associated with lipids, such as dipalmitoylphosphatidylcholine (DPCC) and sphingomyelin. SP-A is considered to play an important role in surfactant secretion and uptake by type II alveolar cells, and also in the organisation of tubular myelin. Both SP-A and SP-D appear to provide innate immunity against lung pathogens by binding to carbohydrates on the pathogens, agglutinating them and triggering effector mechanisms which kill and opsonise the pathogens. SP-A can also interact with macrophages and increase their chemotactic, phagocytic and oxidative properties. It is therefore considered that SP-A may play an important role in the rapid recognition and clearance of pathogens, especially in immunodeficient individuals (Wright 1997).

3.1 SP-A Suprastructure and Assembly

SP-A has a hexameric structure in which six structural subunits of 105 kDa associate to yield a molecule of 630 kDa. Each subunit is composed of three 35-kDa polypeptide chains which are held together by disulphide bonds located in the N-terminal halves of the chains. The overall shape of SP-A is very similar to that of the complement protein C1q, both molecules appearing in the electron microscope as a bouquet-like structures with six globular heads linked by collagen-like strands to a fibril-like central core. The mature forms of the two SP-A polypeptide chains designated α_2 (product of the SP-A II gene)

and α_3 (product of the SP-A I gene) are both composed of 248 residues which include: an N-terminal segment (7 residues), a collagen-like region (73 residues), the neck region (26 residues) and a CRD domain (123 residues). There is one *N*-linked glycosylation site at Asn-198 in each chain. There are seven cysteine residues in the mature α_3 polypeptide and six in the mature α_2 polypeptide. The cysteines at positions 6, 48 and 65 in the α_3 polypeptide are considered to form inter-chain disulphide bonds and are involved in oligomer formation (Elhalwagi et al. 1997). The remaining four cysteine residues in each chain are the conserved intra-chain cysteine residues found within the CRD domain. The collagen region contains 23–24 Gly-Xaa-Yaa repeats. Prolines are present in the X position of 4–5 triplets, and at the Y position of 15–16 triplets, most of which are hydroxylated. These hydroxyprolines greatly enhance the stability of collagen triple-helix. The stability of the triple-helix is also contributed to by the alternating charged residues present in the two halves of the collagen domain of SP-A, the two halves being separated by an interruption following the 12th triplet, called the 'hinge region'. As in the C1q, the hinge region introduces a bend in the collagen segment which tilts the CRD trimers away from the core in the whole SP-A molecule. The 38–40 residue long SP-A α-helical coiled-coil neck region is organised into 'heptad repeats'. The co-operative interactions between the neck and CRDs may contribute to SP-A interactions with type II cells (Sano et al. 1998). The CRDs of SP-A have been shown to mediate a variety of interactions including modulation of alveolar type II cell functions, binding and aggregation of phospholipids, and recognition of bacterial, viral and fungal organisms. Mutational analysis has suggested that the carbohydrate binding site of SP-A co-localises with a major Ca^{2+}-binding site (McCormack et al. 1994; Sano et al. 1998).

3.2 SP-A Gene and Genomic Organisation

Two transcribed SP-A genes (SP-A I and SP-A II) and one pseudogene have been localised to chromosome 10q21–24, within a cluster that includes the SP-D and MBL genes (Hoover and Floros 1998). Human SP-A contains two types of chain (α_2 and α_3) and it has been proposed that each trimeric subunit has one α_2 chain and two α_3 chains. The sequences and genomic organisations of the SP-A I and SP-A II genes are very similar, each being composed of seven exons. The expressed proteins are each encoded within four exons (I-IV): I, covering part of the 5′ untranslated region, the leader peptide, the N-terminal region and part of the collagen-like sequence; II, covering the remainder of the collagen-like sequence; III, the α-helical neck sequence; and IV, the CRD plus the 3′ untranslated sequences. Allelic variants of each gene are generated by splicing variability in the 5′ untranslated regions and by sequence variability in the 3′ untranslated regions (Floros and Hoover 1998).

The SP-A mRNA and protein are expressed in epithelial cells and the non-ciliated bronchiolar cells (Clara cells) of the terminal bronchioles and con-

ducting airways (Khoor et al. 1993). It has also been detected in the serous glands of proximal human trachea, in the endocytic compartment of macrophages, rat intestinal epithelia, human and rat mesentery and human inner ear. SP-A is synthesised as a precursor with a 17- to 28-residue-long leader sequence. After synthesis, SP-A is hydroxylated in the endoplasmic reticulum (ER) by the enzyme prolyl hydroxylase, via the formation of 4-hydroxyproline. SP-A is also co-translationally glycosylated with mannose-rich carbohydrates in the ER, followed by post-translational modifications, such as sialylation and sulphation, contributing to the charge heterogeneity of the molecule (McCormack 1998).

3.3 SP-A-Carbohydrate Interaction

The ability of various monosaccharides to compete for binding of SP-A to mannan, a yeast-derived polymer of mannose, has the order: *N*-acetylmannosamine >L-fucose, maltose >glucose >mannose. Galactose, D-fucose, glucosamine, mannosamine, galactosamine, *N*-acetylglucosamine and *N*-acetylgalactosamine do not inhibit mannan binding. SP-A binds specifically to mannose-rich carbohydrates which are likely components of bacterial lipo-oligosaccharides or capsular oligosaccharides. The CRDs of SP-A bind avidly, in a Ca^{2+} - dependent manner, to LPS via the lipid A domain, whose structure closely resembles that of phosphatidylcholine. SP-A shows an acute phase response to LPS aerosolisation (van Helden et al. 1997), leading to upregulation of SP-A receptor expression on the macrophage surface. SP-A also binds, via its CRDs, to the major surface glycoprotein of *Pneumocystis carinii*, implicated in pneumonia in immunosuppressed subjects (Zimmerman et al. 1992). SP-A is also known to bind to glycolipids, such as lactosylceramide and galactosylceramide (Childs et al. 1992).

3.4 SP-A-Phospholipid Interactions

SP-A forms a part of the mixture of phospholipid and protein which lines the alveolar space and acts to reduce surface tension forces and prevent atelectasis during expiration. It can function as an inhibitor of phospholipid secretion by alveolar type II cells, via interaction with a high affinity receptor. SP-A binds tightly to dipalmitoylphosphatidylcholine (DPCC) and galactosylceramide (GalCer) and preferentially enhances DPCC uptake by type II cells, as well as the incorporation of this lipid into lamellar bodies. Since DPCC is the principal component responsible for the biophysical properties of pulmonary surfactant, SP-A may play an important role in phospholipid homeostasis in the alveolar space. Mutational studies have implicated the CRD as the major phospholipid interaction site (McCormack et al. 1994). The lipid binding site may overlap with the Ca^{2+}/carbohydrate binding site. SP-A is considered essential

for the formation of tubular myelin and other surfactant aggregates, and in concert with surfactant protein B, facilitates rapid adsorption and spreading of surface active phospholipids at the air-liquid interface of the alveoli (Suzuki et al. 1989). SP-A gene-deficient mice have little or no tubular myelin (Korfhagan et al. 1996). SP-A augments the adsorption of phospholipids to an air-liquid interface and improves surface tension of cycled surfactant mixtures *in vitro*. A possible involvement of the N-terminal region, and not the collagenous region, in lipid aggregation, is suggested by the fact that disruption of the interchain disulphide bond at Cys-6 completely blocks lipid vesicle cross-linking by SP-A (McCormack et al. 1994).

3.5 SP-A-Type II Cell Interaction

The alveolar type II cells synthesise and secrete surfactant, and also internalise surfactant from the alveolar space. The interaction of SP-A with type II cells has been shown *in vitro* to inhibit lipid secretion and to promote the uptake of lipid by cells, suggesting a role of SP-A in the regulation of surfactant turnover and metabolism. SP-A interacts with the type II cells via its CRDs. This interaction involves a small disulphide loop containing Cys-204 and Cys-218 (Kuroki et al. 1988) and a region of Glu-202 to Met-207. Mutants of rat SP-A, in which Glu-195 was changed to Gln and Arg-197 to Asp, converted SP-A from a mannose-binding to a galactose-binding lectin (McCormack et al. 1994). These mutations also reduce SP-A binding to the type II cells, confirming the CRD as the interacting region. The CRDs have also been implicated in the ability of SP-A to increase lipid uptake (Kuroki et al. 1988). However, SP-A interaction with type II cells and regulation of type II cell function is complex in nature and needs further investigation.

3.6 Interaction of SP-A with Phagocytes

Binding of SP-A to alveolar macrophages has been found to be calcium-, temperature- and concentration-dependent, with an apparent K_d of 2–4 nm (Kuroki et al. 1988; Wintergerst et al,1989). It is inhibitable by mannosyl-BSA, the CRD of SP-A, and the collagen region of C1q, suggesting the involvement of both collagenous and CRD regions (Wintergerst et al. 1989). Studies on the functional implications of SP-A binding to alveolar macrophages, neutrophils, peripheral blood monocytes, monocyte-derived macrophages, and bone-marrow derived macrophages have formed the basis for the SP-A role in pulmonary defense (Table 1).

SP-A increases intracellular calcium and inositol triphosphate (IP3) concentrations in alveolar macrophages. The calcium response correlates with IP3 generation, and is necessary for SP-A stimulated phagocytosis. SP-A also stimulates chemotaxis via cell interaction involving its collagen region. It also stim-

Table 1. Biological functions proposed to be mediated by SP-A

Surfactant homeostasis
Receptor-mediated inhibition of surfactant secretion from type II cells
Receptor-mediated enhanced uptake of surfactant phospholipids by type II cells
Surfactant biophysical activity
Enhanced phospholipid adsorption to the monolayer
Prevention of protein inhibition by proteinaceous pulmonary oedema
Maintenance of tubular myelin
Phospholipase A_2 inhibition
Myosin clearance
LPS clearance
Host defense functions
Microbial binding and aggregation
Macrophage and neutrophil activation and chemotaxis
Enhanced microbial phagocytosis and killing (via superoxidative burst)
Antiproliferative and antiinflammatory effects on lymphocytes
Modulation of allergic reactions

ulates directional actin polymerisation (Tino and Wright 1996). These two effects are preceded by receptor binding and transmission of intracellular signals. SP-A also modulates the production of several mediators of inflammation, such as cytokines (TNF-α, CSF) which control inflammatory response and recruitment of other immune cells and reactive species, leading to tissue damage (Tino and Wright 1998).

3.7 Interaction of SP-A with Pathogens and Allergens

SP-A has been shown to act as an opsonin for herpes simplex virus type I via alveolar macrophages. Deglycosylated SP-A does not bind to infected HEp-2 cells expressing viral proteins over the cell surface (Van Iwaarden et al. 1992). SP-A has been shown to bind IAV via its sialic acid residues and thereby neutralises the virus (by inhibiting virus-mediated agglutination of RBCs). Deglycosylation of SP-A completely prevents binding to IAV (H3N2)-infected HEp-2 cells. SP-A, but not SP-D, has been shown to bring about phagocytosis of H3N2 by rat alveolar macrophages (Benne et al. 1995). Preincubation of IAV with SP-A also enhances the ability of the virus to stimulate the respiratory burst of neutrophils (Hartshorn et al. 1997), however, without protecting neutrophils against virus-induced deactivation.

Increased attachment of SP-A-coated *Staphylococcus aureus* to macrophages has been reported, but this interaction does not lead to phagocytosis (McNeely and Coonrod 1993). SP-A enhances the binding and opsonisation of *E. coli* J 5 (containing *O*-antigen deficient rough LPS), but not *E. coli* O 111 (with *O*-antigen containing smooth LPS), to macrophages, suggesting that SP-A binding

to Gram-negative bacteria is dependent on LPS structure (Pikkar et al. 1995). SP-A has been reported to bind *Streptococcus pneumoniae* (Group A *Streptococcus* and Group B *Streptococcus*; Tino and Wright 1996). SP-A binds, aggregates, and promotes phagocytosis by macrophages of *H. influenzae*. Low binding to *H. influenzae* type b without agglutination and phagocytosis has also been reported (Tino and Wright 1996). SP-A binds Bacillus Calmette-Guerin (BCG) via CRDs and brings about phagocytosis by several types of phagocytic cells (Weikert et al. 1997). SP-A enhances phagocytosis, agglutination, and killing of *Klebsiella pneumoniae* K21a strain (capsule containing Man α1 Man sequences), either via opsonisation, or through activation of macrophages via the mannose receptor (Kabha et al. 1997). *Mycoplasma pulmonis* is considered to be involved in pneumonia and exacerbation of asthma and chronic obstructive pulmonary disease (COPD). SP-A binds, independent of calcium and sugar, and brings about phagocytosis and mycoplasmal killing by interferon-γ-activated murine alveolar macrophages. It also involves generation of peroxynitrite by alveolar macrophages (Hickman-Davis et al. 1999). SP-A also enhances phagocytosis of *M. tuberculosis* by a direct interaction with human monocyte-derived macrophages and human alveolar macrophages, possibly via upregulation of the macrophage mannose receptor activity. Carbohydrate moieties on SP-A seem important for this interaction. SP-A also enhances the attachment of *M. tuberculosis* to alveolar macrophages, and thus may contribute to the development of tuberculosis in patients with HIV infection (Downing et al. 1995).

SP-A contributes to the clustering of *P. carinii in vivo* by interacting with *gpA*, a mannose- and glucose-rich glycoprotein expressed on cysts and trophozoites of the pathogen (Crouch 1998). SP-A has recently been shown to bind and agglutinate, via CRDs, pathogenic unencapsulated *C. neoformans*, but not the capsulated forms (Schelenz et al. 1995). SP-A and SP-D have been shown to agglutinate *Aspergillus fumigatus* conidia and also to enhance binding, phagocytosis and killing of conidia by human alveolar macrophages and circulating neutrophils (Madan et al. 1997a). It appears that SP-A and SP-D may have an important immunological role in the early antifungal defense response in the lung, through inhibiting infectivity of the conidia by agglutination and by enhancing uptake and killing of conidia as well as hyphae of *A. fumigatus* by phagocytic cells.

SP-A has been reported to bind a variety of allergenic pollens such as Lombardy poplar, Kentucky blue grass, cultivated rye and short ragweed, via CRDs (Malhotra et al. 1993). SP-A and SP-D have been shown to bind whole mite extracts (*Dermatophagoides pteronyssinus*, *Derp*) and the purified glycoprotein allergens, in a carbohydrate-specific and calcium-dependent manner and inhibit specific IgE binding to allergens (Wang et al. 1996). SP-A and SP-D can also bind a range of allergens/antigens present in the 3-week culture filtrate of *A. fumigatus* and to purified allergens, *gp45* and *gp55*, and inhibit the ability of allergen specific IgE from aspergillosis patients to bind these allergens (Madan et al. 1997b). The blocking of IgE binding is probably mediated either

by steric hindrance posed by the surfactant molecules already bound to the allergens' carbohydrate structures, or by the recognition of the same binding site of both IgE and surfactant proteins. The possible protective roles played by SP-A and SP-D against airborne allergens are further supported by their ability to block allergen-induced histamine release from basophils isolated from patients having allergic broncho pulmonary aspergillosis (ABPA) and asthmatic children (Madan et al. 1997b; Wang et al. 1998). SP-A and SP-D have also been shown to have antiproliferative effects on peripheral blood mononuclear cells, which were isolated from asthmatic children and challenged with *Derp* allergens (Wang et al. 1998). It therefore appears that SP-A and SP-D may modulate the development of asthmatic symptoms by both inhibiting histamine release in the early phase of allergen challenge and suppressing lymphocyte proliferation in the late phase of asthmatic attacks where there is bronchial inflammation. The inhibitory effects of SP-A and SP-D against *Derp* and *A. fumigatus* allergens suggest a general defense role in the allergen sensitisation/ allergic reactions. Recently, IgE has been shown to have a central role in the induction of lung eosinophil infiltration and Th2 cytokine production (Coyle et al. 1996). Antigen-IgE complexes, bound to CD23, allow B cells to facilitate antigen presentation to antigen specific T cells, resulting in a greatly amplified T cell response. Thus, through their ability to inhibit IgE binding to allergens, SP-A and SP-D may represent a novel approach for the treatment of asthma (1) by preventing degranulation of mast cells, and also (2) by possibly inhibiting CD23/IgE-enhanced antigen processing and presentation to $CD4^+$ T cells and subsequent activation of Th2 cytokine production.

4 Surfactant Protein D (SP-D)

SP-D is one of the surfactant proteins found in the air space lining material in the lungs. Although it does bind to specific phospholipids in the lung surfactant, it shows quite different properties to those of the hydrophobic peptides, SP-B and SP-C, which are strongly associated with lipids. Pulmonary SP-D is produced by alveolar type II cells and nonciliated bronchiolar alveolar cells. However, SP-D may not be a lung specific protein since low levels of material antigenically similar to SP-D are found in normal human serum and, animal studies indicate the presence of SP-D, or SP-D like proteins, in gastric mucosa, tracheobronchial, lacrymal and salivary glands (Crouch 1998).

4.1 Molecular Structure and Assembly of SP-D

SP-D is composed of oligomers of a 130-kDa subunit formed from three identical polypeptide chains (43 kDa each) which have an *N*-linked oligosaccharide structure at Asn-70. As judged by electron microscopy, human SP-D is

assembled into a 520-kDa tetrameric structure with four of the 130-kDa, homotrimeric subunits linked via their N-terminal regions, but trimers, dimers and monomers of the 130-kDa subunit are also seen in SP-D preparations. The triple-helical arms in each 130-kDa subunit are approximately 46 nm in length and although appearing flexible, show no sharp bends or distribution which is consistent with there being no interruptions to the Gly-Xaa-Yaa repeat in the collagenous region of SP-D. Clusters of three CRDs are held together by the α-helical coiled-coil region found at the C-terminal end of the collagen-like triple helix present in each 130-kDa subunit. Up to eight of the 520-kDa tetrameric structures can undergo further oligomerization to give SP-D multimers having a large array, of up to 96 (8 × 12), CRDs (Crouch 1998). Each chain contains four distinct regions: a 25-residue-long N-terminal distinct region which contains cysteine residues involved in inter-chain disulphide bonding, a 177-residue-long collagen region, a 28-residue-long neck region, and a 125-residue-long CRD region. Unlike SP-A, the SP-D collagen region contains hydroxylysine and hydroxylysylglycosides. An important structural feature is the repeating heptad pattern of hydrophobic residues, in the 'a' and 'd' positions, seen in the α-helical coiled-coil neck region of the SP-D molecule. The alignment of these hydrophobic residues allows the formation of a self-associating triple-stranded parallel α-helical bundle which determines the trimeric orientation of the CRDs and possibly also acts as a nucleation point for triple-helix formation (Hoppe et al. 1994). The two cysteines at positions 15 and 20 are considered to be involved in the inter-chain disulphide bonding while the four within the CRD region are considered to be involved in intra-chain disulphide bonding.

4.2 Interaction of SP-D with Carbohydrate and Lipid Ligands

The order of preference of human SP-D in solid phase competition assays using maltosyl-BSA as the ligand is maltose >glucose, mannose, fucose >galactose, lactose, glucosamine >*N*-acetylglucosamine. SP-D is known to bind to the glucose-containing core oligosaccharides of LPS (Kuan et al. 1992), and mannose-rich *N*-linked oligosaccharides of the haemagglutinin of IAV and the *gpA* of *P. carinii*. SP-D shows high affinity binding to phosphatidylinositol (PI) and glucosyl-ceramide in a calcium- and sugar-dependent manner (Persson et al. 1992). Substituting Glu-321 to Gln and Asn-323 to Asp in the CRDs reverses the relative carbohydrate binding specificity from maltose >glucose >galactose to galactose >maltose, glucose. Phospholipid binding by SP-D appears to involve neck as well as CRD regions (Kishore et al. 1996).

4.3 Interaction of SP-D with Pathogens and Allergens

SP-D binds to carbohydrates/LPS on the surfaces of a variety of pathogens such as IAV, Gram-negative bacteria (*E. coli*, *Salmonella*, *Pseudomonas aeruginosa*,

K. pneumoniae) and fungal organisms (*C. neoformans*, *P. carinii*, *A. fumigatus*). The binding of SP-D to these organisms leads to a reduction in infectivity, agglutination and enhanced killing (Reid 1998).

SP-D CRDs bind the glycoconjugates expressed near the sialic acid binding site on the haemagglutinin (or neuraminidase) of specific strains of IAV (Hartshorn et al. 1996) and act as potent inhibitors of HA-mediated agglutination, thereby causing viral aggregation. SP-D is a more potent inhibitor of IAV infectivity than SP-A or MBL. The susceptibility of various IAV strains to neutralisation by SP-D directly correlates with specific differences in the number of glycoconjugates expressed on the HA (Reading et al. 1997). Inoculation of mice with IAV in the presence of mannan is known to increase viral replication, suggesting involvement of the CRDs.

SP-D binds to glycoconjugates (LPS) expressed by a variety of Gram-negative bacterial strains involved in lung pathogenesis, such as *K. pneumoniae*, *Ps. aeruginosa*, *H. influenzae*, and *E. coli*. In contrast to SP-A, SP-D binding to LPS and subsequent agglutination of bacteria is calcium-dependent and inhibitable by competing sugars, LPS and rough mutant forms of LPS (but not by lipid A; Kuan et al. 1992). SP-D does not recognise the capsular polysaccharides of *K. pneumoniae*, and the presence of a well-formed capsule limits interaction of SP-D with underlying LPS molecules. SP-D contributes to the clustering of *P. carinii in vivo* by interacting with *gpA*, a mannose- and glucose-rich glycoprotein expressed on cysts and trophozoits (Crouch 1998). SP-D has recently been shown to bind and agglutinate, via CRDs, pathogenic unencapsulated *C. neoformans* (but not the capsulated) forms (Schelenz et al. 1995).

4.4 SP-D Gene Organisation and Genetics

Human SP-D is encoded by a single gene with seven exons spanning >11 kb of DNA on the long arm of chromosome 10, at a locus on 10q22.2–23.1, which also includes the SP-A genes. The first protein-encoding exon (exon 2) includes sequences corresponding to the signal peptide, N-terminal region and first seven Gly-Xaa-Yaa triplets. The remainder of the collagen region (the total of 59 Gly-Xaa-Yaa repeats), is encoded by three exons of 117 bp each. The sixth exon encodes the coiled-coil neck region, whereas the seventh exon codes for the CRD.

4.5 SP-D Crystal Structure

The crystal structure of a trimeric fragment of human SP-D, composed of an α-helical coiled-coil 'neck' region and the three CRD domains, have recently been determined at 2.3 Å resolution (Hakansson et al. 1999). The fold of the CRD is similar to that of MBL. The novel central packing of one of the tyro-

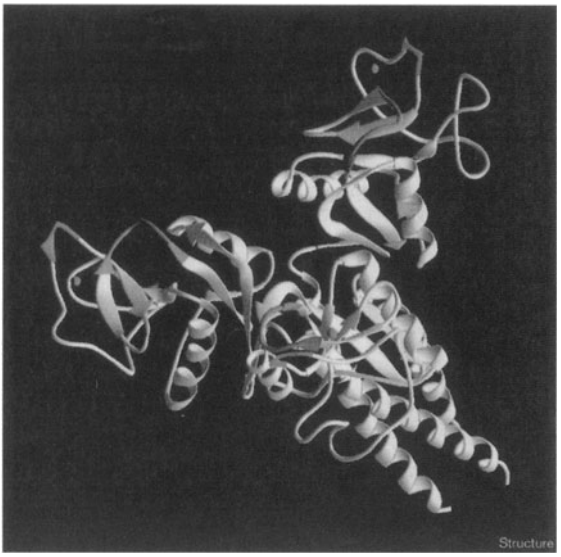

Fig. 4. Ribbon diagram showing the overall main chain structure of the trimeric α-helical coiled-coil and three lectin domains of human lung surfactant protein D. The positions of the three carbohydrate-binding calcium ions, one in each monomer, are shown as black spheres

sine side chains within the coiled-coil results in an asymmetric orientation of the CRDs. There are three calcium ions bound to each SP-D monomer (Fig. 4), one at the carbohydrate binding site and two in a second site previously described for MBL (Weis et al. 1992). The average distance between the two carbohydrate binding calcium ions is 51 Å. The position of the conserved calcium ligands in SP-D (Glu 321, Asn 323, Glu 329, Asn 341 and Asp 342) matches very closely with those of MBL-A. There is a central cavity between the three CRD domains which presents a positively charged surface, which has been suggested to be a putative interacting region for LPS, or cell surface receptors.

5 Cell Surface Receptors for Collectins

SP-A, MBL and conglutinin, but not SP-D, have been shown to bind to a molecule, designated as the cC1qR/collectin receptor, which is now generally regarded as being a membrane associated form of an intracellular, multifunctional Ca^{2+}-binding protein, called 'calreticulin' (Eggleton et al. 1997). However, given its primarily intracellular localisation and lack of membrane anchorage and signal transduction components, its candidature as a receptor molecule is debatable. The binding of C1q, SP-A and MBL, but not SP-D, to another cell surface molecule, defined as $C1qR_p$ has also been demonstrated (Nepomuceno

et al. 1997). *C1qR$_p$*, a 126-kD glycoprotein, is a novel type I membrane protein composed of a C-type CRD, five epidermal growth factor-like domains, a transmembrane domain, and a short cytoplasmic tail. It is expressed on the surfaces of monocytes/macrophages, neutrophils, endothelial cells, and microglia. *C1qR$_p$* is considered to mediate phagocytosis by binding to the collagen regions of C1q, MBL and SP-A. Since ligand–*C1qR$_p$* ligation triggers phagocytosis without inducing release of proinflammatory cytokines, further dissection of the *C1qR$_p$* system may provide a basis for antimicrobial therapy without an inflammatory response. It could be potentially useful in regulating the phagocytic capacity of myeloid cells (a prophylactic treatment for immunocompromised individuals at risk from infection). A putative SP-D receptor, *gp340*, appears to bind SP-D through its CRD regions in a calcium- dependent and sugar- independent manner (Holmskov et al. 1997). The *gp340* has been described as a new member of a scavanger receptor cysteine-rich superfamily containing multiple scavanger receptor type B domains. Although the interaction between *gp340* and SP-D involves the CRD region, the inability of maltose to block the interaction indicates a protein–protein interaction, rather than a carbohydrate–CRD interaction.

6 SP-A and SP-D Gene Knock-out Mice

Mice lacking SP-A mRNA and protein *in vivo* [designated SP-A (–/–)] have been generated using gene knock-out technology (Korfhagen et al. 1996). SP-A knock-out mice survive and breed normally, having normal levels of SP-B, SP-C and SP-D, phospholipid composition, secretion and clearance, and incorporation of phospholipid precursors. Lungs of SP-A (–/–) mice have markedly decreased tubular myelin figures. *P. aeruginosa* (Le Vine et al. 1998) and Group B *Streptococci* (Le Vine et al. 1999a) are cleared less efficiently than the wild-type mice. These mice are also more susceptible to respiratory syncitial virus (RSV) infection than the control mice (Le Vine et al. 1999b). These studies on SP-A knock-out mice demonstrate that SP-A has an important role in the innate immune system of the lung *in vivo*.

Mice bred after disruption of the SP-D gene have shown remarkable abnormalities in surfactant homeostasis and alveolar cell morphology. They also show a progressive accumulation of surfactant lipids and apoproteins in the alveolar space, hyperplasia of type II cells with massive enlargement of intracellular lamellar bodies, and an accumulation of alveolar macrophages (Botas et al. 1998). Thus, SP-D deficient mice resemble mice deficient in granulocyte-macrophage colony stimulating factor (GM-CSF; Dranoff et al. 1994). The SP-D receptor, *gp340*, which is a scavenger receptor, may serve as a route for internalisation of lipids in macrophages and may mediate the macrophage proliferation induced by exposure to oxidised low density lipoproteins. However, an association of SP-D and gp340, the GM-CSF pathway, and surfactant clearance remain to be investigated.

7 SP-A and SP-D in Human Diseases

The expression of SP-A and SP-D within the lung makes these collectins specific markers for lung diseases (Kuroki et al. 1998). The measurement of SP-A and SP-D in amniotic fluids and tracheal aspirates reflects lung maturity and the production levels of the lung surfactant in infants with respiratory distress syndrome (RDS). The SP-A concentrations in bronchoalveolar lavage (BAL) fluids are significantly decreased in patients with acute respiratory distress syndrome (ARDS) and also in patients at risk to develop ARDS. A significant increase of SP-A and SP-D in BAL fluids and sputum is diagnostic for pulmonary alveolar proteinosis (PAP). The BAL fluid from the alveolar proteinosis patients forms a very good source of human SP-A (Strong et al. 1998). The SP-A and SP-D concentrations in BAL fluids from patients with idiopathic pulmonary fibrosis (IPF; McCormack et al. 1995) and interstitial pneumonia with collagen vascular diseases (IPCD) are lower than those in healthy controls. SP-A and SP-D appear in the circulation in specific lung diseases, such as PAP, IPF, IPCD and ARDS (Kuroki et al. 1993). SP-A is also a marker for lung adenocarcinomas and can be used to differentiate lung adenocarcinomas from other types and metastatic cancers, and to detect metastasis of lung adenocarcinomas.

8 Bovine Collectins: Conglutinin (BC) and Collectin-43 (CL-43)

To date, conglutinin (BC) and collectin-43 (CL-43) have only been identified as being present in the serum of Bovidae. BC, the first mammalian lectin to be discovered, is known for its ability to agglutinate complement-coated erythrocytes – a reaction called 'conglutination'. Conglutination is brought about via binding of its CRDs to the complement component, iC3b, covalently attached to the erythrocytes (Hirani et al. 1985). BC is a cruciform-shaped tetramer of identical polypeptides, each being 44 kDa. It binds well to oligosaccharides containing nonreducing terminal GlcNAC. BC binds to the high mannose group on the α-chain of the complement degradation product iC3b – which contains the single high mannose oligosaccharide at Asn-917, covalently attached to another protein/carbohydrate via the activated thiol ester (Holmoskov and Jensenius 1996). This binding is inhibitable by GlcNAC and mannose. BC shows opsonising activity *in vitro* toward *Salmonella typhimurium* and *E. coli* (Friis-Christiansen et al. 1990). This activity depends on the presence of the complement system. It is likely that by binding to iC3b deposited on the bacterial surfaces, BC interacts with macrophages. Subcutaneous injection of BC has been shown to protect mice challenged with *Salmonella typhimurium* (Friis-Christiansen et al. 1990). BC also interacts with IAV, as shown by inhibition of virus agglutinating activity as well as by inhibition of infection *in vitro* (Hartley et al. 1992).

CL-43 (molecular mass of single polypeptide 31.5kDa) is known to exist mostly as monomer, comprising a 28-residue-long N-terminal region, 114 residues of a collagen region (38 Gly-Xaa-Yaa triplets), a 31-residues-long neck region, and a 128-residue-long CRD. CL-43 shows selectivity for L-fucose and mannose, similar to BC. No data concerning the biological role for CL-43 has yet been reported (Holmoskov and Jensenius 1996).

Acknowledgements. We wish to thank Alison Marsland for secretarial help.

References

Anders EM, Hartley CA, Jackson DC (1990) Bovine and mouse serum β-inhibitors of influenza A viruses are mannose-binding lectins. Proc Natl Acad Sci USA 87:4485–4489

Benne CA, Kraaijeveld CA, van-Strijp JA, Brouwer E, Harmsen M, Verhoef J, van Golde LM, van Iwaarden JF (1995) Interactions of surfactant protein A with influenza A viruses: binding and neutralization. J Infect Dis 171:335–341

Botas C, Poulain F, Akiyama J, Brown C, Allen L, Goerke J, Clements J, Carlson E, Gillespie AM, Epstein C, Hawgood S (1998) Altered surfactant homeostasis and alveolar type II cell morphology in mice lacking surfactant protein D. Proc Natl Acad Sci USA 95:11869–11874

Childs RA, Wright JR, Ross GF, Yuen CT, Lawson AM, Chai W, Drickamer K, Feizi T (1992) Specificity of lung surfactant protein SP-A for both the carbohydrate and the lipid moieties of certain neutral glycolipids. J Biol Chem 267:9972–9979

Coyle AJ, Wagner K, Bertrand C, Tsuyuki S, Bews J, Heusser C (1996) Central role of immunoglobulin (Ig) E in the induction of lung eosinophil infiltration and T helper 2 cell cytokine production: inhibition by a non-anaphylactogenic anti-IgE antibody. J Exp Med 183:1303–1310

Crouch EC (1998) Collectins and pulmonary host defense. Am J Respir Cell Mol Biol 19:177–201

Downing JF, Pasula R, Wright JR, Twigg H, Martin W (1995) Surfactant protein A promotes attachment of *Mycobacterium tuberculosis* to alveolar macrophages during infection with human immunodeficiency virus. Proc Natl Acad Sci USA 92:4848–4852

Dranoff G, Crawford AD, Sadelain M, Ream B, Rashid A, Bronson RT, Dickersin GR, Bachurski CJ, Mark EL, Whitsett JA (1994) Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis. Science 264:713–716

Drickamer K (1992) Engineering galactose-binding activity into a C-type mannose-binding protein. Nature 360:183–186

Eggleton P, Reid KBM, Kishore U, Sontheimer RD (1997) Clinical relevance of calreticulin in systemic lupus erythematosus. Lupus 6:564–571

Elhalwagi BM, Damodarasamy M, McCormack FX (1997) Alternate amino terminal processing of surfactant protein A results in cysteinyl isoforms required for multimer formation. Biochemistry 36:7018–7025

Ezekowitz RAB, Kuhlman M, Groopman JE, Byrn RA (1989) A human serum mannose-binding protein inhibits *in vitro* infection by the human immunodeficiency virus. J Exp Med 169: 185–196

Floros J, Hoover RR (1998) Genetics of the hydrophilic surfactant proteins A and D. Biochem Biophys Acta 1408:312–322

Friis-Christiansen P, Thiel S, Svehag SE, Dessau R, Svendsen P, Andersen O, Laursen SB, Jensenius JC (1990) *In vivo* and *in vitro* antibacterial activity of conglutinin, a mammalian plasma lectin. Scand J Immunol 31:453–460

Hakansson K, Lim NK, Hoppe HJ, Reid KBM (1999) Crystal structure of the trimeric α-helical coiled-coil and the three lectin domains of human lung surfactant protein D. Structure 7:255–264

Hartley CA, Jackson DC, Anders EM (1992) Two distinct serum mannose-binding lectins function as β-inhibitors of influenza virus: identification of bovine serum β-inhibitor as conglutinin. J Virol 66:4358–4363

Hartshorn KL, Sastry KN, White MR, Anders EM, Super M, Ezekowitz RAB, Tauber AI (1993) Human mannose-binding protein functions as an opsonin for influenza A viruses. J Clin Invest 91:1414–1420

Hartshorn KL, Reid KBM, White MR, Jensenius JC, Morris SM, Tauber AI, Crouch E (1996) Neutrophil deactivation by influenza A viruses: mechanisms of protection after viral opsonization with collectins and hemagglutination-inhibiting antibodies. Blood 87:3450–3061

Hartshorn KL, White MR, Shepherd V, Reid KBM, Jensenius JC, Crouch EC (1997) Mechanisms of anti-influenza activity of surfactant proteins A and D: comparison with serum collectins. Am J Physiol 273:L1156–L1166

Haurum JS, Thiel S, Jones IM, Fischer PB, Laursen SB, Jensenius JC (1993) Complement activation upon binding of mannan-binding protein to HIV envelope glycoproteins. AIDS 7: 1307–1313

Hickman-Davis J, Gibbs-Erwin J, Lindsey JR, Matalon S (1999) Surfactant protein A mediates mycoplasmacidal activity of alveolar macrophages by production of peroxynitrite. Proc Natl Acad Sci USA 96:4953–4958

Hirani S, Lambris JD, Müller-Eberhard HJ (1985) Localization of the conglutinin binding site on the third component of human complement. J Immunol 134:1105–1109

Holmskov U, Jensenius JC (1996) Two bovine collectins: conglutinin and CL-43. In: Ezekowitz RAB, Sastry KN, Reid KBM (eds) Collectins and innate immunity. Landes Company, Chapman and Hall, Austin, USA

Holmskov U, Lawson P, Teisner B, TornØe I, Willis AC, Morgan C, Koch C, Reid KBM (1997) Isolation and characterization of a new member of the scavenger receptor superfamily, glycoprotein-340 (gp-340), as a lung surfactant protein-D binding molecule. J Biol Chem 272: 13743–13749

Hoover RR, Floros J (1998) Organisation of the human SP-A and SP-D loci at 10q22-q23. Physical and radiation hybrid mapping reveal gene order and orientation. Am J Respir Cell Mol Biol 18:353–362

Hoppe HJ, Reid KBM (1994) Collectins – soluble proteins containing collagenous regions and lectin domains – and their roles in innate immunity. Protein Sci 3:1143–1158

Hoppe HJ, Barlow PN, Reid KBM (1994) A parallel three-stranded α-helical bundle at the nucleation site of collagen triple-helix formation. FEBS Lett 344:191–195

Iobst ST, Wormald MR, Weis WI, Dwek RA, Drickamer K (1994) Binding of sugar ligands to Ca^{2+}-dependent animal lectins. I. Analysis of mannose binding by site-directed mutagenesis and NMR. J Biol Chem 269:15505–15511

Jack DL, Dodds AW, Anwar N, Ison CA, Law A, Frosch M, Turner MW, Klein NJ (1998) Activation of complement by mannose-binding lectin on isogenic mutants of *Neisseria meningitidis* serogroup B. J Immunol 160:1346–1353

Kabha K, Schmegner J, Keisari Y, Parolis H, Schlepper-Schaeffer J, Ofek I (1997) SP-A enhances phagocytosis of *Klebsiella* by interaction with capsular polysaccharides and alveolar macrophages. Am J Physiol 272:L344–L352

Khoor A, Gray ME, Hull WM, Whitsett JA, Stahlman MT (1993) Developmental expression of SP-A mRNA in the proximal and distal respiratory epithelium in the human fetus and newborn. J Histochem Cytochem 41:1311–1319

Kishore U, Wang JY, Hoppe HJ, Reid KBM (1996) The α-helical neck region of human lung surfactant protein D is essential for the binding of the carbohydrate recognition domains to lipopolysaccharides and phospholipids. Biochem J 318:505–511

Kishore U, Eggleton P, Reid KBM (1997) Modular organisation of carbohydrate recognition domains in animal lectins. Matrix Biol 15:583–592

Korfhagen TR, Bruno MD, Ross GF, Huelsman KM, Ikegami M, Jobe AH, Wert SE, Stripp BR, Morris RE, Glasser SW, Bachurski CJ, Iwamoto HS, Whitsett JA (1996) Altered surfactant function and structure in SP-A gene targeted mice. Proc Natl Acad Sci USA 93:9594–9599

Kuan SF, Rust K, Crouch E (1992) Interactions of surfactant protein D with bacterial lipopolysaccharides. Surfactant protein D in an *E. coli*-binding protein in bronocho-alveolar lavage. J Clin Invest 90:97–106

Kuhlman M, Joiner K, Ezekowitz RAB (1989) The human mannose-binding protein functions as an opsonin. J Exp Med 169:1733–1745

Kuroki Y, Mason RJ, Voelker DR (1988) Alveolar type II cells express a high-affinity receptor for pulmonary surfactant protein A. Proc Natl Acad Sci USA 85:5566–5570

Kuroki Y, Tsutahara S, Shijubo N, Takahashi H, Shiratori M, Hattori A, Honda Y, Abe S, Akino T (1993) Elevated levels of lung surfactant protein A in sera from patients with idiopathic pulmonary fibrosis and pulmonary alveolar proteinosis. Am Rev Respir Dis 147:723–729

Kuroki Y, Takahashi H, Chiba H, Akino T (1998) Surfactant proteins A and D: disease markers. Biochem Biophys Acta 1408:334–345

LeVine AM, Kurak KE, Bruno MD, Stark JM, Whitsett JA, Korfhagen TR (1998) Surfactant protein-A-deficient mice are susceptible to *Pseudomonas aeruginosa* infection. Am J Respir Cell Mol Biol 19:700–708

LeVine AM, Kurak KE, Wright JR, Watford WT, Bruno MD, Ross GF, Whitsett JA, Korfhagen TR (1999a) Surfactant protein A binds group B *Streptococcus* enhancing phagocytosis and clearance from lungs of surfactant protein-A-deficient mice. Am J Respir Cell Mol Biol 20:279–286

LeVine AM, Gwozdz J, Stark J, Bruno M, Whitsett J, Korfhagen T (1999b) Surfactant protein-A enhances respiratory syncytial virus clearance in vivo. J Clin Invest 103:1015–1021

Lu J, Thiel S, Wiedemann H, Timpl R, Reid KBM (1990) Binding of the pentamer / hexamer forms of mannan-binding protein to zymosan activates the proenzyme $C1r_2$ $C1s_2$ complex, of the classical pathway of complement, without involvement of C1q. J Immunol 144:2287–2294

Madan T, Eggleton P, Kishore U, Strong P, Aggrawal SS, Sarma PU, Reid KBM (1997a) Binding of pulmonary surfactant proteins A and D to *Aspergillus fumigatus* conidia enhances phagocytosis and killing by human neutrophils and alveolar macrophages. Infect Immun 65:3171–3179

Madan T, Kishore U, Shah A, Eggleton P, Strong P, Wang JY, Aggrawal SS, Sarma PU, Reid KBM (1997b) Lung surfactant proteins A and D can inhibit specific IgE binding to the allergens of *Aspergillus fumigatus* and block allergen-induced histamine release from human basophils. Clin Exp Immunol 110:241–249

Madsen HO, Garred P, Kurtzhals J, Lamm LU, Ryder LP, Thiel S, Svejgaard A (1994) A new frequent allele is the missing link in the structural polymorphism of the human mannan-binding protein. Immunogenetics 40:37–44

Madsen HO, Garred P, Thiel S, Kurtzhals JA, Lamm LU, Ryder LP, Svejgaard A (1995) Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. J Immunol 155:3013–3020

Malhotra R, Haurum J, Thiel S, Jensenius JC, Sim RB (1993) Pollen grains bind to lung alveolar type II cells (A549) via lung surfactant protein A (SP-A). Biosci Rep 13:79–90

Matsushita M, Fujita T (1992) Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like serine protease. J Exp Med 176:1497–1502

McCormack FX (1998) Structure, processing and properties of surfactant protein A. Biochem Biophys Acta 1408:109–131

McCormack FX, Kuroki Y, Stewart JJ, Mason RJ, Voelker DR (1994) Surfactant protein A amino acids Glu195 and Arg197 are essential for receptor binding, phospholipid aggregation, regulation of secretion, and the facilitated uptake of phospholipid by type II cells. J Biol Chem 269:29801–29807

McCormack FX, King T, Bucher BL, Nielsen L, Mason RJ (1995) Surfactant protein A predicts survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 152:751–759

McNeely TB, Coonrod JD (1993) Comparison of the opsonic activity of human SP-A for *Staphylococcus aureus* and *Streptococcus pneumoniae* with rabbit and human macrophages. J Infect Dis 167:91–97

Nepomuceno RR, Henschen-Edan AH, Burgess WH, Tenner AJ (1997) cDNA cloning and primary structure analysis of $C1qR_p$, the human C1q/MBL/SPA receptor that mediates enhanced phagocytosis *in vitro*. Immunity 6:119–129

Ohata M, Okada M, Yamashina I, Kawasaki T (1990) The mechanism of carbohydrate-mediated complement activation by the serum mannan-binding protein. J Biol Chem 265:1980–1984
Persson AV, Gibbons BJ, Shoemaker JD, Moxley MA, Longmore WJ (1992) The major glycolipid recognised by SP-D in surfactant is phosphatidylinositol. Biochemistry 31:12183–12189
Pikkar JC, Voorhout WF, van Golde LMG, Verhoof J, Vanstrijp JAG, Van Iwaarden JF (1995) Opsonic activities of surfactant proteins A and D in the phagocytosis of Gram-negative bacteria by alveolar macrophages. J Infect Dis 172:481–489
Rademacher TW, Parekh RB, Dwek RA (1988) Glycobiology. Annu Rev Biochem 57:785–838
Reading PC, Morey LS, Crouch EC, Anders EM (1997) Collectin-mediated anti-viral host defense of the lung: evidence from influenza virus infection of mice. J Virol 71:8204–8212
Reid KBM (1998) Interactions of surfactant protein D with pathogens, allergens and phagocytes. Biochem Biophys Acta 1408:290–295
Sano H, Kuroki Y, Honma T, Ogasawara Y, Sohma H, Voelker DR, Akino T (1998) Analysis of chimeric proteins identifies the regions in the carbohydrate recognition domains of rat lung collectins that are essential for interactions with phospholipids, glycolipids, and alveolar type II cells. J Biol Chem 273:4783–4789
Sastry KN, Herman GA, Day L, Deignan E, Bruns G, Morton CC, Ezekowitz RA (1989) The human mannose-binding protein gene. Exon structure reveals its evolutionary relationship to a human pulmonary surfactant gene and localisation to chromosome 10. J Exp Med 170:1175–1189
Schelenz S, Malhotra R, Sim RB, Holmskov U, Bancroft GJ (1995) Binding of host collectins to the pathogenic yeast *Cryptococcus neoformans*: human surfactant protein D acts as an agglutinin for acapsular yeast cells. Infect Immun 63:3360–3366
Sherrif S, Chang CY, Ezekowitz RAB (1994) Human mannose-binding protein carbohydrate recognition domain trimerises through a triple α-helical coiled-coil. Nature Struct Biol 1:789–794
Strong P, Kishore U, Morgan C, Lopez Bernal A, Singh M, Reid KBM (1998) A novel method of purifying lung surfactant proteins A and D from the lung lavage of alveolar proteinosis patients and from pooled amniotic fluid. J Immunol Methods 220:139–149
Suzuki Y, Fujita Y, Kogishi K (1989) Reconstitution of tubular myelin from synthetic lipids and proteins associated with pig pulmonary surfactant. Am Rev Respir Dis 140:75–81
Thiel S, Vorup-Jensen T, Stover CM, Schwaeble W, Laursen SB, Poulsen K, Willis AC, Eggleton P, Hansen S, Holmskov U, Reid KBM, Jensenius JC (1997) A second serine protease associated with mannan-binding lectin that activates complement. Nature 386:506–510
Tino MJ, Wright JR (1996) Surfactant protein A stimulates phagocytosis of specific pulmonary pathogens by alveolar macrophages. Am J Physiol 270:L677–L688
Tino MJ, Wright JR (1998) Interaction of surfactant protein A with epithelial cells and phagocytes. Biochem Biophys Acta 1408:241–263
Turner MW (1996a) Mannose-binding lectin: the pleuripotent molecule of the innate immune system. Immunol Today 17:532–540
Turner MW (1996b) Functional aspects of mannose-binding protein. In: Ezekowitz RAB, Sastry KN, Reid KBM (eds) Collectins and innate immunity. Landes Company, Austin, USA
Van Helden HP, Kuijpers C, Steenvoorden D, Go C, Bruijnzeel PL, van Eijk M, Haagsman HP (1997) Intratracheal aerosolisation of endotoxin (LPS) in the rat: a comprehensive animal model to study adult (acute) respiratory distress syndrome. Exp Lung Res 23:297–316
Van Iwaarden JF, van Strijp JA, Visser H, Haagsman HP, Verhoef J, van Golde LM (1992) Binding of surfactant protein A (SP-A) to herpes simplex virus type 1-infected cells is mediated by the carbohydrate moiety of SP-A. J Biol Chem 267:25039–25043
Wang JY, Kishore U, Lim BL, Strong P, Reid KBM (1996) Interaction of human lung surfactant proteins A and D with mite (*Dermatophagoides pteronyssinus*) allergens. Clin Exp Immunol 106:367–373
Wang JY, Shieh CC, You PF, Lei HY, Reid KBM (1998) Inhibitory effect of pulmonary surfactant proteins A and D on allergen-induced lymphocyte proliferation and histamine release in children with asthma. Am J Respir Crit Care Med 158:510–518

Weikert LF, Edwards K, Chroneos ZC, Hager C, Hoffman L, Shepherd VL (1997) SP-A enhances uptake of bacillus Calmette-Guerin by macrophages through a specific SP-A receptor. Am J Physiol 272:L989–L995

Weis WI, Drickamer K (1994) Trimeric structure of a C-type mannose-binding protein. Structure 2:1227–1240

Weis WI, Kahn R, Fourme R, Drickamer K, Hendrickson WA (1991) Structure of the calcium-dependent lectin domain from a rat mannose-binding protein determined by MAD phasing. Science 254:1608–1615

Weis WI, Drickamer K, Hendrickson WA (1992) Structure of a C-type mannose-binding protein complexed with an oligosaccharide. Nature 360:127–134

Weis WI, Taylor ME, Drickamer K (1998) The C-type lectin superfamily in the immune system. Immunol Rev 163:19–34

Wintergerst E, Manz-Keinke H, Plattner H, Schlepper-Schafer J (1989) The interaction of a lung surfactant protein (SP-A) with macrophages is mannose dependent. Eur J Cell Biol 50:291–298

Wright JR (1997) Immunomodulatory functions of surfactant. Physiol Rev 77:931–962

Zimmerman PE, Voelker DR, McCormack FX, Paulsrud JR, Martin WJD (1992) The 120 kDa surface glycoprotein of *Pneumocystis carinii* is a ligand for surfactant protein A. J Clin Invest 89:143–149