CHAPTER 6

Lung Surfactant Proteins A and D as Pattern Recognition Proteins

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Abstract

Lung surfactant proteins A and D belong to a group of soluble humoral pattern recognition receptors, called collectins, which modulate the immune response to microorganisms. They bind essential carbohydrate and lipid antigens found on the surface of microorganisms via low affinity C-type lectin domains and regulate the host's response by binding to immune cell surface receptors. They form multimeric structures that bind, agglutinate, opsonise and neutralize many different pathogenic microorganisms including bacteria, yeast, fungi and viruses. They modulate the uptake of these microorganisms by phagocytic cells as well as both the inflammatory and the adaptive immune responses. Recent data have also highlighted their involvement in clearance of apoptotic cells, hypersensitivity and a number of lung diseases.

Introduction

Nonclonal germline encoded pattern recognition receptors (PRR) that bind common essential constituents of pathogenic organisms such as lipopolysaccharide, peptidoglycan, lipoteichoic acid, mannan, bacterial and viral DNA or RNA, and fungal glucans were first proposed by Charles Janeway in the early 1990s.[1,2] These broad-spectrum innate immune molecules are thought to be a first line of defense against invading pathogens, involved in controlling pathogen number and minimizing tissue damage caused by the inflammatory response, while allowing time for activation of the adaptive immune response. Since then, a large number of PRR's have been described and can be classified either functionally as endocytic or signaling, or by their subcellular localization: cytosolic, membrane-bound or soluble.[2-6] One family of predominantly soluble PRRs is called collectins, which bind carbohydrate and lipid structures found on microbial surfaces.[5-8] These oligomeric molecules are characterized by the presence of four structural elements: a short cysteine containing N-terminus, a collagen domain, a coiled-coil domain and a C-terminal Ca^{2+} dependent C-type lectin domain that, apart from collectin placenta-1 (CL-P1), have a preference for mannose type monosaccharide subunits. At present nine mammalian collectins have been described: mannan-binding lectin (MBL), collectin liver-1 (CL-L1[9]), collectin placenta-1 (the only membrane-bound collectin; CL-P1 [10,11]), collectin kidney-1 (CL-K1[12]), surfactant protein A (SP-A[13]), surfactant protein D (SP-D[14,15]), conglutinin, collectin of 43 kD (CL-43[16]), and collectin of 46 kD (CL-46[17]).

SP-A and SP-D are two of the best characterized of these collectins. Apart from their homeostatic role in the regulation of lung surfactant, they are effective PRRs, employing a variety of mechanisms to kill, or reduce the infectivity of different pathogens. Initially these include

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Target Pattern Recognition in Innate Immunity, edited by Uday Kishore.

bacteriostatic and fungistatic mechanisms, agglutination of bacteria, fungus, and viral particles, opsonization, and enhancement of phagocytosis. As the adaptive immune response matures the collectins direct the helper T-cell polarization, and can alter the activity of a variety of other immune cells depending on the pathogen involved.[18]

In this chapter we will briefly describe the domain structure of mammalian surfactant proteins A and D and look at the details of the active sites which give the molecules their fine specificities that enable them to carry out their different effector functions. We will highlight the range of microorganisms and immune receptors they interact with and illustrate the different mechanisms by which they help clear pathogens and carry out their roles as pattern recognition receptors.

Collectin Structure Overview

Members of the collectin family have specific single chain architecture (Fig. 1). They have a short N-terminal segment of 7 to 25 amino acids that includes 1 to 3 cysteine residues followed by a Gly-X-Y rich collagen-like sequence of between 66 and 177 residues long. A single exon encodes the short 30 amino acid neck region which is followed by the carbohydrate recognition domain (CRD; ~125 amino acids). These individual chains form trimers in all collectins and can form higher order multimers via disulphide bonds. They are divided into two groups based on their multimeric structure, MBL and SP-A have a sertiform or 'bouquet-of-tulips' structure with six trimers, while conglutinin, CL-46 and SP-D form a second group that assembles into a cruciform-like structure (Fig. 2). SP-D can form even higher-order multimers, so-called fuzzy balls, with a mass of several million kDa.[19]

N-Terminal Region

The N-terminal noncollagenous sequence of collectins shows limited similarity within the family and no homologous regions have been found in other proteins. However the conservation of the cysteine residues indicates that they may play an important role in collectin structure and function. Mutation of the cysteine residues within the N-terminal region of SP-D to serine residues led to the formation of trimers only. However, more importantly, the transgenic mice with these two mutations developed a phenotype similar to the SP-D-/- mice with emphysema and foamy macrophages, highlighting not only their importance in the multimerization of the trimers of SP-D, but also the vital role the quaternary structure of SP-D plays in lung homeostasis.[20]

It is thought that in order to form multimers of the trimeric subunits, at least two cysteine residues have to be present in the N-terminal domain. This view is supported by the fact that CL-L1, which has only one cysteine residue in this domain, exists as a trimer.[9] However, CL-43 is also secreted as a trimer, despite having two N-terminal cysteine residues that are found in exactly the same positions as in the highly multimerized SP-D. Others have demonstrated with chimeric collectins that the N-terminal cysteine rich domain along with the collagen domain determines their level of mutlimerization.[21-24] This was confirmed by the SP-A allele SP-A1. It contains a cysteine residue in its collagen region (residue 85) that permits inter-trimer disulphide bond formation and the creation of larger multimers than the SP-A2 allele, which lacks this essential cysteine.[25]

Collagen Region

The collagen regions of SP-A and SP-D, which form triple helices, are thought to be aligned by their trimerising neck regions.[26,27] Each of the three chains forms a left-handed polyproline II like helix that are coiled in a right-handed manner with the glycine residues in the interior of the helix. Interchain hydrogen bonds between N-H groups of glycine and the C=O groups of the amino acid in the X position of the Gly-X-Y motif stabilize the structure. Interruptions in the Gly-X-Y triplet within the collagen domain results in a destabilization of the collagen region, and can introduce a hinge point as can be seen in SP-A (see Fig. 2).[28,29]

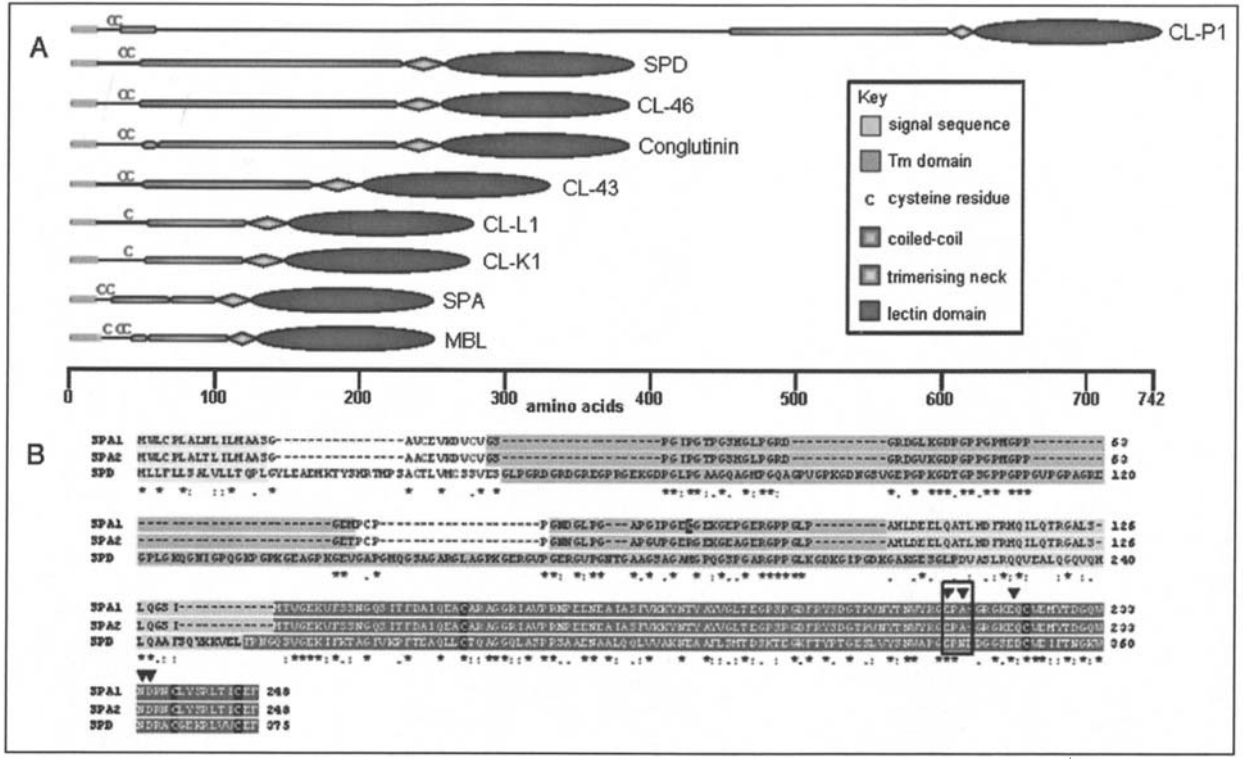

Figure 1. A) Schematic overview of the domain architecture of the mammalian collectins. The conserved N-terminal cysteine residues are coloured red, the collagen domain is blue, the neck domain is green and the lectin domain is red. B) A ClustalW alignment of SP-A1, SP-A2 and SP-D, colour coded in a similar manner to (A). The five residues involved in the coordination of the calcium ion are marked with an arrow-head (▼), the four conserved lectin domain cysteine residues are coloured in dark red and the tripeptide involved in saccharide specificity is boxed. A color version of this figure is available online at www.landesbioscience.com/curie.

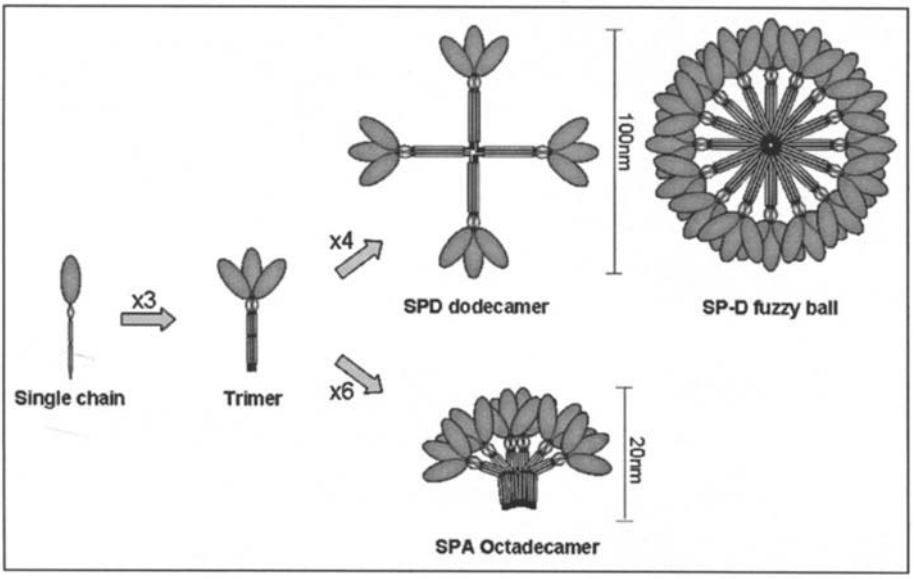

Figure 2. The single chain architecture of the collectins is composed of a cysteine containing N-terminus, a collagen-like region, a trimerising neck domain and spherical calcium dependent sugar binding domain. All collectins can form trimers. SP-D can further multimerize into dodecamers and fuzzy balls, while SP-A has a sertiform shape.

The collagen domain of collectins is thought to have several distinct functions. As previously mentioned, it is involved in determination of the mutlimericity of the different collectins, which has significant effects on its role in lung homeostasis. It also separates trimers to allow the cross-linking of microorganisms, leading to their subsequent aggregation and neutralization.[21] Another interesting structural feature of the collagen domain is their glycosylation,[26,27] which has important immune implications as the carbohydrate moiety of SP-A provides sialic acids that act as competitive targets for Herpes simplex virus (HSV) and Influenza A virus (IAV) binding, reducing viral infectivity and expediting their removal by macrophage and neutrophils.[30] Finally, CL-P1 was shown to bind negatively charged molecules including oxidized LDL via the exposed positive charges of the collagen domain.[31] It is not clear if the soluble collectins can bind other molecules in a similar fashion.

Neck Region

The collagen region is attached to the CRD via a short sequence of approximately 30 residues.[32] This sequence is characterized by conserved hydrophobic residues in a pattern oXooXoooXooXoooXooXoooXooXooo, where o denotes any residue; X denote hydrophobic residue like Leu, Val, Phe or Tyr. Each copy of this sequence forms an alpha helix and three alpha helices form a coiled-coil bundle structure that is held together via interhelix hydrophobic interactions.[32] As previously mentioned the neck domain is considered to assist in formation of collagen like triple helices and also trimerises the CRD's. Recombinant proteins consisting only of the neck and CRD region are still assembled as trimers, whereas isolated CRDs lacking the neck domain are secreted as monomers[33,34] or in the case of MBL as dimers.[35]

Carbohydrate Recognition Domain (CRD)

The highly conserved calcium-dependent lectin domain found at the N-terminus of all collectins is composed of about 125 amino acids and has a characteristic motif that includes 3

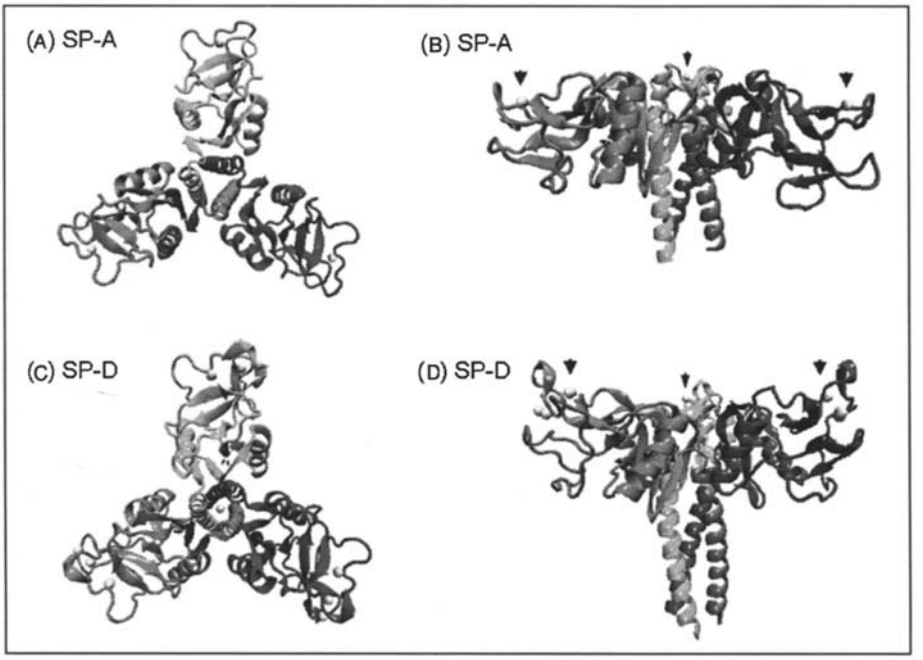

Figure 3. Ribbon diagrams of the neck and CRD domains of SP-A (A,B) and SP-D (C,D). Figures B and D are rotated 90° from figures A and C respectively. The individual chains are coloured red, green and blue, with the calcium ions, one in SP-A and 4 in SP-D coloured yellow. The sugar binding sites are highlighted with arrow-heads. A color version of this figure is available online at www.landesbioscience.com/curie.

of the 4 cysteine residues that form the intradomain disulphide bonds (see Fig. 3 for X-ray crystal structures of the CRD and neck region of SP-A and SP-D).[36] Within this motif is a tripeptide, which despite their broad monosaccharide specificity is thought to divide C-type lectins into mannose/glucose-type or galactose-type lectins (see Table 1 for a summary of the relative carbohydrate affinities of the collectins). These conserved residues are Glu-Pro-Asn at positions 239-241 in SPD and Glu-Pro-Ala at positions 212-214 in human SPA. Although Ala is found at position 3 in this motif in human SP-A or Arg at the same position in rat SP-A, they still have a preference for mannose over galactose, indicating that the conservation of the last amino acid of the triplet is not critical in determining relative saccharide affinity. In fact apart from the membrane bound CL-P1, which has a motif predicted to bind galactose (Gln-Pro-Asp), all collectins have mannose-type CRDs.[31] The specificity of CL-P1 was confirmed by showing a soluble form of its CRD bound GalNAc-conjugated gel in a Ca^{2+}-dependent manner, and this binding was inhibited by free GalNAc, L-, D-fucose, D-galactose, and lactose.[37] In addition to these studies, substitution of the motif of SP-A or SP-D with Gln-Pro-Asp changes their CRD specificity from mannose-type to galactose- type.[36,38] CL-L1 has Ser at position 3 of the triplet, but the effect on its monosaccharide specificity is not known.

The molecular basis on which the CRDs discriminate between mannose- and galactose-type ligands lies in the presentation of two vicinal hydroxyl groups on the 3' and 4' position of the sugar ring of hexoses. For ligand binding in mannose-type CRDs, these hydroxyl groups need to have an equatorial position, whereas for high affinity binding by galactose-type CRDs, at least one of the hydroxyl groups have to be placed axially. Mannose-type CRDs are thought to bind fucose in a slightly different manner, as this molecule has equatorial hydroxyl groups on

Table 1. The mammalian collectin family gene names, chromosomal location, size, expression pattern, carbohydrate preferences and structure

Collectins*	Gene Name Chromosome	Residues in Monomer	Mol. wt (Da)	Expression and Distribution	Monosaccharide Preference	Oligomeric Structure
Conglutinin (bovine)	CGN1 28q1.8-1.9	371	37,973	Liver, plasma	GlcNac>Man, Fuc Glc>ManNac, Mal	Tetramer of trimers (cruxiform)
Collectin-43 (bovine)	CL-43 28q1.8-1.9	321	33,607	Liver, plasma	Man>ManNac>Fuc, glcNac>glc, Mal>Gal	Monomeric and trimeric
Collectin-46 (bovine)	CL-46 28q1.8-1.9	371	37,450	Liver, thymus, plasma	Man>ManNac>Fuc, GlcNac>Glc, Mal>Gal	Monomeric and trimeric
MBL	COLEC1 10q11.2-q21	248	26,143	Liver, plasma and amniotic fluid	GlcNac>Fuc, Man, ManNac>Mal>Glc>Gal	Hexamer of trimers (C1q like)
SP-A	SFTPA (2 expressed genes, 1 pseudogene) 10q22.2-q23.1	248	26,242	Type 2 alveolar cells, clara cells, lungs, amniontic fluid, GIT, generalized mucosal occurrence	ManNac>Fuc, Mal> Glc>Man>Gal, GlcNac	Hexamer of trimers (C1q like)
SP-D	SFTPD 10q22.2-q23.1	375	37,728	Type 2 alveolar cells, clara cells, lung, amniotic fluid, GIT, generalized mucosal occurrence	Mal>Man, Glc>Lac, Gal>GlcNac>Fuc	Tetramer of trimers (cruxiform)
Collectin-L1 (Liver)	COLEC10 8q23-q24.1	277	30,733	Ubiquitous, mainly liver, placenta adrenal gland, cytosolic only.	weakly binds Gal, Man, Glc, GlcNac	At least trimeric
Collectin-K1 (Kidney)	COLEC11 (4 splice variants) 2p25.3	271	28,660	Ubiquitous, high in kidney, liver, small intestine, thymus, sc, placenta, adrenal gland, placenta, plasma	Fuc> weak Man	Trimers and larger on nonreducing PAGE.
Collectin-P1 (Placenta)	COLEC12 18pter-p11.3	742	81,516	Mainly placenta, retina, and also mammary gland, heart, skeletal muscle, lung, tonsils, predominantly cytosolic	GalNac>weak Fuc>Gal	Likely to be trimeric

*Homo sapiens unless otherwise indicated. GlcNac: N-acetyl glucosamine; Fuc: Fucose; Glu: Glucose; Man: Mannose; Gal: Galactose; GalNac: N-acetyl galactosamine Lac: Lactose; ManNac: N-acetyl mannosamine; Mal: Maltose.

its 2' and 3' positions of the sugar ring which in molecular models can be superimposed on the hydroxyl groups at the 3' and 4' position of the sugar ring of mannose.[39,40] In addition to fucose, α-D-glucose also appears, by molecular modeling, to dock into the SP-D CRD via vicinal equatorial hydroxyl groups on the 2' and 3' position of the sugar ring.[41] Even though MBL has a low affinity for galactose, there are X-ray crystal structures of MBL complexed with galactose. They show that the sugar is coordinated via its equatorial hydroxyl groups at positions 1' and 2'. This excludes the possibility of MBL binding to galactose residues that are part of galactosides, as the hydroxyl group at the 1' position is involved in glycosidic bonding.[40]

SP-A and SP-D Bind Lipid via Their CRDs

The pulmonary surfactant molecules also recognize specific lipids via their CRDs. SP-A binds to DPPC[42] and galactosylceramide, while SP-D associates with phosphatidylinositol[43,44] and glucosylceramide.[45,46] The CRD of SP-A appears important in the association of SP-A with tubular myelin, a unique structure formed by secreted surfactant phospholipids.[47] The lipid binding capabilities of the surfactant proteins may be of greater importance in their homeostatic role in lung surfactant rather than as PRRs.[48,49]

Calcium Ions Are Required for CRD Function

Although the CRDs are calcium dependent, both for their correct folding and physiological function, the number of calcium ions bound to each CRD under physiological conditions is as not clear. A different number of $Ca2^+$ ions are present in X-ray crystal structures of SP-A and SP-D depending on the concentration of $Ca2^+$ employed during their crystallization.[50] Three $Ca2^+$ ions are commonly found in X-ray crystal structures of the CRD of SP-D while a fourth calcium ion has been reported in the presence of 2.5 mM calcium in the funnel formed by the three CRDs and close to the neck–CRD interface.[51] It is known that the Ca2+ ion coordinated by Glu321, Asn323, Glu329, Asn341 and Asp342 is the active site calcium involved in binding sugar residues,[35,39,40,50] but the function of the other two calcium ions located near the active site is not known (see Fig. 4 for the location of the calcium ions and the carbohydrate binding site of SP-A and SP-D). There are indications that at least one of them is involved in the correct folding of the CRD, while others have attempted to show by computer simulation that they may be involved in binding extended ligands. Docking studies of SP-D showed that flanking saccharide residues in trisaccharides form additional hydrogen bonds with amino acids outside the CRD binding pocket, and thereby contribute to overall binding energy.[41,51,52] But the majority of the X-ray crystal structures show interaction solely between the two equatorial hydroxyl groups on a single monosaccharide, even though many of them are extended structures.[50] Shrive et al showed that the fourth calcium ion, located at the top of the funnel between the three monomers causes a change in charge distribution between Glu232 and Lys246, which allows a conformational change that opens the cleft between the CRDs and the neck. They hypothesized that this may be important in binding either ligands or immune cells.[51]

Factors Affecting Interaction with Polysaccharides

Collectins bind the (poly)saccharide ligands present on the surface of microorganisms via their CRDs. The affinity of an individual CRD binding a monosaccharide is low (in the order of 10^{-3} M), hence the concerted binding of two or more CRDs is necessary for biologically relevant interactions. Collectin trimers and higher-order multimers bind to polyvalent ligands in the order of 10^{-8} or 10^{-11} M, respectively.[34] These interactions depend on the density of carbohydrate ligands on the microbial surface, the degree of oligomerization of the collectin and the flexibility of both.[23] As collectins predominantly bind terminal saccharides, their position within the carbohydrate structure and their chemical bonding are also important. The collectins can bind either multiple monosaccharides on a single glycolipid or glycoprotein, or individual saccharide moieties present on different surface ligands.

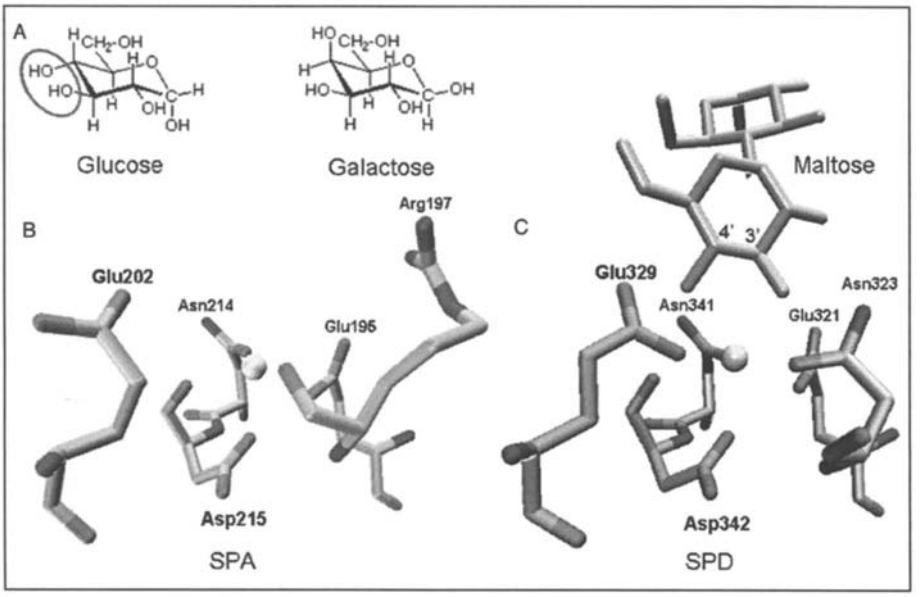

Figure 4. A) The equatorial 3′ and 4′ hydroxyl groups that are bound by SP-A and SP-D are highlighted in red in the glucose molecule. Galactose has an axial hydroxyl group at the 4′ position. Line diagrams of the sugar binding site of SP-A (B) and SP-D with a maltose in the active site (C) highlight the residues involved in coordinating the active site calcium ions. There is no difference in the position of the side-chains with and without bound sugar in SP-D, but water molecules complete the calcium ion coordination in the unbound structure (not shown). A color version of this figure is available online at www.landesbioscience.com/curie.

As we have described in the structure of the collectins, SP-D and SP-A form multimeric structures with at least 12 or 18 chains respectively, and can form much higher order polymers that allow higher affinity binding to pathogens. Genetic studies have highlighted allelic variations that can influence the quantity and mutlimericity of SP-D produced in the serum. These changes have differential effects on binding. A change at position 11 in the coding region of SP-D from methionine to threonine resulted in the production of low levels of only monomeric SP-D in the serum of homozygotes. As predicted by their structure their SP-D binding to intact influenza A virus, Gram-positive and Gram-negative bacteria was weaker, but surprisingly it did bind isolated LPS better than native SP-D.[53] Polymorphisms in receptor molecules that bind SP-A or SP-D are associated with an increased risk of infection as well as variety of disorders including autoimmune diseases, asthma, and atherosclerosis.[54-56]

The X-ray crystal structures of SP-A and SP-D show no interaction between individual CRDs, but there is substantial interaction between the CRD and neck regions, hinting at limited mobility of this region. However, electron microscopy pictures of dodecameric SP-D and conglutinin revealed great flexibility of the trimeric subunits within higher-order multimers.[57] It is thought that the kink in the collagen stalk of SP-A provides it with additional flexibility to bind microbial surfaces and is necessary for lung lipid homeostasis.[58] Also, it is well known that carbohydrates are relatively mobile structures. They are generally removed for crystallography studies, and NMR studies have shown them to have considerable flexibility, which may be of importance for collectin binding.

Finally, carbohydrate binding of collectins is usually to terminal carbohydrate residues. However, recently SP-D has been shown to bind to nonterminal glucosyl residues of polysaccharides, and binding was shown to be dependent on the nature of the glycosidic linkage

between monosaccharide units, as the hydroxyl groups on the 2' and 3' or on the 3' and 4' position had to be available to dock into the CRD.[41,59] It is not known if the ability to bind internal saccharide units is a property of all collectins, or specific to SP-D.

Directing the Immune Response

As innate pattern recognition molecules SP-A and SP-D are first line defense molecules that attempt to regulate the immune response in an appropriate manner against a multitude of pathogens (see Table 2 for a list of microbes that SP-A and/or SP-D have been shown to interact with). They are capable of stimulating or dampening the inflammatory response.

They can reduce the inflammatory response in many ways: by binding to membrane bound PRR and altering their activity or surface expression, by binding inhibitory regulatory proteins, reducing C1q mediated complement activity and aiding in the removal of cellular debris. SP-A binds CD14 on alveolar macrophages and inhibits its binding to smooth LPS, reducing the production of the pro-inflammatory cytokine TNF-α.[60] Similarly, by binding to TLR2, SP-A can inhibit its activation by peptidoglycan and zymosan.[61,62] Both SP-A and SP-D bind the signal inhibitory regulatory protein alpha (SIRPα), which stops pro-inflammatory mediator production via the activation of the tyrosine phosphatase SHP-1 and its subsequent blockade of signaling through src-family kinases and the p38 MAP kinase. Recent studies suggest that SP-D, and SP-A to a lesser extent, binds to the DNA on the surface of apoptotic cells as well as bacterial cellular fragments. They act as opsonins speeding up the clearance of dying cells and cellular debris by phagocytic cells.

As activators of the inflammatory response the lung collectins act similarly, by binding to surface receptors and changing their activity or expression. Although SP-A inhibits the activation of CD14 by smooth LPS, it enhances the inflammatory response induced by rough LPS through the same receptor. It binds rough LPS via its CRD and can interact with CD14 via its neck domain forming an LPS-SP-A-CD14 complex, which is thought to activate the receptor and increase the expression of TNF-α. Although, a direct interaction between SP-A and TLR-4 has not been reported, a study by Guillot et al, 2002, demonstrated that SP-A induced the NF-κB pathway and induced TNF-α secretion both of which were critically dependent on a functional TLR-4 complex.[63] The collagenous tails of SP-A and SP-D were also shown to induce pro-inflammatory responses including stimulating phagocytosis via an interaction with calreticulin/CD91 after binding to foreign material or damaged cells. SP-A and SP-D are involved in increasing the number of PRR on the surface of different immune cells including the mannose receptor on monocyte derived macrophage, and the scavenger receptor A (SR-A) on alveolar macrophage after exposure to S. pneumoniae, which increases their phagocytic capabilities.[64-66] A list of receptors or binding proteins that interact with SP-A and SP-D can be found in Table 3.

Thus, the lung surfactant proteins interact with many different microorgamisms and can activate or dampen the immune response depending on the pathogen type, quantity and the state of activation at the site of infection. The roles of SP-A and SP-D as PRRs against three different pathogens are described in more detail below in order to highlight differences both in their functions and the specific surfactant-pathogen interactions, which can lead to different outcomes.

Specific Examples of SP-A and SP-D as PRRs

Bacteria

There is a wealth of information on the interaction of the pulmonary surfactant proteins with different pathogenic organisms (Table 2). They predominantly bind surface glycoproteins via their CRDs. For example, SP-A and SP-D bind both rough and smooth forms of LPS although to different sites on the rough LPS molecule: SP-A interacts with the lipid-A moiety of LPS, whereas SP-D binds to the LPS core saccharides. SP-A binds to *Haemophilus influenza*

Table 2. SP-A and SP-D bind a variety of fungi, bacteria, yeast and viruses

	SP-A	SP-D
Fungus/Yeasts		
Acapsular Cryptococcus neoformans	√	√
Aspergillus fumigatus	√	√
Candida albicans	+/-	+/-
Encapsulated Cryptococcus neoformans	+/-	√
Histoplasma capsulatum	√	√
Pneumocystis carinii	√	+/-
Saccharomyces cerevisae	x	√
Gram-negative bacteria		
Enterobacter aerogenes		
Klebsiella pneumoniae	√	√
Chlamydia trachomatis	√	√
Chlamydia pneumoniae	√	√
Escherichia coli	√	√
Haemophilus influenzae	√	x
Helicobacter pylori	x	√
Pseudomonas aeruginosa	+/-	√
Gram-positive bacteria		
Alloiococcus otitis	√	x
Bacillus subtilis	x	√
Group A Streptococcus	√	x
Group B Streptococcus	√	x
Staphylococcus aureus	√	√
Streptococcus pneumoniae	+/-	√
Type 25 pneumococci	√	x
Mycobacteria		
Mycobacterium avium	+/-	√
Mycobacterium bovis (BCG)	√	x
Mycobacterium tuberculosis	√	√
Mycoplasma		
Mycoplasma pneumoniae	√	√
Mycoplasma pulmonis	√	x
Viruses		
Adenovirus	√	x
Herpes simplex virus	√	x
Human immunodeficiency virus	√	√
Influenza A virus	√	√
Respiratory syncytial virus	+/-*	√
Rotavirus	x	√

√: The collectin in question binds the microorganism. *: Binding occurs here, but there is a question over its effect on RSV infection. x: No information on binding between the collectin and the microorganism, or they do not interact. +/-: Disagreement on whether binding between the collectin and the microorganism occurs or not.

not via its LPS, but instead via its glycosylated major outer membrane protein P2,[59] while lipoteichoic acid (LTA) of *Bacillus subtilis* and peptidoglycan of *Staphylococcus aureus* represent ligands on Gram-positive bacteria for SP-D, but not for SP-A. SP-D also binds to *Mycoplasma pneumoniae* via interactions with its membrane glycolipids.[67]

Table 3. Receptors and binding proteins for SP-A and SP-D

Receptor/Binding Protein	Interacting Protein and Domain	Cells Expressing the Receptor	Proposed Functions
56 kDa	SP-A	Monocytes, macrophages, neutrophils, endothelial cells, platelets, microglia	Phagocytosis
C1qRp (CD93)	SP-A (collagen region)	Monocytes, macrophages, neutrophils, endothelial cells, platelets, microglia	Originally proposed to have a role in phagocytosis
SPR210	SP-A (36 residue long collagen region containing RGD motif)	Bone marrow derived macrophages, alveolar macrophages, Type II cells, peripheral blood mononuclear cells	Phagocytosis, regulation of phospholipid secretion, regulation of cytokine production, inhibition of T-cell proliferation, antibody to the receptor inhibits T cell mediated responses against Mtb
55 kDa	SP-A	Alveolar Type II cells	Phospholipid uptake
SIRP_α (CD 172)	SP-A, SP-D (CRD)	Myeloid cells, neurons, endothelial cells, B cells, antigen presenting cells, macrophages	Inhibition of pro-inflammatory cytokine production, suppression of clearance of apoptotic cells
Calreticulin and CD91	SP-A, SP-D (collagen region)	Ubiquitous	Phagocytosis and production of pro-inflammatory cytokines
gp340	SP-A, SP-D (CRD region)	Soluble opsonin	Stimulation of alveolar macrophage migration; an opsonin
CD14	SP-A (neck region) binds the peptide portion containing the leucine-rich repeats of CD14; SP-D (CRDs) binds the carbohydrate moiety of CD14	Alveolar macrophages	Modulation of the CD14-LPS interaction and probably release of pro-inflammatory cytokines

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Table 3. Continued

Receptor/Binding Protein	Interacting Protein and Domain	Cells Expressing the Receptor	Proposed Functions
TLR-2	SP-A, SP-D	Monocytes/macrophages, dendritic cells, epithelial cells, B cells	Inhibits activation by peptidoglycan and zymosan
TLR-4/MD-2	SP-A	Monocytes/macrophages, dendritic cells, epithelial cells, B cells	Attenuated cell surface binding of smooth LPS and smooth LPS-induced NF-kappaB activation
TLR-2/TLR-4	SP-D	Monocytes/macrophages, dendritic cells, epithelial cells, B cells	-
TLR-2/TLR-4	SP-A	Macrophages	Up-regulate TLR2 expression; dampens TLR2 and TLR4 signaling by decreasing the phosphorylation of IkappaBα
Desmin and Vimentin	SP-A	Myometrial cells	Inhibits in vitro the polymerization of desmin filaments
$\alpha_2\beta_1$ integrin	SP-A	Peritoneal mast cells	
p63 (CKAP4/ERGIC-63/CLIMP-63)	SP-A	alveolar Type II cells	Inhibition of ATP-stimulated phospholipid secretion

The effect of these different interactions depends both on the pathogen and the collectin involved. To highlight both the different roles played by SP-A and SP-D as innate immune molecules as well as some unique mechanisms that pathogens have developed to avoid their elimination we attempt to collate the information available on their role in the protection against three pathogens: Mycobacterium tuberculosis (Mtb), *Pneumocystis carinii* and IAV.

Mtb, the airborne etiological agent of pulmonary tuberculosis, is one of the leading causes of death in the developing world due to an infective agent.[68] This pathogen of mononuclear phagocytes attempts to attach to, and enter alveolar macrophage as an initial step in its life cycle. It encounters this cell within the complex mixture of lipids (90%) and proteins (10%) that make up pulmonary surfactant. The two major hydrophilic surfactant proteins, SP-A and SP-D, bind the bacterium when it enters lung surfactant, but these interactions lead to very different outcomes.

Mtb attempts to enter AM through an interaction of its surface glycoproteins e.g., lipoarabinomannan (LAM) or alanine- and proline-rich antigenic glycoprotein (APA) with the macrophage mannose receptor.[69] It is believed that SP-A, the most abundant surfactant protein, binds Mtb via α1-2 linked mannosyl residues on two of its major envelope lipoglycans, LAM and lipomannan (LM), with a Kd of 10^{-7}-10^{-9} M.[68,70] SP-A is thought to act as a chemoattractant for macrophage, and to upregulate the mannose receptor (MR; CD14) on the macrophage surface, facilitating the attachment of LAM on the Mtb surface to the MR. Entry via this receptor is thought to prevent phagosome-lysosome fusion, providing a permissive environment for bacterial growth. It does not stimulate the production of intracellular oxidative species.[71] However, when SP-A is bound to Mtb, the effective reduction in free SP-A may reduce its interaction with SIRPα and activate inflammation via the production of the proinflammatory cytokines IL-6, IL-8, IL-1b and TNF-α.[72,73] The SP-A-mtb complex may also promote inflammation by presenting clustered collagen tails to the calreticulin receptor (CD91).

At the same time the second surfactant PRR, multimeric SP-D, also binds Mtb via the terminal mannosyl residues of LAM, but with a greater affinity than SP-A (in the order of 10^{-10} M).[74] This binding leads to agglutination of the bacteria, and a reduction of Mtb uptake and growth within AM.[75] Both binding and agglutination have different protective effects. SP-D binding to both Mtb and to the MR reduces infectivity by reducing the bacteria-macrophage interaction. This leads to a reduction in bacterial uptake and a decline in bacterial multiplication within AM. However, Mtb can be taken up via other macrophage receptors e.g., gp340 or complement receptors (CR1 or CR3), permitting phagosome-lysosome fusion and its degradation.[76] This still does not lead to the production of intracellular oxidative species. The agglutination is not thought to enhance phagocytosis, but may aid mucociliary clearance.

The level of SP-A in the lungs may be a key regulator of inflammation upon Mtb infection. Patients with HIV have increased SP-A levels, which is thought to reduce pulmonary inflammation and increase pathogen burden, while patients with pulmonary tuberculosis have reduced SP-A levels and initiate an inflammatory response augmenting pathogen clearance.[77]

Fungus and Yeast

The number of immunocompromised individuals worldwide is increasing due to HIV infection, transplants and treatment with steroids or chemotherapy.[78] These patients cannot clear opportunistic pathogens such as *Pneumocystis carinii*; hence there has been a renewed interest in the molecules involved in regulating the immune response to these pathogens.[79-81] It is known that SP-A and SP-D bind a variety of fungi and yeast via at least two different types of surface molecule. Structural polysaccharides that consist of repetitions of the same oligosaccharide, for example the *Saccharomyces cerevisiae* cell wall component mannan and glycosylated proteins like gp55 and gp45 found on the surface of *Aspergillus fumigatus*.

Here we examine the role of SP-A and SP-D against one of these opportunistic pathogens: *P. carinii*. After entry into the upper airways, it forms a proteinaceous foamy exudate that includes lipids and surfactant proteins, which can restrict respiration and lead to pneumonia.

SP-A (Kd: 10^{-9} M) and SP-D bind to *P. carinii* via the surface glycoprotein gp120 (also known as gpA, gp95 or major surface glycoprotein).[78,82,83] SP-D aids its attachment to alveolar macrophage, but does not seem to increase its uptake,[84] while SP-A reduces *P. carinii* binding to AM and hence its phagocytosis,[85] but may potentiate its binding to alveolar epithelium.[83] Their effects in vivo have been examined in two mouse models: SP-A-/- mice and SP-D-/- mice, both of which required the depletion of CD4+ cells to develop disease.[80,81] Without $CD4^+$ depletion only the SP-A-/- mice showed some lung burden after *P. carinii* infection. These SP-A-/- CD4-depleted mice did show a greater lung burden, an increase in numbers of alveolar macrophage, inflammation and lung injury.[80,81] The SP-D-/- CD4-depleted mice showed a more rapid onset in disease, with increased lung burden and inflammation, but the levels were similar to wt CD4-depleted mice after 4 weeks. Both SP-A and SP-D modified the production of oxidative species. The higher oxides, which are involved in pathogen killing, were reduced in the lungs of the SP-A-/- mice, while the higher levels of all oxidative species in the lungs of the SP-D-/- animals was thought to increase lung injury. These models also highlight the interdependence of SP-A and SP-D. A reduction in the post-infection level of SP-D in the lungs of the SP-A-/- mice was thought to be due to the lower IL-4 and IL-5 levels, while the SP-D-/- mice had a 40-50% decrease in SP-A levels at baseline. These observations emphasize the difficulty in separating the effects of these two proteins during *P. carinii* infection, while demonstrating that they are involved in its clearance both during the early stage of infection, modifying the inflammatory response, and later regulating the adaptive immune response.

Viruses

Generally, SP-A and SP-D are thought to bind viruses or virally infected cells in a manner that involves an interaction between their CRDs and surface-exposed glycoproteins that contain oligosaccharides of the high-mannose type. Their different modes of binding and their roles in defense against viral pathogens are highlighted here in their interaction with IAV.[86]

The IAV enters the human host via mucosal surfaces, predominantly the lungs. Its envelope protein hemagglutinin binds to sialic acids that are typically linked α2-6 or α2-3 to galactose residues on surface glycoproteins of lung epithelia. After their uptake and replication the majority of particles bud-off the apical surface and reenter the alveolar airspace without killing the epithelial cell. As the inflammation develops, the release of cytokines enhances a profound infiltration of neutrophils and macrophage into the lungs. The neutrophils release defensins and reactive oxidative species in an attempt neutralize and kill the pathogens, but their large numbers and the quantity of oxidative species released are thought to cause lung damage.

SP-A and SP-D strongly agglutinate and neutralize viral particles via different mechanisms. SP-D binds via its CRD to the oligosaccharide moieties of viral envelope proteins including hemagglutinin and neuraminidase, while IAV binds the sialic acid residues on the carbohydrate moiety of SP-A.[86,87] Although SP-A2 has greater activity than SP-A1 in almost all aspects of SP-A function, both isoforms perform equally well as soluble competitive targets for hemaglutinin binding.[88] Since SP-A and SP-D interact with IAV in different ways, they agglutinate and neutralize different strains of IAV. Further highlighting the roles of SP-A and SP-D in host defense against IAV infection the SP-A-/- and SP-D-/- animals have increased viral titres, increased neutrophil influx, inflammation and increased reactive oxidative species in their lungs compared to their wild-type counterparts, with SP-D-/- animals more strongly affected.[89,90] IAV inhibit neutrophil activity and augments its apoptosis, but SP-D reduces this inhibition.[91,92] It also increases IAV uptake by neutrophils, without altering the release of reactive oxidative species.

Although we still do not fully understand the mechanism that has led to the death of millions of people due to the different influenza pandemics, the research on SP-A and SP-D is shedding some light on these key innate molecules and the mechanisms they use to clear viral pathogens (Table 4).

Table 4. Effector Mechanisms of SP-A and SP-D as pattern recognition molecules

Mechanism	Effect
Microbiostatic	SP-A and SP-D increase the surface permeability of bacterial pathogens. They increase the lag time of fungal growth and inhibit hyphal and pseudohyphal outgrowth. They inhibit bacterial, fungal and viral entry into host tissue.
Aggregation/Agglutination	SP-A and SP-D bind to the surface glycoproteins and cross-link pathogens.
Opsonization and Phagocytosis	The binding of SP-A and SP-D on the surface of bacteria, fungus, yeast and viral particles enhances the respiratory burst and pathogen uptake by macrophage and neutrophils.
Adaptive immune modulation	SP-A and SP-D bind allergens, which has the effect of inhibiting IgE binding, reduces B and T lymphocyte proliferation, suppresses histamine release from basophils and mast cells, and directs the polarization of T cells towards Th1. Modulate the maturation of dendritic cells. SP-A binds C1q, blocking its binding to C1r and C1s, reducing complement mediated damage.
Chemotaxis	The chemotactic domains (for phagocytes) can be localized to the CRD for SP-D and the collagen tail for SP-A.

SP-A and SP-D in Protection against Allergens and Pulmonary Hypersensitivity

Owing to their presence in the lung, SP-A and SP-D, were investigated for their role in allergic hypersensitivity. Being lectins in nature, initial studies explored their affinity towards glycosylated allergens. SP-A and SP-D can bind via their CRD region to allergenic extracts derived from pollen grains,[93] house dust mite,[94] and *Afu*.[95] This binding resulted in inhibition of specific IgE binding to allergens, allergen-induced histamine release from sensitized basophils[94,96] and proliferation of PBMC's isolated from mite-sensitive asthmatic children.[97] Madan et al observed that Asp f1, a nonglycosylated major allergen of *A. fumigatus*, showed binding to SP-A and SP-D leading to inhibition of ribonuclease activity of Asp f1 (unpublished data). Recently Deb et al, 2007, showed that two mite allergens with cysteine protease activity degrade SP-A and SP-D.[98]

Another set of studies are on levels of SP-A and SP-D in murine models and allergic patients. Cheng et al, 2000, reported increased levels of surfactant protein A and D in bronchoalveolar lavage fluids in patients with bronchial asthma.[99] However, Wang et al, 2001 showed that murine model of asthma had decreased levels of SP-A and SP-D.[100] Atochina et al, 2003 associated attenuated AHR of C57BL/6 mice in comparison to Balb/c mice to the 1.5 fold increased levels of SP-D in C57Bl/6 mice.[101] Schmeidl et al, 2005 showed that SP-A specifically decreases in allergen sensitized and provocated rat lungs.[102] Erpenbeck et al, 2006, showed that allergen challenge of patients resulted in increased BAL levels of SP-D and correlated with eosinophil numbers but not with levels of IL-5 and IL-13.[103] These patients however, showed a decrease in SP-A on allergen challenge.

In vivo therapeutic trials of SP-A, SP-D, and rhSP-D in murine models of *Afu*-, mite- or ovalbumin- induced pulmonary hypersensitivity yielded interesting results.[100,104-106] *Afu* is an opportunistic fungal pathogen that is most commonly implicated in causing both

IgE-mediated and non IgE-mediated hypersensitivity in immunocompetent human subjects, leading to development of allergic bronchopulmonary aspergillosis (ABPA). ABPA is clinically characterized by episodic bronchial obstruction, positive immediate skin reactivity, elevated *Afu*-specific IgG and *Afu*-specific IgE antibodies in serum, peripheral and pulmonary eosinophilia, central bronchiectasis, and expectoration of brown plugs or flecks. Other important features of ABPA are activated Th2 cells and asthma, and patients may develop localized pulmonary fibrosis at later stages of the disease. The murine model resembles the human disease immunologically, exhibiting high levels of specific IgG and IgE, peripheral blood and pulmonary eosinophilia, and a Th2 cytokine response.

Intranasal administration of SP-A, SP-D, and rhSP-D using 3 doses on consecutive days significantly lowered eosinophilia and specific IgG and IgE antibody levels in the mice. This therapeutic effect persisted up to 4 days in the SP-A-treated ABPA mice and up to 16 days in the SP-D- or rhSP-D-treated ABPA mice. Lung sections of the ABPA mice showed extensive infiltration of lymphocytes and eosinophils, which were considerably reduced following treatment with SP-D or rhSP-D. The levels of IL-2, IL-4, and IL-5 were decreased, while IFN-γ levels increased in supernatants of the cultured spleen cells, suggesting a shift in the cytokine profile from pathogenic Th2 to protective Th1 response.[107] Thus, SP-A and SP-D appear to suppress the Th2 responses, probably via their ability to modulate functions of antigen-presenting cells, such as macrophages[106] and dendritic cells,[108-110] which may eventually lead to an induction of IL-12-dependent Th1 responses. In addition, a significant inhibition of nitric oxide production has been reported when alveolar macrophages, derived from Derp mice, are preincubated with rhSP-D, resulting in reduced levels of TNF-α in the rhSP-D treated Derp mice.[111]

Very recently, Mahajan et al, 2008, have reported that SP-D induces apoptosis in activated eosinophils and significantly increased phagocytotic clearance of the apoptotic eosinophils.[112] Since IgE cross-linking, histamine release, lymphocyte proliferation, persistent activated eosinophilia and antigen presentation are central steps in the development of allergic asthma, the possibility of using exogenous SP-A and SPD (or their recombinant fragments) as therapy for allergic disorders merits further investigation.[34,107]

Phenotype and Susceptibility of SP-A and SP-D Gene Deficient Mice

The distinct phenotypes and variable susceptibility of SP-A-/-, SP-D-/- and SP-A-/-SP-D-/- mice to allergens and pathogens have elucidated the distinct roles of these two collectins in airway remodeling, inflammation and host defense.

SP-A Gene Deficient Mice

Mice lacking SP-A mRNA and protein in vivo, generated by gene knock-out technology, survive and breed normally. They have normal levels of SP-B, SP-C, and SP-D, phospholipid composition, secretion and clearance, and incorporation of phospholipid precursors. Although there is a complete absence of tubular myelin in SP-A-/- mice, it does not appear to have a significant physiologic effect.[113,114] SP-A-/- mice show increased bacterial proliferation and systemic dissemination following intratracheal inoculation with Group B *Streptococci*, and defective clearance of *S.aureus*, *P. aeruginosa* and *K. pneumoniae*.[115] These mice also show increased susceptibility to RSV, *M. pneumoniae*, and pneumocystis than the wild-type mice. Killing of group B *Streptococcus* and *H. influenza* is significantly reduced in SP-A-/- mice, which is accompanied by increased inflammation of the lung, decreased oxidant production, and decreased macrophage phagocytosis.[79,89] SP-A-/- mice exposed to LPS have elevated levels of TNF-α and these levels get normalized on exogenous administration of SP-A, confirming an association of SP-A and the control of inflammation.[116,117] Zhang et al 2005, reported two SP-A-sensitive *P. aeruginosa* mutants exhibited reduced susceptibility in SP-A gene deficient mice.[118] Later in 2007, they showed that a flagellar-deficient *P. aeruginosa* mutant harbors inadequate amounts of LPS required to resist membrane permeabilization by SP-A and was

preferentially cleared by the SP-A+/+ mice, but survived in the SP-A-/- mice.[119] These studies on SP-A-/- mice essentially reaffirm the role of SP-A as an important regulator of pulmonary infection and inflammation.

SP-D Gene Deficient Mice

Mice, bred after disruption of the SP-D gene (SP-D -/- mice), have shown remarkable abnormalities in surfactant homeostasis and alveolar cell morphology. The SP-D-/- mice exhibit a progressive accumulation of surfactant lipids and apoproteins in the alveolar space, hyperplasia of alveolar Type II cells with massive enlargement of intracellular lamellar bodies, and an accumulation of foamy alveolar macrophages.[120] These mice spontaneously develop emphysema and fibrosis of the lungs, which suggests continuous inflammatory reaction associated with abnormal oxidant metabolism and MMP activity.[49] Expression of SP-D in adult lungs restored alveolar SP-D levels and corrected pulmonary lipid abnormalities but emphysema persisted.[121] The SP-D-/- phenotype appears to share selected features of both GM-CSF-/- as well as GM-CSF over-expressing phenotypes.[122-124] Experiments involving cross out-breeding of compound heterozygous mice have suggested that the mechanisms underlying the alveolar surfactant accumulation in the SP-D-/- and GM-CSF-/- mice are different, and that GM-CSF might mediate some of the changes associated with macrophages and Type II cells seen in SP-D-/- mice.[125-127] SP-D deficiency results in a low SP-A pool size, rapid conversion from large-aggregate to small-aggregate surfactant, increased uptake into alveolar Type II cells and recycling.[49,120]

When exposed to 80% or 21% oxygen, SP-D-/- mice had 100% survival vs. 30% in SP-D+/+.[128] Biochemically, in contrast to SP-D+/+, SP-D-/- mice had higher levels of surfactant phospholipid and SP-B accompanied by a preservation of surfactant biophysical activity. From a multiplex assay of nine cytokines, we found elevated levels of IL-13 in BAL fluid of normoxic SP-D-/- mice compared with SP-D+/+. SP-D-/- mice infected with bacteria or viruses have increased lung inflammation compared with infected wild-type strains, suggesting an anti-inflammatory role for SP-D. SP-D-/- mice show a decreased clearance of RSV, an increase of recruitment of inflammatory cells, and a reduced level of phagocytosis and oxygen radical production by alveolar macrophage.[89]

When wild-type, C1q-/-, SP-A-/-, and SP-D-/- mice were compared for their ability to clear exogenously instilled apoptotic PMN, only SP-D altered apoptotic cell clearance from the naive lung, emphasizing a major role for SP-D in the clearance of apoptotic and necrotic cells in vivo.[129] Thus, SP-A-/- and SP-D-/- mice have distinct phenotypes with respect to microbial challenge and the inflammatory response. This is further evident by the distinct response of SP-A and SP-D gene deficient mice to allergen challenge. SP-D-/- mice show higher intrinsic hyper-eosinophilia and a several fold increase in levels of IL-5 and IL-13, with a lowering of the IFN-γ to IL-4 ratio in the lungs in comparison to SP-A-/- and wild type mice.[130] Similarly, Haczku et al, 2006 also showed that mice lacking SP-D had increased numbers of CD4+ cells with elevated IL-13 and thymus- and activation-regulated chemokine levels in the lung and showed exaggerated production of IgE and IgG1 following allergic sensitization.[131] The hyper-eosinophilia and Th2 predominance is partly reversible by treating SP-A-/- or SP-D-/- mice with SP-A or SP-D, respectively.[130] SP-D-/- mice are more susceptible than wild-type mice whereas SP-A-/- mice have been found to be nearly resistant to *A. fumigatus* sensitization. Intranasal treatment with SP-D or rhSP-D can rescue the *A. fumigatus* sensitized SP-D-/- mice, while SP-A treated *A. fumigatus* sensitized SP-A-/- mice show several fold elevated levels of IL-13 and IL-5, resulting in increased pulmonary eosinophilia and damaged lung tissue. This suggests differential mechanisms involved in SP-A and SP-D mediated resistance to allergen challenge. Hypereosinophilia exhibited by both SP-A-/- and SP-D-/- mice, probably due to significantly raised levels of IL-5 and IL-13 in these mice, suggests that SP-A and SP-D have a role in regulating eosinophil infiltration

and modulation in the lung in response to environmental stimuli. It is interesting to note that similar to SP-D-/- mice, IL-13 over-expressing mice have characteristic foamy macrophages, Type II cell hypertrophy, fibrosis, massive inflammation involving eosinophilia, protease-dependent acquired emphysema, and AHR.[132] Given the involvement of IL-13 in processes such as mucus production and AHR, as well as eosinophil survival, activation and recruitment, it is likely that certain physiological effects in SP-A-/- as well as SP-D-/- mice arise due to over-expression of IL-13.[130,132]

In another study involving a murine model of lung inflammation induced by ovalbumin, SP-D-/- mice have been shown to express increased BAL eosinophils, IL-13 and IL-10, and lowered IFN-γ at early time points (1–3 days) compared with wild-type mice.[133] Ovalbumin-induced TLR- 4 expression in the lungs has been shown to be increased in the wild-type, but not in SP-D-/- mice. Spleen cells, when stimulated in vitro, showed increased lymphocyte proliferation and reduction in IFN-γ in the SP-D-/- mice. These studies highlight that SP-D is involved in the early resistance to allergen challenge and its deficiency leads to default Th2 response. A recent study by Brandt et al, 2008, showed that SP-D gene deficient mice have increased CD4+ T cell, macrophage and neutrophil levels in bronchoalveolar lavage fluid, increased large airway mucus production and lung CCL17 levels. However, 4- to 5-week-old SP-D-/- mice showed significantly lower levels of IgG1 and IgE and splenocytes of these mice on anti-CD3/CD28 stimulation released significantly less IL-4 and IL-13 ($P < .01$)[134]. After intranasal allergen exposures, a modest decrease in bronchoalveolar lavage fluid eosinophilia and IL-13 levels was observed in association with decreased airway resistance.

SP-A-/-SP-D-/- Mice

Mice deficient in both SP-A and SP-D genes (double knock-out) show a progressive increase in BAL phospholipid, protein, and macrophage content through 24 weeks of age.[135] The double knock-out phenotype is characterized by the excessive accumulation of surfactant lipid in the alveolar space, increased numbers of foamy alveolar macrophages with up-regulation of MMP-12, and emphysema.[135] The absolute increase in macrophage number and the extent of MMP up-regulation by macrophages was greater in the double knock-out mice compared with the SP-D-/- mice. Jung et al 2005 showed that A-/-D-/- mice in comparison with wild-type, have fewer and larger alveoli, an increase in the number and size of Type II cells, as well as more numerous and larger alveolar macrophages.[136] More surfactant-storing lamellar bodies are seen in Type II cells, leading to a threefold increase in the total volume of lamellar bodies per lung, but the mean volume of a single lamellar body remains constant. These results demonstrate that chronic deficiency of SP-A and SP-D in mice leads to parenchymal remodeling, Type II cell hyperplasia and hypertrophy, and disturbed intracellular surfactant metabolism. Using SP-A-/-, SP-D-/- and double knock-out mice, Hawgood et al, 2004, have shown that SP-D, but not SP-A, is important in restricting IAV replication and spread in vivo, which is crucially dependent on the glycosylation of residue 165.[137]

Conclusion

The pulmonary collectins SP-A and SP-D not only function as regulators of lung surfactant, but also as innate pattern recognition molecules. They bind pathogenic microorganisms predominantly via their multimeric low-affinity Ca^{2+}-dependent lectin domains that cause their agglutination, opsonization and clearance via phagocytosis. They modulate the immune system via interactions with membrane bound PRRs and other receptors on immune cells, which can lead to an increase or decrease in inflammation depending on the microbe involved. They also act to clear up cellular debris and dying cells in order to reduce inflammation and help avoid autoimmune pathology.

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