

# Surfactant Collectins and Innate Immunity

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## Key Words

Lung · Pulmonary surfactant · Innate immunity · Collectins · Fungi · Bacteria · Viruses · Alveolar macrophages · Immunomodulation · Surfactant therapy

## Abstract

Respiratory pathogens encounter various lines of defenses before infection of the host is established. The innate immune response represents an important first-line protection mechanism against potentially pathogenic microorganisms during early stages of infection of the naive host. Important players in this host defense system are ‘collectins’, a class of soluble innate immune proteins. Well-characterized members of the collectin family are the surfactant proteins A (SP-A) and D (SP-D). These collectins are expressed in the lung and also in extrapulmonary mucosal tissues. Collectins are secreted as multimers resulting in trimeric clustering of the lectin domains which enables recognition of evolutionary conserved sugar patterns present on the surface of a large variety of pathogens. Binding to collectins may lead to direct agglutination and neutralization of pathogens, to opsonization in order to present bound microbes directly to phagocytes, to modulation of the inflammatory response and to regulation of dendritic cell and T cell functions. In pulmonary tissue, this early acute-phase-like response can be regarded as a crucial layer of protection against a vast array of pathogens that escape the physical barriers and threaten to infect the delicate respiratory epithelium. An important clinical application may be the inhalation, or instillation of collectin-based drugs as part of surfactant therapy, to prevent and treat infectious and inflammatory diseases of newborn infants.

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## Introduction

A major function of pulmonary surfactant is to reduce surface tension at the air-liquid interface of the alveoli, thereby preventing alveolar collapse at end-expiration. Pulmonary surfactant is a mixture of lipids (90%) and proteins (10%). The surfactant lipids are mainly phospholipids (90%), of which phosphatidylcholine represents 70–80%. The major phospholipid component of lung surfactant is dipalmitoylphosphatidylcholine, which is responsible for the low surface tension at end-expiration [1]. Besides the two hydrophobic surfactant proteins, SP-B and SP-C, which are important for the adsorption and spreading of the surfactant film at the air-liquid interface [2], pulmonary surfactant comprises two hydrophilic proteins SP-A and SP-D [3–5].

Innate immunity provides a first-line defense against potential pathogens, bridging the interval between exposure to the pathogen and the specific response of the adaptive immune system. The importance of this evolutionary ancient defense system cannot be overestimated and is particularly very important in newborn infants where the adaptive immune system has not been developed yet. The innate immune system may be divided into a recognition system (sensing or afferent arm) and an effector system (efferent arm). Members of the former system comprise membrane-bound molecules like Toll-like receptors (TLRs) and lectin receptors such as DC-SIGN. SP-A and SP-D, members of the ‘collectin family’, and also small antimicrobial peptides, such as defensins, are effector molecules of the innate immune system. These latter molecules play an important role in the protection of mucosal surfaces of the body. Effector molecules ex-

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press either direct antimicrobial activity, or may facilitate the elimination of infectious agents by phagocytic cells. Furthermore, the cellular and molecular constituents of the innate immune defense are not only important for rapid killing and clearance of pathogens, but they also play a critical role in fine-tuning the inflammatory response in such a way, that containment of infection is sufficient while damage to the delicate respiratory epithelium is averted. Thus, the innate immune system comprises a variety of antimicrobial and anti-inflammatory molecules produced by cells of the upper and lower respiratory tract. The pulmonary surfactant proteins SP-A and SP-D are important players of this innate immune system of the lung. Their properties and functions are discussed below.

### SP-A and SP-D

SP-A and SP-D are synthesized and secreted by epithelial type II cells of the alveoli and by nonciliated bronchiolar cells (Clara cells) [6]. In Clara cells, both proteins are found in secretory granules and can potentially be secreted in the acute-phase reaction upon challenge with infectious agents. Therefore, it is assumed that SP-A and SP-D are also involved in innate host defense of the upper airways. Although SP-A and SP-D are referred to as 'lung collectins', especially SP-D is also expressed in many other tissues including gastric and intestinal mucosae [7, 8]. The expression of SP-D at many extrapulmonary mucosal sites suggests that this collectin plays a more general role in local innate host defense.

The primary structure of a collectin is composed of four domains: (1) a cysteine-rich N-terminal domain, (2) a collagenous domain, (3) an  $\alpha$ -helical neck domain and (4) a lectin or carbohydrate recognition domain (CRD), which mediates the calcium-dependent carbohydrate binding. The basic structural unit of a collectin is a trimer, either composed of three identical polypeptides or a combination of two identical polypeptides and a closely related monomer (fig. 1).

In general, these trimers multimerize to varying degrees, resulting in differently shaped multimeric forms. In the case of SP-D, trimers assemble into cruciform dodecamers which extend the spatial range of their CRDs considerably. SP-D can assemble into even higher-order multimers, which are often referred to as fuzzy ball-like structures. SP-A multimerizes into octadecamers, in which the six trimers are arranged into a bouquet-shaped hexamer, resembling the complement protein C1q. The

collagen domain is composed of repeating tripeptide motifs of Gly-X-Y, where X or Y can be any amino acid, but most frequently proline or hydroxyproline. In SP-A, the collagen domain is interrupted, resulting in a kink that causes the trimers to angle away from the central core (fig. 1). As the affinity of a single CRD for carbohydrates is low, the basic requirement for carbohydrate recognition is for the collectins to trimerize, which allows for simultaneous and multivalent interactions with carbohydrates. The clustering of the CRDs ensures that collectins can bind with high affinity, and in a calcium-dependent fashion, to oligosaccharide complexes, which are typically found on the surface of microorganisms such as bacteria, viruses, fungi, and yeasts. Below, an overview is given of the interactions of SP-A and SP-D with pathogens and of the interactions of these collectins with other components of the immune system.

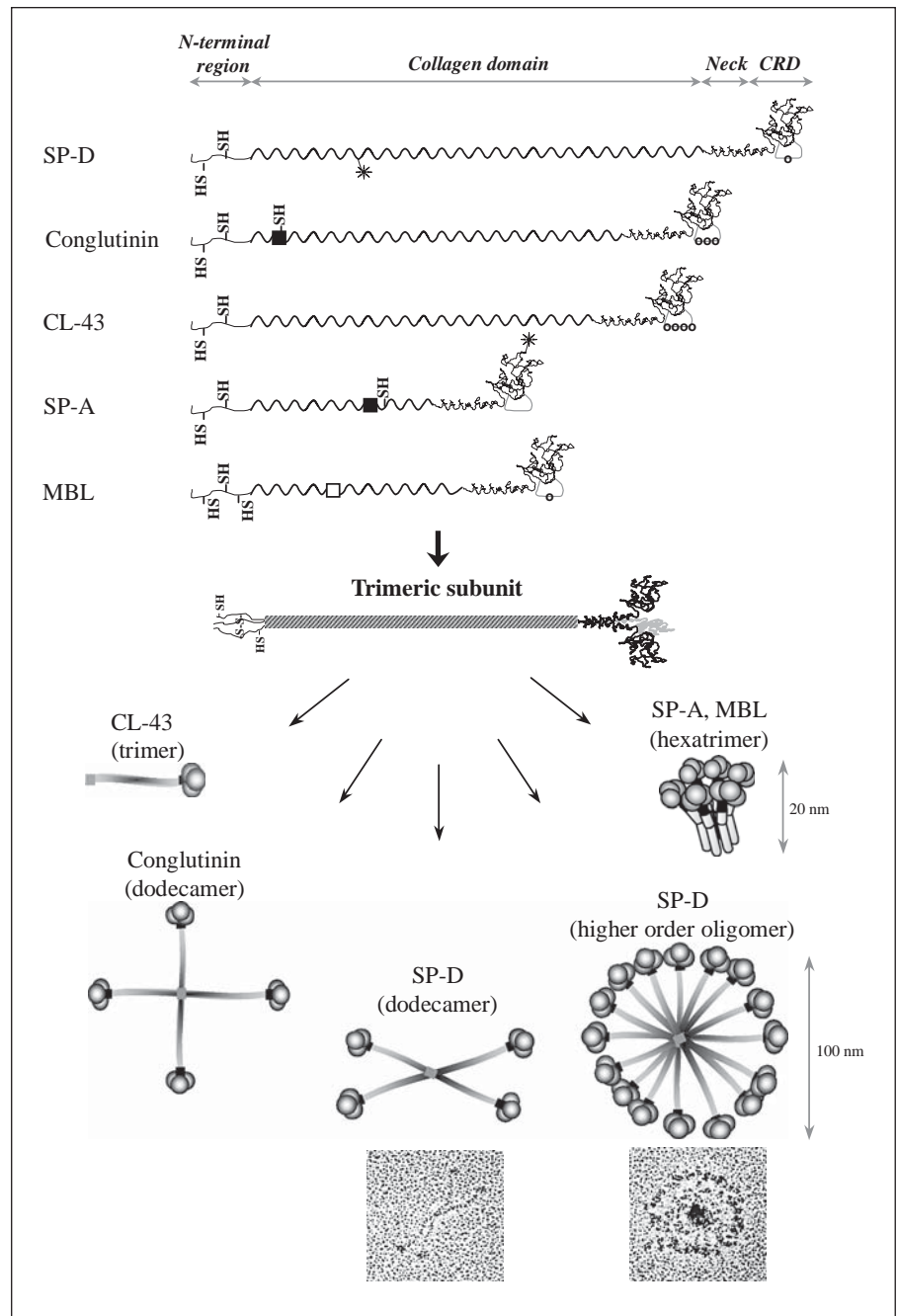
### Interactions of SP-A and SP-D with Pathogens

SP-A and SP-D bind to a variety of pathogens and protect the host from infection by (1) aggregation of pathogens, thus preventing dissemination; (2) stimulation of phagocytotic activity and subsequent killing of microorganisms; (3) modulation of the inflammatory response. The reader is referred to extensive reviews about collectin-pathogen interactions [9–11] and immunomodulatory functions of collectins [4, 5, 12]. Here, we briefly discuss interactions of SP-A and SP-D with representative pathogens from several classes. In table 1, interactions of SP-A and SP-D with various pathogens are listed.

#### *Fungi and Yeasts*

Both SP-A and SP-D bind to pathogenic fungi and yeasts and have shown to protect the lungs from infection. As an example, interactions with *Candida albicans* are discussed. *C. albicans* is a pathogen which can alter its cell morphology from yeast to hyphae, enabling this fungus to penetrate host tissues. *C. albicans* can germinate in the respiratory tract after entering the lung via inhaled air, or via aspiration. SP-A can bind to *C. albicans*, and phagocytosis of serum-opsonized *C. albicans* by alveolar macrophages is inhibited by SP-A [13]. SP-A downregulates proinflammatory cytokine production evoked by *C. albicans* in alveolar macrophages and monocytes, but release of anti-inflammatory cytokines is not influenced by SP-A [14]. This was still observed in the presence of surfactant lipids or serum components, indicating that SP-A may elicit this response in vivo, even

**Fig. 1.** Primary and tertiary structures of the collectins. All collectins depicted are from humans, except conglutinin and CL-43 which are only found in Bovidae. The domain organization of the polypeptide chains of the 5 well-characterized members of the collectin family is drawn to scale (top). The neck CRD is illustrated as the main chain structure, as determined for human SP-D; open circles on CRD indicate number of extra amino acid residues compared to SP-A that are present in loop 4 of the mannose-binding lectin (MBL) crystal structure. Asterisks indicate conserved N-glycosylation sites; squares in collagen domain represent incomplete (white) or interruption in (black) triplet sequence. Triple helix formation over the collagen-like region results in the clustering of 3 CRDs. This trimer is the subunit structure which is assembled into oligomeric forms (bottom), except for CL-43 which is only present as a trimer. Oligomerization involves stabilization by interchain disulfide bonds (-SH). SP-D oligomers, purified from porcine bronchoalveolar lavage, are also shown as visualized by rotary shadowing and electron microscopy.



under conditions of plasma leakage resulting from lung damage. *C. albicans* is bound and aggregated by SP-D in a lectin-like manner [15]. Interestingly, incubating *C. albicans* with SP-D leads to profound fungal growth inhibition and a decreased hyphal outgrowth. Decreased uptake of radiolabeled precursors is also observed, indicating that protein synthesis is inhibited. It is unclear whether this is a direct effect of SP-D, or if SP-D-mediated

aggregation leads to a reduced access to the precursors. Furthermore, SP-D inhibits phagocytosis of *C. albicans* by alveolar macrophages, which is probably due to the size of the aggregates [15].

#### Bacteria

Collectins have been shown to interact with many different bacteria [11] (table 1). The most important target,

**Table 1.** Interactions of surfactant collectins with pathogens

Species	SP-A	SP-D
Fungi and yeasts		
<i>Aspergillus fumigatus</i>	×	×
<i>Pneumocystis carinii</i>	×	×
<i>Cryptococcus neoformans</i>	×	×
<i>Candida albicans</i>	×	×
<i>Histoplasma capsulatum</i>	×	×
Gram-negative bacteria		
<i>Escherichia coli</i>	×	×
<i>Haemophilus influenzae</i>	×	×
<i>Klebsiella pneumoniae</i>	×	×
<i>Pseudomonas aeruginosa</i>	×	×
<i>Helicobacter pylori</i>		×
<i>Chlamydia trachomatis</i>	×	×
<i>Chlamydia pneumoniae</i>	×	×
Gram-positive bacteria		
<i>Staphylococcus aureus</i>	×	×
<i>Streptococcus pneumoniae</i>	×	×
Group B Streptococcus	×	
<i>Alloicoccus otitis</i>	×	
Mycobacteria		
<i>Mycobacterium tuberculosis</i>	×	×
<i>Mycobacterium avium</i>	×	×
<i>Mycobacterium bovis</i> BCG	×	
Mycoplasmas		
<i>Mycoplasma pneumoniae</i>	×	×
<i>Mycoplasma pulmonis</i>	×	
Viruses		
Herpes simplex virus	×	
Rotavirus		×
Respiratory syncytial virus	×	×
Human immunodeficiency virus		×
Influenza A virus	×	×

for Gram-negative bacteria, is lipopolysaccharide (LPS); for Gram-positive bacteria, the main targets are lipoteichoic acid and peptidoglycan. The C1q-receptor may be involved in the SP-A-mediated uptake of bacteria by monocytes [16]. As an example for a Gram-negative bacterium, the interactions of pulmonary collectins with *Pseudomonas aeruginosa* are discussed. SP-D can bind to *P. aeruginosa*, which stimulates phagocytosis of the bacteria by alveolar macrophages in vitro. SP-D may function as an opsonin, since macrophage internalization of *P. aeruginosa* is increased by approximately 50% when the bacteria are preincubated with SP-D [17]. In addition, SP-D is capable of stimulating phagocytosis of the bacteria by human monocytes [18], and SP-D-deficient mice have been shown to clear *P. aeruginosa* at a slower rate than wild-type mice [19]. Also SP-A-deficient mice are

susceptible to *P. aeruginosa* infection, as bacteria are cleared from the lungs of SP-A-deficient mice at a much slower rate than from wild-type mice [20]. Alveolar macrophages show a decreased uptake of bacteria in SP-A-deficient mice, supporting earlier observations that SP-A enhances uptake of *P. aeruginosa* by alveolar macrophages [21]. The protective function of SP-A and SP-D can be impaired by the bacterium because *P. aeruginosa* produces elastase which can cleave these collectins [22]. This degradation will eliminate many of the collectins' normal immune functions, which may represent a strategy of *P. aeruginosa* to evade immune defenses.

#### Viruses

SP-A and SP-D bind to many viruses and thus protect the lungs from infections [11] (table 1). In contrast to most bacteria, viruses must infect host cells in order to replicate. The surfactant collectins are present in the mucous and surfactant layers and consequently are very well situated to prevent infection of the epithelia. In vitro and in vivo infection models have indeed indicated that SP-A and SP-D are antiviral molecules. As an example, the effects of surfactant collectins on respiratory syncytial virus (RSV) infections are discussed. RSV causes bronchitis and pneumonia. Its two integral envelope glycoproteins mediate cell attachment (G glycoprotein) and fusion of the virion with the cell membrane (F glycoprotein).

SP-A binds to the F glycoprotein of RSV in a calcium-dependent manner, which neutralizes virion infectivity [23]. SP-A-deficient mice have been shown to clear RSV at a slower rate than wild-type mice and infiltration of neutrophils after RSV administration is more severe, consistent with an observed increase in macrophage inflammatory protein-2. Administration of human SP-A promoted viral clearance, and pulmonary infiltration by inflammatory cells is reduced to levels comparable to those of wild-type mice [24].

Native human SP-D, as well as a recombinant trimeric form of SP-D consisting of the neck domain and the CRD, are capable of binding to the G glycoprotein of RSV in a calcium-dependent manner [25]. SP-D-deficient mice clear RSV from the lungs at a slower rate than wild-type mice [26]. SP-D deficiency was associated with increased lung inflammation, higher levels of proinflammatory cytokines, and increased inflammatory cell recruitment after infection. Phagocytosis of RSV by alveolar macrophages isolated from SP-D-deficient mice is decreased compared to that of alveolar macrophages isolated from wild-type mice. In vitro, SP-D enhanced phagocytosis of RSV by alveolar macrophages (but not peritoneal macro-

phages) and neutrophils isolated from wild-type mice [26]. Trimeric SP-D decreased RSV infection in a human larynx carcinoma cell-line, and therapeutic administration of trimeric SP-D to mice infected with RSV led to decreased RSV replication in the lung, suggesting that SP-D can block RSV infection by coating the G glycoprotein of the virus [25].

### Immunomodulatory Effects of SP-A and SP-D

The surfactant collectins do not only serve to bind pathogens in order to ensure efficient removal of microorganisms. Both SP-A and SP-D also have direct, nonopsonic, effects on phagocytic cells that result in an increased cell surface expression of phagocytic receptors. Furthermore, surfactant collectins have been shown to regulate cytokine and free radical production and to modulate the allergic response. Many more functions have been attributed to SP-A and SP-D including clearance of apoptotic cells and DNA, direct microbicidal activity, and chemotaxis. An important function of the surfactant collectins is the modulation of the inflammatory response. Finally, SP-A and SP-D may also modulate the adaptive immune response. Both surfactant collectins have been shown to inhibit T cell proliferation. In addition, SP-A inhibits dendritic cell maturation and SP-D stimulates antigen presentation by dendritic cells. Since it was recently reported that SP-A preparations may contain transforming growth factor- $\beta$  (TGF- $\beta$ ) [27], many studies that involve SP-A cell interactions must be repeated to discern the effects of SP-A from that of TGF- $\beta$ . Below, a few examples of immunomodulatory effects of SP-A and SP-D are given. For more information about the immunomodulatory effects of surfactant collectins, the reader is referred to recent review articles [3–5, 12].

#### *Direct Effects on Phagocytic Cells*

SP-A stimulates the expression of the scavenger receptor A on the surface of alveolar macrophages [28]. In addition, the expression of the mannose receptor is also increased by SP-A [29, 30]. It is not clear via which mechanisms SP-A enhances the surface localization of these receptors. The fact that mannose-binding lectin, a collectin that is produced in the liver, has similar effects on Kupffer cells (liver macrophages) suggests that induction of phagocytic surface receptors by collectins is a general phenomenon [31]. Besides the induction of phagocytic receptors by SP-A, this collectin also has been reported to stimulate cytokine and free radical production. It should

be pointed out that, just like the recent discovery that SP-A preparations may contain TGF- $\beta$ , in the older studies SP-A (and SP-D) preparations were used that were not free of LPS. LPS is a potent activator of phagocytic cells. Therefore, the results from these older studies should be interpreted with caution. Many putative SP-A and SP-D receptors have been identified. Since surfactant collectins bind avidly to a wide variety of molecules, the physiological significance of receptor binding is not always clear. However, with respect to the modulation of inflammation by the surfactant collectins a comprehensive picture starts to emerge.

#### *Modulation of Pulmonary Inflammation*

The lungs are constantly exposed to agents that may elicit an inflammatory response. Yet, in normal individuals these agents are generally removed without inflammation. Pulmonary surfactant and its constituents contribute to the anti-inflammatory environment. For example, intratracheally instilled LPS is rapidly bound by SP-D and delivered to alveolar macrophages [32] and instillation of LPS into SP-A-deficient mice causes increased inflammation compared to wild-type mice [33]. When LPS was instilled together with exogenous SP-A, the inflammation in the lungs of SP-A-deficient mice was less severe.

TLRs are essential components of the innate immune system; they sense microorganisms and represent a link to the adaptive immune system [for a recent review, see 34]. The surfactant collectins directly interact with TLRs. Both SP-A and SP-D bind to TLR2 and TLR4 [35, 36]. TLR2 is the receptor for peptidoglycan. SP-A binds to the extracellular domain of TLR2 via the neck domain [37]. Coincubation of TLR2 with SP-A reduced the binding of a recombinant soluble form of TLR2 to peptidoglycan, and peptidoglycan-induced nuclear factor- $\kappa$ B activity is reduced in the presence of SP-A. Furthermore, SP-A significantly reduced the peptidoglycan-elicited TNF- $\alpha$  secretion by rat alveolar macrophages and U937 cells, a macrophage-like cell line [37]. This indicates that direct interaction of SP-A with TLR2 alters peptidoglycan-induced cell signaling, resulting in a reduced inflammatory response.

TLR4 is the receptor for LPS. SP-A binds to the recombinant soluble extracellular domain of TLR4 and its accessory protein MD-2 [36]. Furthermore, the binding of smooth LPS to the cell surface of TLR4/MD-2-expressing HEK293 cells and subsequent nuclear factor- $\kappa$ B activation was inhibited by SP-A. This mechanism may explain the observed anti-inflammatory role of SP-A that

was observed in vivo [33]. Inflammation of the fetal lung results in increased SP-A expression which may be considered as a feedback mechanism [38]. However, the regulation of the balance between the inflammatory and anti-inflammatory state of the fetus and the resulting effects on lung maturation are far from clear [39].

An interesting hypothesis was put forward by Gardai et al. [40] with respect to the immunomodulatory effects of SP-A and SP-D. Both surfactant collectins may initiate a signal pathway that blocks proinflammatory mediator production by binding with their C-type lectin domains to signal inhibitory regulatory protein- $\alpha$ . Via this mechanism activation of the tyrosine phosphatase SHP-1 leads to a blockade of signaling through src-family kinases and P38 mitogen-activated protein kinase, thus resulting in an anti-inflammatory response. The authors propose that upon binding pathogens, apoptotic cells or cell debris, the surfactant collectins cannot maintain the anti-inflammatory state because under these conditions the collectins do not bind signal inhibitory regulatory protein- $\alpha$  anymore. Instead, the presentation of the collagenous tails in an aggregated state to calreticulin/CD91 on the cells would initiate phagocytosis and a proinflammatory response. The validity of this model is dependent on many assumptions, including relative affinities of the different collectin domains for the various ligands. It will be interesting to further investigate whether surfactant collectins indeed act as dual function surveillance molecules in the lung.

## Perspectives

A multitude of data from experiments in vitro and in vivo demonstrate that SP-A and SP-D are important contributors to innate pulmonary defense. These surfactant collectins can interact with a wide variety of respiratory pathogens, regulate the immune cell response to these microorganisms, and play a modulatory role in lung inflammation. The importance of SP-A and SP-D to protect the newborn may not be limited to the lungs; the surfactant collectins may also protect other organs like the intestine [41, 42]. SP-A-deficient neonatal mice that were reared in a bacterium-laden environment had a significant higher mortality compared to both wild-type and SP-D-deficient mice. Interestingly, the mortality of the SP-A-deficient mice was not so much associated with little lung pathology but with gastrointestinal tract pathology.

SP-A and SP-D might be used as a paradigm to develop drugs to prevent or treat lung infections, because of their ability to protect from infection by a wide variety of microorganisms and their capacity to regulate the inflammatory response. It is envisaged that new generations of pulmonary surfactants, developed to treat neonatal respiratory distress syndrome, contain collectins or collectin-based molecules.

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