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Protective role of the lung collectins surfactant protein A and surfactant protein D in airway inflammation

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Abstract

The acute inflammatory airway response is characterized by a time-dependent onset followed by active resolution. Emerging evidence suggests that epithelial cells of the proximal and distal air spaces release host defense mediators that can facilitate both the initiation and the resolution part of inflammatory airway changes. These molecules, also known as the hydrophilic surfactant proteins (surfactant protein [SP]–A and SP-D) belong to the class of collagenous lectins (collectins). The collectins are a small family of soluble pattern recognition receptors containing collagenous regions and C-type lectin domains. SP-A and SP-D are most abundant in the lung. Because of their structural uniqueness, specific localization, and functional versatility, lung collectins are important players of the pulmonary immune responses. Recent studies in our laboratory and others indicated significant associations of lung collectin levels with acute and chronic airway inflammation in both animal models and patients, suggesting the usefulness of these molecules as disease biomarkers. Research on wild-type and mutant recombinant molecules *in vivo* and *in vitro* showed that SP-A and SP-D bind carbohydrates, lipids, and nucleic acids with a broad-spectrum specificity and initiate phagocytosis of inhaled pathogens as well as apoptotic cells. Investigations on gene-deficient and conditional overexpresser mice indicated that lung collectins also directly modulate innate immune cell function and T-cell–dependent inflammatory events. Thus, these molecules have a unique, dual-function capacity to induce pathogen elimination and control proinflammatory mechanisms, suggesting a potential suitability for therapeutic prevention and treatment of chronic airway inflammation. This article reviews evidence supporting that the lung collectins play an immune-protective role and are essential for maintenance of the immunologic homeostasis in the lung.

Keywords

Macrophage; dendritic cell; surfactant; SP-D; SP-A; innate immune regulation

The pulmonary immune system is faced with the dual task of elimination of inhaled pathogens and maintenance of an inflammation-free mucosal environment. Although physical barriers filter out most of the inhaled material, a large amount of small, potentially toxic, infectious or allergenic particles (<5 μm in diameter) still reaches the distal air spaces,

where they encounter components of the innate immune system. Under normal, nondiseased conditions, these include the alveolar macrophages, dendritic cells, and lung collectins.

DISCOVERY OF THE IMMUNE REGULATORY AND PATHOGEN SENSING ACTIVITIES OF THE LUNG COLLECTINS: A HISTORICAL PERSPECTIVE

Lung collectins were originally identified as surfactant associated proteins. Surfactant, a phospholipid material that lines the airliquid interface in the distal air spaces, was discovered by Clements and colleagues^{1,2} in the early 1960s. To date, 4 unique proteins have been found related to surfactant: surfactant protein (SP)–A, SP-B, SP-C, and SP-D. The small, hydrophobic molecules SP-B and SP-C are essential in the structural organization of surfactant and maintenance of low surface tension. On the other hand, SPA and SP-D, the focus of this review, are large, hydrophilic molecules with host defense and immune regulatory functions.

SP-A was discovered by King et al³ in 1973 and was termed a *surfactant protein* because of its close association with pulmonary surfactant. By the mid 1980s, cDNA and genomic sequencing revealed that, unlike SP-B and SP-C, SP-A contains collagenous sequences.^{4–7} SP-D was described in 1988 as a collagenous glycoprotein⁸ with primary sequence similarities to SP-A. In 1989, Tenner et al⁹ showed that the triple-helical collagen structure of purified SP-A is strikingly similar to the structure of the first component of complement *C1q* and that it could mediate phagocytosis through the C1q-receptor by monocytes/macrophages.¹⁰ The C1qR was later shown to be identical to cell surface calreticulin—a multifunctional, low-affinity, and high-capacity calcium-binding and inactivating protein with various chaperone functions—and to recognize the amino terminal collagen tail of SP-A, SP-D, and other collagenous lectin structures.^{11–13} Although binding to calreticulin did not explain all their (the lung collectins SP-A and SP-D) biological activities, these discoveries prompted a large array of studies into the capability of SP-A and SP-D to modulate innate immune cell function directly.

Amino acid sequencing studies in the late 1980s also demonstrated a remarkable homology of SP-A with soluble mannosebinding proteins and revealed residues common to the carbohydrate recognition domains (CRDs) of other mammalian lectins.^{14,15} Although the first collagenous lectin (collectin), bovine conglutinin was discovered more than 100 years ago, the significance of carbohydrate recognition was recognized only in the 1950s (reviewed by Gupta and Surolia¹⁶). The first direct demonstration of lectin-mediated binding of SP-D to gram-negative bacteria and resultant bacterial aggregation was published in 1992 by Kuan et al,¹⁷ suggesting possible roles for collectins in pulmonary host defense. The capability to recognize carbohydratemoieties differentially on pathogen surfaces is today considered an important common function of all collectins.

Together with conglutinin, to date 9 different members have been identified in the collectin family: *mannose-binding lectin (MBL)*, SP-A, SP-D, collectin (CL)–43, CL-46, CL-P1 (placenta), CL-L1 (liver), and CL-K1 (kidney). In spite of structural similarities, C1q does not show C-type lectin activities and is not considered a collectin. MBL, conglutinin, CL-43, CL-46, CL-K1, SP-A, and SP-D are secreted extracellularly, whereas CL-L1 and CL-P1 are

nonsecreted collectins. With the exception of conglutinin, CL-43, and CL-46 (which were only found in cattle¹⁸), collectins are widely distributed among different species. MBL and CL-L1 are produced by the liver, and CL-K1 is found in most organs. CL-P1 is released by the placenta, whereas the majority of SP-A and SP-D is synthesized in the distal airways. Each of these molecules can be detected in the human serum.

Collectins form oligomeric structures similar to the ficolins (L-ficolin, M-ficolin, and H-ficolin) and *adiponectin*, relatively recently identified members of the C-type lectin and defense collagen superfamilies (see reviews^{13,19–21}). On pathogen recognition, C1q, MBL, and the ficolins are capable of initiating the complement activation pathway. However, unlike C1q, which is the first component of the classic complement cascade, MBL and ficolins initiate the complement system through attached MBL-associated serine proteases. Binding to lectins by MBL and ficolins triggers activation/cleavage of C4, C2, and C3 without the presence of C1q.^{13,22–24} This third type of complement activation pathway is called the “lectin pathway.”¹⁹

There is no indication so far that SP-A or SP-D could activate the complement system.²⁵ Nevertheless, we have compelling evidence today that both of these collectins are potent cellular modulators of immune responses in the lung (see reviews^{26–29}). The most recent piece of this evidence comes from studies on various genetically modified mice. In the late 1990s, mice lacking or overexpressing lung collectins were generated in several laboratories.^{30–35} Even though there were discrepancies related to the different background strains or the methods used for genetic manipulations, studies on SP-A and SP-D gene-inactivated mice generally reported increased susceptibility to infectious agents, enhanced acute inflammatory response to pathogen exposure, and vulnerability to chronic inflammation.^{30,32,34–50}

The relevance of these findings to human disease is supported by a high degree of evolutionary conservation among different classes of vertebrates.^{51,52} Given their specific localization to the proximal and distal airway epithelium, uniqueness of their structure, and functional versatility, SP-A and SP-D play immunoprotective roles at multiple levels. The following parts of this review discuss the innate immune role and regulation of lung collectins in the course of the inflammatory airway response.

REGULATION OF SP-A AND SP-D EXPRESSION

The collectin gene

The genes for SP-A and SP-D are clustered on the long arm of chromosome 10q22-23.⁵³ The SP-D gene is linked to SP-A and is located proximally to the centromere at about 80 to 100 kb from the SP-A2 gene. The collagen-like sequences are encoded by several short exons.^{5,14,54,55} The overall length of the collagen domain in each specific collectin is determined by the number of tandem *exon duplications*. The intron-exon organization (1 intron is inserted between 2 exon duplicates) resembles the genes of nonfibrillar collagens,¹⁴ suggesting an evolutionary relationship.

The neck domain and the CRD are each encoded by single discrete exons. The similar structure of the collectin genes indicates that they evolved by duplication from a common ancestral gene, which was probably formed by *exon shuffling* between genes encoding nonfibrillar collagens and a primordial lectin.⁴²

Cell- and tissue-specific SP-A and SP-D expression

The majority of SP-A and SP-D is expressed on the luminal surface of the pulmonary epithelial cells (Fig 1, *A and B*). Although the most important source of SP-A and SP-D is the lung,⁵⁶ transcripts for these molecules were also detected in mesothelial tissues (mesentery, peritoneum, and pleura), synovial cells, the eustachian tube and nasal sinuses, the gastrointestinal tract, and the genitourinary tract.^{56–62} The importance of extrapulmonary collectin expression was supported by studies showing that specific SP-A haplotypes differ between children susceptible to recurrent otitis media⁶³ and that SP-D expression in the human gastric mucosa is significantly increased in *Helicobacter pylori* infection.⁶⁴ Collectins were also demonstrated to enhance phagocytosis of chlamydial pathogens⁶⁵ and to inhibit *Chlamydia trachomatis* infection (a frequent cause of infertility) in the female reproductive tract.⁶⁶ SP-A and SP-D are detected in the amniotic fluid in large amounts (2–8 µg/mL) by 40 weeks of gestation⁶⁷ and were suggested to play a role in protection from infections, inflammation, and initiation of parturition (see review²⁷). Thus, nonsurfactant-associated expression of lung collectins in the proximal airways (Fig 1, *C*) and in mucosal surfaces of various organs indicates generalized host defense significance of these molecules. Importantly, collectin levels can be measured in serum and reflect the extent of lung injury. Thus, they may be used as biomarkers.⁶⁸

Transcriptional regulation of lung collectins during fetal lung development and airway inflammation

Relatively short promoter sequences control cell/tissue-specific expression and developmental regulation of the surfactant protein genes, which are also influenced by transcriptional and/or posttranscriptional mechanisms affecting mRNA stability. Gene expression of the lung collectins during embryogenesis parallels increases of the surfactant phospholipids and the hydrophobic surfactant proteins (SP-B and SP-C). SP-D mRNA expression at 10 weeks of gestation precedes that of SP-A,⁶⁹ but both collectins increase exponentially during the third trimester (see review²⁷). Although surfactant protein promoter function depends on multiple transcription factors, thyroid transcription factor (TTF)–1 (Nkx2.1) appears to be a common positive regulator^{70,71} during lung development and under baseline conditions in the adult lung.

In response to inflammation and injury however, transcriptional regulation of SP-A and SP-D appears to fall under differential regulatory pathways. SP-A protein levels transiently decreased during the allergic airway response in mice⁷² and in patients with asthma.⁷³ In contrast, SP-D protein levels increased in a time-dependent fashion, preceded by SP-D mRNA upregulation after allergen challenge^{74,75} (Fig 2, *A*). Although the mechanism is not well understood, differential SP-A and SP-D gene activation can be induced by cyclic AMP,^{69,76} *IFN*-γ,^{69,75} TNF-α, TGF-β, and glucocorticoids.^{69,77} Because of a **negative glucocorticoid response element** in the SP-A1 promoter, SP-A is inhibited by

glucocorticoids. Interestingly, there is no glucocorticoid response element in the +3-kb human SP-D 5' flanking sequence⁷⁸ (Fig 2, D), yet there is a significant SP-D promoter activity in response to glucocorticoids.^{69,79} The significance of the differential transcriptional pathways probably is to ensure the possibility of a competent immune response in a wide variety of pathological conditions. In addition, selective regulation of SP-A and SP-D would allow these collectins to exert specific functions, thereby reducing immune regulatory redundancy.

Lung collectins are upregulated during the T-cell-dependent allergic airway changes in BALB/c, C57BL/6, and 129J inbred mouse strains.^{74,80} Interestingly, both the baseline SP-D expression level and the enhanced SP-D production in response to inflammation were selectively higher in C57BL/6 mice than in BALB/c mice.^{81,82} These data indicated that a relative strain resistance to develop allergic sensitization and airway hyperresponsiveness⁸³ is associated with an increased capability to produce SP-D and that promoter regulation of SP-D gene expression is genetically determined.

It is possible that specific upregulation of SP-D is induced by TH2-type cytokines in response to allergen challenge. Early support for a role of TH2 cytokines came from studies on mice overexpressing IL-4,⁸⁴ IL-5,⁸⁵ and IL-13⁸⁶ because these animals had markedly elevated SP-D expression in the lung. Direct cytokine regulation was confirmed in a type II alveolar epithelial cell culture system in which SP-D (but not SP-A) was enhanced by IL-4,⁷⁹ IL-13⁷⁵ (Fig 2, B and C), and IL-6.⁸²

Sequence analysis of the SP-D gene promoter suggested potential regulatory roles for **signal transducers and activators of transcription (STAT)**, CCAAT/enhancer-binding protein (C/EBP), **activator protein-1 (AP-1)**,^{78,87,88} TTF-1, and **nuclear factor of activated T cells (NFAT)**⁷¹ binding sites (Fig 2,D). The AP-1 complexes⁸⁷ and NFAT in cooperation with TTF-1⁷¹ were shown to activate SP-D mRNA. These sites are clustered in the proximal SP-D gene promoter and are well aligned among the murine, rat, and human sequences, indicating important, shared pathways in SP-D transcription. In addition to the AP-1/NFAT/TTF cluster, the SP-D promoter contains binding sites for C/EBP. Apart from regulating tissue development and cell differentiation, these basic leucine zipper domain (bZip) class DNA-binding proteins are important for the regulation of the acute phase response.⁸⁹⁻⁹¹ C/EBP (C/EBP α), C/EBP β (inducible nuclear factor of IL-6 [NF-IL6]), and C/EBP δ (NF-IL6- β)⁹² are expressed by lung epithelial cells, and each of them was shown to induce SP-D transcription.⁸⁸ Further, the mouse and rat promoter have an identical nonspecific STAT-binding site in a prominent location, proximal to the AP-1 site. This STAT-binding site has a nonspecific 5-base spacing motif⁹³ and could mediate the effects of IL-4/IL-13 and IL-6.^{75,79,82} Although this site is missing from the human SP-D promoter, there are other STAT-like binding motifs both proximally and distally, indicating that the human SP-D gene can be regulated by cytokines that use either the C/EBP or STAT transcription pathways. IL-6 for instance, is known to use both pathways.⁹⁴

Taken together, these studies indicate that the expression of lung collectins is regulated by multiple, complementary pathways that can result in significant changes in the local

collectin levels. The extent of expression in turn influences the outcome of the inflammatory airway response.

SENSING AND CLEARANCE OF PATHOGENS, ANTIGENS, AND APOPTOTIC BODIES

The collectin structure

The significant functional complexity of collectins is based on their structural characteristics. Collectins contain 4 analogous regions: (1) CRD (also called C-terminal lectin or globular head), (2) the α -coiled neck, (3) the collagenlike domain, and (4) the cysteine-rich N-terminal^{20, 95–99} (Fig 3, A).

The collectins are assembled as trimeric subunits, which multimerize to varying degrees. The CRDs are held in trimeric arrays at the end of each collagenous stalk and are responsible for recognition of carbohydrate molecules commonly present on the surface of microbes or pollens but not usually found on mammalian cells. This common element of lectins is characterized by the presence of 14 invariant amino acid residues and 18 conserved residues, including 4 cysteine residues that form a conserved disulfide-bonding pattern. The CRDs have relatively low affinities for a single monosaccharide but have higher affinities for clustered oligosaccharides.¹⁰⁰ Crystal structure analysis of maltose-bound recombinant fragments^{101,102} revealed the presence of a calcium ion located in the trimeric axis in a pore at the bottom of the funnel formed by 3 CRDs, close to the neck-CRD interface.⁹⁶ Sequence alterations showed that presence of this calcium ion and the structural organization of the CRD are essential for a relative saccharide selectivity and the differential quaternary arrangement of the different collectins.

A short trimeric helical coil bridges the collagenlike arm to the globular CRD at the carboxy-terminal domain of the collectins.^{14,100,103} An important spatial relationship between the neck and the CRDs is maintained by coordinated interactions between glutamate and lysine residues.¹⁰⁴ This relationship suggested the possibility of immune cell surface receptor binding in the presence of bound, extended natural *LPS* and phospholipid ligands. The neck region between the CRD and collagen domain is also responsible for the assembly of the 3 identical polypeptide chains to form the characteristic collectin homotrimers.

In the collagen region, an amino terminal disulfide-rich domain of 7 and 25 amino acids in SP-A (Fig 3, B) and in SP-D (Fig 3, C), respectively, contributes to interchain covalent interactions that stabilize the basic trimeric organization of the collagenlike domains. The second domain contains the collagenlike sequence and forms an extended fibrillar triple helix that is 20 nm long in SP-A and 46 nm long in SP-D.⁹⁹ MBL and SP-A have a single interruption in the Gly-X-Y tripeptide repeat pattern leading to a kink in the fibrillar domain (Fig 3, B), whereas SP-D and conglutinin have uninterrupted repeats throughout the fibrillar domain. SP-D has the most extended collagen domain, which allows it to form an interlinked network with bound inhaled particles.¹⁰⁵ Conglutinin and SP-D have a characteristic cruciform structure, assembled as tetramers of 3 identical polypeptides (Fig 3, C). The wild-type form of both of these molecules presented a much higher agglutination

activity than their truncated form that lacked the collagen domain.^{106,107} SP-A and MBL have much smaller collagen domains, with a configuration resembling a bouquet of tulips (Fig 3, B).¹⁰⁵ These collectins are less efficient to aggregate pathogens than SP-D.^{108,109} Chimeric collectins using the N-terminal collagen domains of SP-D coupled to the neck and CRD of MBL aggregated bacteria more strongly than wildtype MBL,¹¹⁰ emphasizing the importance of long collagen stalks. SP-D trimers without the collagen region aggregated pathogens to a smaller degree than the full-length molecule,¹⁰⁶ and a single-arm SP-D mutant was not able to aggregate pollen starch granules (whereas the wild-type, fully assembled molecule did), suggesting that multimerization of SP-D is also required for proper collectin function.¹¹¹ Indeed, trimeric SP-D could not aggregate bacteria,^{112–114} whereas there was a several-fold greater agglutination activity by a multimer cluster of dodecamer SP-D than by single dodecamers.^{115,116} SP-D can naturally form multimers, so-called “fuzzy balls” with a total mass of several million kilodaltons and a size of about 100 nm.¹¹⁷

Carbohydrate specific binding: now collectins distinguish between self and nonself

A large body of evidence suggests that CRD-dependent attachment is important between collectins and inhaled particles (see review⁹⁷) and that there is an essential role for the collagenlike domain and the degree of oligomerization for pathogen aggregation and subsequent phagocytosis.¹¹⁸

To recognize a wide variety of pathogen surfaces, collectins should have a broad specificity that is achieved by a very open binding pocket in the CRD. Collectin pattern recognition entails the ability to bind LPS, lipoteichoic acid, and mannan over the less suitable terminal monosaccharides and macropatterns of mammalian glycoconjugates. Distinguishing between self and nonself can also be achieved through recognition of a specific orientation of hydroxyl groups of hexoses predominantly found in mannose, fucose, and N-acetyl-D-glucosamine and glucose,¹¹⁹ which are often located in repeating structures on the surface of microbes as a result of glycosylation enzymes that differ from higher organisms. Further, organization of the individual low-affinity sugar-binding sites into trimeric arrays in the collectin CRD allows multivalent ligands to have a matching ligand topology (bridging a distance of 45–53 Å), which is important to achieve high-affinity binding.^{13,27,42}

In addition to common carbohydrate binding activities, collectins have a relative monosaccharide preference.¹²⁰ For instance, although both SP-A and SP-D could recognize the carbohydrate structure of *Aspergillus fumigatus*,^{121–123} the interaction with SP-A was inhibited with mannose, but with SP-D it was blocked with maltose.¹²⁴ As assessed by saccharide competition in solid phase-binding assays using adsorbed mannan as the ligand,¹²⁵ the order of carbohydrate preference for SP-A was established as N-acetylmannosamine > L-fucose > maltose > glucose > mannose (no inhibition by galactose, N-acetylglucosamine, and N-acetylgalactosamine). The order of preference of human SP-D in solid-phase competition assays using maltosyl-BSA as the ligand was approximately maltose (inositol) > glucose, mannose, fucose > galactose, lactose, glucosamine > N-acetylglucosamine (see review¹²⁶). Saccharide specificity may be determined by the trimeric

neck + CRD domains, given that bacterially expressed trimeric CRDs show the same saccharide inhibition profile as natural human SP-D.¹²⁷

Binding to lipids

Interactions with surfactant lipids are required to orient the CRDs optimally in relation to the air/lipid or lipid/hypophase (the aqueous lining of the pulmonary epithelium) interface to facilitate uptake of foreign materials embedded in the surfactant layer of the proximal and distal air spaces. Exposed hydrophobic sites could also mediate interactions with hydrophobic domains displayed on various microorganisms, and perhaps stabilize relatively weak lectin-dependent interactions.¹²⁶ Purified SP-A binds to specific surfactant-associated phospholipids, primarily dipalmitoylphosphatidylcholine (DPPC) and *galactosylceramide* *in vitro*.¹²⁸ SP-A aggregates surfactant phospholipids in the presence of calcium^{96,129,130} and regulates the uptake and secretion of surfactant lipids by alveolar type II cells *in vitro*. DPPC binding depends on the collagenase-resistant neck-CRD region, whereas phospholipid aggregation requires oligomerization of SP-A trimers.¹³¹ This function is important for conversion of surfactant secreted from type II cells in lamellar bodies into the latticelike tubular myelin structure. During surface film construction, the tubular myelin is disaggregated and disassembled, followed by a process of vesicle formation dependent on ventilation of the air-filled lung. Lungs of SP-A deficient mice were shown to have markedly decreased tubular myelin formation, emphasizing the significance of this collectin in proper organization of pulmonary surfactant.^{132,133}

SP-A also binds to several glycolipids and neutral glycosphingolipids involving recognition of both the ceramide and saccharide moieties. It is possible that this binding activity can compete for microbial binding to glycolipid receptors recognized by various bacteria and viruses or bacterial toxins.^{42,126}

The major surfactant-associated ligand of SP-D is phosphatidylinositol, but it also binds to glucosylceramide. The physiological significance of this binding is supported by a compelling demonstration that SP-D^{-/-} mice have striking pathological alterations in the lung, including increased surfactant DPPC pool size and abnormalities in type II cells, alveolar macrophages, pulmonary-associated lymphoid tissue, and lung structure.^{30,42} Because it is unlikely that SP-D has a primary role in normal surfactant metabolism, these changes could be secondary to an enhanced inflammatory state of the SP-D^{-/-} lung.

Binding to nucleic acids and removal of apoptotic cells

Lung collectins also bind nucleic acids from a variety of origins, including components of disrupted cells (cell debris) and liberated and cell-surface DNA, thereby contributing to general clearance activities. Binding occurs through both the CRDs and the collagenlike regions. SP-D effectively binds and aggregates alveolar macrophage DNA, and it enhances the uptake of DNA by human monocytic cells. Binding of the collectins to cell-surface DNA might be 1 mechanism by which collectins mediate enhanced phagocytosis of apoptotic cells. Indeed, SP-A and SP-D are both capable of recognizing and binding to apoptotic cells and facilitate their removal by macrophages.¹³⁴⁻¹³⁸ Collectins may recognize damaged cells via their CRD¹³⁹ or collagen region¹³⁵ or independently from those.¹⁴⁰ Several receptors

were proposed as candidates to mediate this function; however, the calreticulin/CD91 complex appears to be the most accepted.¹³⁷ The role of the collagen domain-mediated calreticulin/CD91 binding, however, was contradicted by an *in vivo* study in which treatment with a C-terminal mutant SP-D molecule (that does not contain the collagen domain) restored the macrophage defect and reduced the number of apoptotic cells in the lung of SP-D^{-/-} mice.⁴⁸

Role of lung collectins in pathogen uptake

The consequence of pathogen binding and aggregation by lung collectins is a more efficient phagocytic elimination. Collectin-enhanced particle and pathogen uptake by phagocytes may occur through at least 3 different mechanisms: by regulating cell-surface pattern recognition receptor expression, by opsonizing pathogens, and by functioning as activation ligands.²⁶

Pattern recognition receptors play a major part in host defense and regulate tissue homeostasis within multicellular hosts.¹⁴¹ Interactions between collectins and these receptors may be significant during both infectious and allergen-induced challenges in the lung. Beharka et al¹⁴² showed, for instance, that SP-A selectively enhanced mannose receptor expression on human monocyte-derived macrophages, a process that involved both the attached sugars and the collagenlike domain of SP-A. In contrast, alveolar macrophages from SP-A^{-/-} mice had reduced mannose receptor expression compared with wild-type mice.¹⁴² Such positive regulatory action between collectins and pattern recognition receptors was further suggested in another study, using a model of allergic sensitization. Lack of SP-D in gene-deficient mice resulted in a failure to increase *Toll-like receptor (TLR)-4* expression during allergic inflammation in comparison with wild-type mice.¹⁴³

Phagocytosis of bacteria, viruses, and fungi

Collectin-enhanced phagocytosis was demonstrated for a number of bacteria. Aggregation before uptake was important for *Escherichia coli*,¹⁷ *Streptococcus pneumoniae*, *Staphylococcus aureus*,¹¹² and *Cryptococcus neoformans*.¹⁰⁸ Phagocytosis through direct SP-D binding was shown for *Pseudomonas aeruginosa*,¹⁴⁴ *Haemophilus influenzae*,³⁶ and the unencapsulated variants of *Klebsiella pneumoniae*.¹⁴⁵ On the other hand, SP-A binding significantly enhanced phagocytosis of *Mycobacterium tuberculosis* and *Mycobacterium bovis* BCG by alveolar macrophages.^{146,147} Critical evidence for the significance of collectin-mediated phagocytosis of bacteria was provided by numerous studies on gene-targeted mice.³⁹ These animals were highly susceptible to infection with group B *Streptococcus*,^{148,149} *H influenzae*,³⁶ and *P aeruginosa* infections.¹⁵⁰

Both lung collectins are also involved in neutralization and clearance of inhaled endotoxin.¹⁵¹ Gram-negative LPS polysaccharide side chains are known as O-antigens. Full length O-chains would render the LPS smooth, whereas the absence or reduction of O-chains would make the LPS rough. SP-D prefers rough LPS to the smooth LPS,¹⁵² and unlike SP-A, SP-D does not recognize lipid A as a ligand.¹⁵³ In addition, the LPS component of the pathogens has been identified as a major ligand for SP-D on *E coli*,¹⁷ *K*

pneumoniae, and *P aeruginosa*.¹²⁷ The binding occurs via the CRD and accordingly is calcium-dependent.

Defense against different strains of influenza A virus involves agglutination mediated through sialic acid residues on the SP-A CRD,^{154,155} or viral hemagglutinin and neuraminidase envelop glycoprotein binding by SP-D.¹⁵⁶ The collectins, especially SP-D, are potent inhibitors of influenza virus–hemagglutinin—mediated red blood cell agglutination.¹⁵⁷ Accordingly, SP-D gene-targeted mice show increased susceptibility to influenza A virus infection.³⁸ Lung collectins also bind to respiratory syncytial virus (RSV) through the F (fusion; SP-A) and G (attachment; SP-D) glycoproteins in a calcium-dependent fashion and inhibit viral infectivity *in vitro* and *in vivo*.^{158,159}

Fungal pathogens frequently gain access to the host via the respiratory tract. Under normal conditions, fungal infections of the lung are very rare, although fungi like *C neoformans* and *A fumigatus* are commonly found in the environment and are continuously inhaled. This suggests that efficient defense mechanisms must exist to protect against these pathogens. The acidic capsule of the fungi is the most important in their pathogenesis because it inhibits phagocytosis and is poorly immunogenic, so it enables the infectious agents to persist for extended periods in the host.^{160,161} Mannan and $\beta(1\text{--}6)$ linked glucan represent the major ligands for collectins on the cell walls of fungal pathogens. Although SP-A can bind to both of the aforementioned organisms, it stimulates the phagocytosis of *A fumigatus*^{108,124} but not *C neoformans*.¹⁶² Interestingly, SP-A inhibits phagocytosis of *Candida albicans*¹⁶³ and *Pneumocystis carinii*,¹⁶⁴ and yet SP-A^{−/−} mice showed a heightened susceptibility to *P carinii* pneumonia.¹⁶⁵ Both rat and human SP-D can bind to *P carinii* via its CRD¹⁶⁶ and mediate its aggregation *in vitro*.¹⁶⁷ Unlike SP-A, SP-D enhances agglutination of unencapsulated *C neoformans*.¹⁰⁸

The clinical importance of these observations is illustrated by the fact that a heightened susceptibility of children with cystic fibrosis to a range of infectious and allergenic pathogens is linked with extremely low levels of SP-A and SP-D in the bronchoalveolar lavage (BAL) fluid.¹⁶⁸

Allergen clearance

Depending on the host's immunologic and genetic status, a range of *A fumigatus*–associated respiratory diseases have been recognized, the most common of which is allergic bronchopulmonary aspergillosis (ABPA). When the lung of an immunocompromised subject is exposed to *A fumigatus*, a systemic infection, invasive aspergillosis ensues. In an immunocompetent subject, however, it often results in IgE mediated–asthma. A number of studies using murine models of ABPA or allergic airway sensitization have emphasized the importance of lung collectins in protection against *A fumigatus*–induced pathologies.^{75,169–173} Treatment with recombinant collectins not only protected against mortality but also inhibited the immunoglobulin, eosinophil, and TH2 cytokine responses in murine models. Interestingly, in patients with ABPA, polymorphisms in the collagen region of SPA2 were associated with increased levels of total IgE antibodies and eosinophilia.¹⁷⁴

An important functional consequence of carbohydrate recognition is the ability of collectins to bind inhaled allergenic material. SP-A readily associates with water-extractable particles of pollen grains, including pollen from *Populus nigra italica* (Lombardy poplar), *Poa pratensis* (Kentucky blue grass), *Secale cereale* (cultivated rye), and *Ambrosia elatior* (short ragweed).¹⁷⁵ Further, pollen starch granules extracted from *Dactylis glomerata* (cocksfoot grass) were aggregated by the CRD of SP-D,^{111,176} and this interaction inhibited the release of β -hexosaminidase by peritoneal mast cells. Similarly, both SP-D and SP-A were shown to bind to whole mite extracts (*Dermatophagoides pteronyssinus*) and the purified allergen *Der p 1* in a carbohydrate-specific and calcium-dependent manner.^{177,178} Lung collectins also inhibited mite extract-specific IgE binding and histamine release *in vitro*,¹⁷⁹ indicating a protective importance of calcium and sugar-dependent allergen binding by collectins.¹⁸⁰

MODULATION OF INNATE AND ADAPTIVE IMMUNITY

Binding to cell surface receptors

The first indications of collectin binding to immune cells came from studies investigating the C1q receptor function on alveolar macrophages.⁹ However, the details of cell function modulation by SP-A and SP-D are still not well understood. Because these proteins are sticky and strongly bind carbohydrate, lipid, and protein ligands, promiscuous binding partners and several putative receptors have been identified (Table I).

The amino terminal collagen tail of the collectins may serve as a ligand for collectin receptors.^{175,181} SP-A, SP-D, MBL, and collectinlike C1q were reported to enhance pathogen phagocytosis and removal of apoptotic cells by binding to C1q receptor. This receptor (originally termed cC1qR), was later shown to recognize all the collectin tails and to be identical to calreticulin. Because the endoplasmic reticulum (ER) membrane fuses with the plasma membrane during phagocytosis, calreticulin (which is a cytoplasmic molecule with an ER retention sequence) is expressed on the cell surface during this process. Calreticulin does not have a transmembrane domain but appears to mediate signaling through the low density lipoprotein–receptor related protein, CD91,¹² which is found on macrophages.^{27,182,183} Because calreticulin, CD91, SP-A, and SP-D can all bind multiple ligands in a promiscuous manner, the importance and specificity of this receptor-ligand mechanism needs further investigations. Although the calreticulin/CD91 complex explains some of the biological actions mediated by SP-A and SP-D, it is still unclear whether ER recruitment and calreticulin binding is necessary and/or sufficient for phagocytosis.^{12,137,181,184,185}

Not only the collagenous tail can act as a ligand for collectin receptors. SP-A for, instance has another receptor on alveolar macrophages called SP receptor 210 (SP-R210, named for its 210-kd size), to which it binds via its globular head. SP-R210 binding mediated ingestion of SP-A–BCG complexes and the ensuing release of TNF- α and nitric oxide and mycobacterial killing by alveolar macrophages and monocytes.^{147,186,187}

A 340-kd glycoprotein (gp-340) was originally identified as a pulmonary SP-D binding protein,¹⁸⁸ but later it was shown to bind SP-A also¹⁸⁹ in the presence of calcium but independently of the CRD region. This protein has both a soluble and a membrane-

associated form and functions as an opsonin receptor for various pathogens.^{190–192} Its presence on the membrane surface was not necessary to mediate collectin function (chemotaxis) on macrophages.¹⁸⁹

Carbohydrate recognition domain–specific interactions with lung cells may be mediated through a number of receptors. Apart from pathogen binding, the CRD region has been involved in modulating functions of various cell types of the immune system. The ability to exert chemotactic activity on neutrophils and monocytes, for instance, was suggested to depend on its lectin activity.¹⁹³ The importance of CRD in immunomodulation was corroborated by studies that used calcium chelation or treatment with sugars that inhibited SP-D–mediated neutrophil phagocytosis¹¹² as well as lymphocyte immunosuppression.¹⁷⁹

Through CRD, SP-A and SP-D can recognize, bind, and modulate function of *CD14*,^{194,195} TLR-2,^{196,197} and TLR-4.^{197,198} Murakami et al¹⁹⁶ showed that peptidoglycan-induced TNF- α secretion by U937 cells and rat alveolar macrophages was inhibited by SP-A in a dose-dependent manner. Peptidoglycan, which is not a ligand for SP-A, attenuated this inhibitory effect, while SP-A inhibited peptidoglycan binding to soluble TLR-2, indicating a direct interaction. Using macrophages from C3H/HeJ mice, which carry an inactivating mutation in the TLR-4 gene, and TLR-4–transfected Chinese hamster ovary cells, Guillot et al¹⁹⁸ showed that SP-A–induced activation of the NF- κ B signaling pathway and upregulation of TNF- α and *IL-10* were critically dependent on the TLR-4 functional complex. SP-D was also shown to bind both TLR-2 and TLR-4 in a concentration-dependent and calcium-dependent manner. Epitope mapping with recombinant proteins consisting of the CRD and the neck domain plus CRD indicated that human SP-D binds the extracellular domains of TLR-2 and TLR-4 through its CRD by a mechanism different from its binding to phosphatidylinositol and LPS.¹⁹⁷

Gardai et al¹⁸⁵ provided *in vitro* as well as *in vivo* evidence that SP-A and SP-D (but not MBL or C1q) binding to signal inhibitory regulatory protein (SIRP- α) on macrophages through their globular head inhibits LPS-stimulated and H₂O₂-stimulated cytokine production¹⁸⁵ as well as phagocytosis.¹⁹⁹ Their results confirmed previous studies in which SP-A inhibited *C albicans*-induced cytokine production by macrophages²⁰⁰ that was not reproduced by C1q and type IV collagen and was independent of *Candida*–SP-A binding and phagocyte C1q receptor occupancy. These data suggest that inhibition occurs by direct binding to the cell through the globular head of SP-A.

Importance of a dual biological activity

Gardai et al¹⁸⁵ further showed that collectin binding to pathogens via CRD and presenting their collagen tail to calreticulin/CD91 on macrophages induced proinflammatory cytokine release and put forward the elegant ‘‘head or tail hypothesis’’ (Fig 4) that lung collectins are capable of differential binding through either their CRD or their collagen domain to cell membrane receptors and eliciting respective anti-inflammatory or proinflammatory signaling pathways. Early indications of dual collectin activity on macrophages came from studies on SP-A and mycobacterial infections: SP-A enhanced phagocytosis of *M tuberculosis* and *M bovis* BCG by alveolar macrophages^{146,147} and augmented production of IL-6 in the presence of infection. In the absence of infection, however, SP-A inhibited

IL-6.²⁰¹ These studies suggested that the nature of collectin-mediated immune functions may depend on whether an immune intervention is needed after infection or allergen exposure or whether an immunosuppressive/anti-inflammatory action would be necessary to prevent chronic tissue damage during inflammatory changes.

According to the “head or tail hypothesis,” SP-A and SP-D interacting with pathogens via their globular head, and presenting their collagenous tail to phagocytes to calreticulin/CD91, induce phagocytosis and a proinflammatory response.¹⁸⁵ In subsequent studies, the Gardai group demonstrated that SP-A or SP-D binding to SIRP- α on residential alveolar macrophages via the CRD suppressed phagocytic function of these cells.¹⁹⁹ Thus, presence of SP-A and SP-D is necessary for pulmonary immune homeostasis and an immune inhibitory effect on these cells can be mediated through SIRP- α binding and Src homology phosphatase-1 signaling.¹⁹⁹

Collectin regulation of macrophage function

Unlike the SP-D^{-/-} mice that show serious signs of constitutive alveolar macrophage activation,^{30,35,41,202,203} under normal conditions, SP-A^{-/-} mice display no pathological features in the lung. Under proinflammatory circumstances, however, SP-A enhanced production of TNF- α by macrophages incubated with BCG¹⁸⁷ and by PBMCs and U937 cells in the presence of RSV.²⁰⁴ SP-A, on the other hand, downregulated TNF- α and other proinflammatory cytokines and chemokines by human alveolar macrophages induced by *C albicans*²⁰⁰ as well as LPS.²⁰⁵ Inhibition of the LPS effect on TNF- α by SP-A was specific to smooth LPS and was mediated by blocking CD14.²⁰⁶ These data indicate that SP-A has potent pathogen specific regulatory effects on macrophage cytokine production.

In a model of allergic sensitization, *in vitro* culture of alveolar macrophages together with SP-D and allergen resulted in increased production of the immunosuppressive IL-10 as well as *IL-12*, and IFN- γ cytokines unfavorable for development of TH2-type changes.²⁰⁷ The protective effect of SP-A and SP-D against allergen-induced TH2-type activation was confirmed by direct inhibition of TH2 cytokine release in allergen-stimulated splenic mononuclear cells *in vitro*.^{72,75}

The effects of lung collectins on free radical production are also pathogen-dependent. SP-D was shown to enhance the amount of oxygen radicals produced by alveolar macrophages and neutrophils,²⁰⁸ and 2 independent studies showed that SP-A stimulated the release of reactive oxygen metabolites.^{209,210} In spite of these reported effects, SP-D knockout mice have increased oxidant production.^{36,211} Further, in a recent study on allergic sensitization in mice, recombinant SP-D significantly inhibited allergen-induced and LPS-induced nitric oxide production by isolated alveolar macrophages.²¹² Interestingly, lung collectins showed additional protective effects because they appeared to be potent endogenous inhibitors of lipid peroxidation and oxidative cellular injury.²¹³

Alveolar macrophages need to home to the lung constitutively to perform their immune surveillance function without the presence of inflammation. Lung collectins have significant chemotactic activities on alveolar macrophages. Both SP-A and SP-D stimulated chemotaxis in a concentration-dependent manner²¹⁴ and induced directional actin polymerization in

alveolar macrophages *in vitro*.¹⁸⁹ In this latter study, it was also demonstrated that this effect is cell-specific and collectin-specific, because SP-A did not stimulate the chemotaxis of monocytes,²¹⁵ whereas in another study, SP-D attracted these cells.¹⁹³ Binding of collectins to macrophages probably occurred independently from the C1q receptor, because C1q did not show the same chemotactic effect on macrophages.

Taken together, these studies indicate that lung collectins recognize many pathogens by using various binding motifs that are frequently, but not always, targets for the collectin CRD. Pathogen sensing then leads to aggregation, phagocytosis, and regulated release of inflammatory mediators (Fig 4, *B*). The importance of this mechanism is supported by evidence obtained using gene-targeted mice in models of infection and allergic sensitization. In spite of an exaggerated inflammatory response, these animals show impaired clearance of a number of bacteria, viruses, and fungal pathogens. These studies also strongly suggest that direct binding of collectins to phagocytes in the presence and absence of pathogens may result in different (proinflammatory or anti-inflammatory) outcomes (Fig 4, *A and B*).

Effects of lung collectins on dendritic cells

An important implication of both phagocytosis and direct binding of collectins to antigen presenting cells is the ensuing regulation of adaptive immune response (Fig 5, *A*). Studies investigating the effects of collectins on dendritic cells showed that SP-A inhibited whereas SP-D augmented antigen presentation by bone marrow–derived (myeloid) dendritic cells.^{216,217} SP-D, however, inhibited antigen presentation when dendritic cells were isolated from the lung.²¹⁸ Under baseline conditions, dendritic cells reside in the lung tissue in an immature state. These resting cells are scattered throughout in the bronchial and alveolar wall, capturing inhaled pathogens but unable to present them to T cells. In response to danger signals such as pathogens, proinflammatory cytokines, or necrotic cells, dendritic cells start to mature and switch from an antigen-capturing to an antigen-presenting and T-cell–stimulating proinflammatory state.²¹⁹ Although all mature dendritic cells share a common ability to process and present antigen to naive T cells for the initiation of an immune response, they differ in origin, migratory patterns, localization, and cytokine production.²²⁰ Plasmacytoid dendritic cells make up the majority in the lung under baseline conditions,²²¹ and these cells were shown to be tolerogenic in allergen-induced inflammation. On the other hand, myeloid dendritic cells migrate rapidly to the lung during a TH2-type inflammation and exert potent TH2-cell activation.^{222–224}

Dendritic cells in the BAL of SP-D^{−/−} mice had a myeloid (bone marrow–derived) phenotype and constitutive TNF- α expression. In bone marrow–derived dendritic cell cultures, administration of SP-D suppressed both the activation marker and autocrine TNF- α expression. Thus, SP-D can alter the differentiation and inhibit activation of immature dendritic cells through inhibition of TNF- α (Fig 5, *A*).²⁰³ Interestingly, dendritic cells in the lung of SP-D^{−/−} mice were unable to migrate to the regional lymph nodes and accumulated instead in the airway submucosal tissue (S. Kierstein and A. Haczku, unpublished data, June 2006), possibly because of a markedly increased expression of *thymus and activation-regulated chemokine (TARC)*.⁷⁵ Activated myeloid dendritic cells themselves release large amounts TARC, which selectively attracts more activated dendritic cells and TH2

lymphocytes into the lung, creating a vicious TH2-type inflammatory circle. TNF- α is a potent activator of TARC release. By suppressing TNF- α , SP-D provides an efficient lock on the autocrine TNF- α /TARC proinflammatory pathway.

Effects of lung collectins on the adaptive immune response

Pulmonary lymphocytes are hyporesponsive to a variety of antigenic stimuli compared with peripheral blood leukocytes. There is also evidence that the alveolar lining fluid contributes to the induction and maintenance of such cellular hyporesponsiveness *in vivo* and *in vitro*.^{225–227} These early studies, which suggested a role for the alveolar lining fluid in immune defense, were more recently confirmed by the use of collectin-deficient mice. Investigations of SP-A gene-targeted mice (infected with influenza A virus) showed an increase of B and activated T lymphocytes in the lung compared with wild-type mice.²²⁸ Similarly, there was marked T-cell activation observed in the lungs of SP-D-deficient mice, as reflected by an increased percentage of both CD4+ and CD8+ T cells expressing the activation markers *CD69* and *CD25* in the BAL.^{33,75} Binding and sequestering of the inhaled antigens may be 1 way to interfere with T-cell activation. However, because lung collectins were also capable of inhibiting mitogen-induced T cells,^{72,75} blocking of antigen–T-cell receptor interaction may not be the major pathway for inhibition of lymphocyte function.

Surfactant protein A inhibited T-cell proliferation and decreased IL-2 production²²⁹ via its collagenlike domain through SP-R210.²³⁰ SP-D also inhibited T-cell proliferation²³¹ in response to both antigenic and mitogenic activation,²³² but no receptor has been identified to mediate this function. Further, an IL-2-independent mechanism may be also involved, but viability was not affected.²³³ However, the inhibitory effects of surfactant proteins on PHA-stimulated and *Der p*-stimulated lymphocytes were attenuated when cells were derived from children with asthma with acute attacks.¹⁷⁹ These results indicated that T cells that are already activated may be resistant to the inhibitory effects of collectins.

In summary, lung collectins directly inhibit T-cell activation stimulated by either mitogens or antigens (Fig 5, *B*). This effect does not depend on interfering with antigen–T-cell interaction and does not alter viability. In addition to inhibition of T-cell function, SP-A and SP-D can influence neutrophils, monocytes, basophils, and mast cells (see review¹⁸⁰).

The *in vivo* consequences of collectin interaction with T-cell–dependent inflammatory events were demonstrated in various models of allergic airway sensitization. Treatment of allergen-sensitized mice with recombinant or purified collectins was effective in reducing the eosinophilic inflammation and specific IgE production.^{169–172,212,234} Furthermore, airway hyperresponsiveness was also inhibited, and there was a shift from a TH2 cytokine pattern toward a TH1 response *ex vivo*^{169,170} and *in vitro*.^{75,207} SP-A and SP-D-deficient mice exhibited intrinsic hypereosinophilia,⁵⁰ increased IL-13,^{50,75,143} and TARC^{75,235} levels in the BAL, indicating a diminished control of inflammation in these animals.

CLINICAL SIGNIFICANCE

Lung collectin gene polymorphisms in bacterial sensing and disease susceptibility

The association of qualitative and quantitative differences in gene expression and their correlations with airway disease have been extensively studied, and polymorphic lung collectin alleles were identified and associated with susceptibility to a number of diseases^{236–239} (Table II).

The human SP-A locus has 2 functional genes, SP-A1 and SP-A2 in opposite transcriptional orientation, and a nonfunctional gene^{240,241} in addition to sequence variability within the 3'-untranslated region.^{77,242,243} Although SP-A1 and SP-A2 are both detected in the human BAL and have a 96% protein sequence identity, SP-A2 is more effective in stimulating phagocytosis,^{244,245} binding carbohydrates,²⁴⁶ inhibiting surfactant secretion,²⁴⁷ and inducing TNF- α release by macrophagelike THP-1 cells.^{248,249}

Using coding sequence differences, a number of SP-A1 and SPA2 alleles have been identified as frequent (>1%) in the general population.^{250,251} Amino acid changes occur in the mature secreted SP-A1 alleles at positions 19, 50, and 219, and in the SP-A2 alleles at amino acids 9, 91, and 223. The major SP-A1/SP-A2 haplotype (1A0/6A2) represents 55% to 57% of all SPA alleles, was shown to result in reduced SP-A mRNA expression,²⁵² and is associated with various respiratory illnesses in independent populations.^{63,253} The main SP-A haplotype, interactively with the SP-B Ile131Thr polymorphism, also influences the risk of respiratory distress syndrome and may be also important in susceptibility to influenza virus.²⁵⁴

Respiratory syncytial virus can directly alter SP-A gene and protein expression in type II alveolar epithelial cells²⁵⁵; therefore, it is important that the SP-A2 Gln223Lys allele (1A1) is overrepresented whereas the SP-A2 Ala91Pro allele is underrepresented in infants with severe RSV infections.²⁵⁶ These mutations affect SP-A binding to RSV (the Gln223Lys mutation is in the CRD of the molecule) as well as the function of the collagenous region (which contains the SP-A2 Ala91Pro), resulting in an impaired host defense capability. The SP-A2 1A1 allele also increases susceptibility to *Neisseria meningitidis*,²⁵⁷ supporting the importance of CRD. Mutations in SP-A2 in addition may play a role in susceptibility to *Aspergillus*-mediated allergies¹⁷⁴ and *M tuberculosis* infection,²⁵⁸ whereas the SP-A1 6A4 allele was found with higher frequency in idiopathic pulmonary fibrosis.²⁵⁹

Four polymorphisms have been identified within the SP-D gene, including the **nonsynonymous** Met11Thr, Ala160Thr, Ser270Thr, and a **synonymous** Ala286Ala **mutation**. The Met11Thr and Ala160Thr polymorphisms in the amino terminal and collagen domain have a frequency exceeding 20%, whereas the Ser270Thr and Ala286Ala polymorphisms are relatively rare. To date, only the polymorphism in amino acid 11 has been associated with disease.^{250,260} Importantly, individuals with the Met11Thr-encoding genotype had significantly lower SP-D serum levels than individuals with the Met11Met genotype.²⁶¹ The Met11Thr allele is linked with protection from severe RSV infection in infants, whereas the Met11Met allele is associated with RSV bronchiolitis, and the Thr11-coding allele is associated with susceptibility to *M tuberculosis* infection.^{260,262} Studies

have suggested that the Met11Met allele produces SP-D of both low-molecular-weight and high-molecular-weight forms (monomers, trimers, and dodecamers), whereas the Thr11 produces mainly low-molecular-weight structures (monomers).²⁶¹ It was also shown that the high-molecular-weight form of SP-D has an increased binding affinity to complex microorganisms, whereas low-molecular weight SP-D preferentially bound LPS. Further, in a recent study, significantly more atopic black children had the Met allele compared with nonatopic black children, indicating differential regulation of allergic sensitization.²³⁵ It appears therefore that the different alleles of codon 11 can influence SP-D oligomerization, which can result in markedly different SP-D serum levels and function.²⁶¹

Nucleotide polymorphisms that do not result in amino acid changes were also identified in the lung collectin genes. These are called *silent* or, if exons are involved, *synonymous* mutations and generally thought to be evolutionarily neutral because these polymorphisms do not alter protein function. However, because of possible *codon usage biases*, *translational stability*, splicing, or *transcriptional control* of expression of the lung collectins may be significantly affected. Indeed, chronic obstructive pulmonary disease susceptibility was associated with such polymorphisms in the SP-A and SP-D genes.^{263,264}

SUMMARY

During the inflammatory airway response, the lung collectins play immune-protective roles in multiple levels. The mechanistic significance of these molecules from the onset to resolution of the inflammatory airway response is illustrated by the fact that lung collectins are capable of differential (proinflammatory or anti-inflammatory) regulation of innate immune cell function. The outcome of collectin-cell interactions may be dictated by binding orientation (either via the CRD or through the collagen region^{13,185}), receptor signaling (Table I), and the cytokine/inflammatory mediator milieu of the local environment.²⁶⁵ The ability to achieve differential effects on the innate immune response by the same molecules carries obvious benefits. On the basis of recent human studies on genetic polymorphisms and chronic inflammatory lung disease, it appears that deficiency in expression and/or function of lung collectins is associated with enhanced susceptibility to infections and inflammation, particularly in severe conditions such as cystic fibrosis^{168,266} and chronic obstructive pulmonary disease.²⁶⁷ Further studies are needed, however, to understand better the clinical significance of allelic variations in collectin expression and function and to aid future clinical investigations into the use of collectin preparations for therapeutic manipulations of the pulmonary immune system.

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Abbreviations usedGLOSSARY

ABPA Allergic bronchopulmonary aspergillosis

BAL	Bronchoalveolar lavage
C1	Complement component 1
C1qr	Complement 1q-receptor
C/EBP	CCAAT/enhancer-binding protein
CL	Collectin
CRD	Carbohydrate recognition domain
DPPC	Dipalmitoylphosphatidylcholine
ER	Endoplasmic reticulum
MBL	Mannose-binding lectin
NFAT	Nuclear factor of activated T cells
NF-κB	Nuclear factor- κ B
NKT	Natural killer T
RSV	Respiratory syncytial virus
SIRP	Signal inhibitory regulatory protein
SP	Surfactant protein
SP-R210	Surfactant protein receptor 210
STAT	Signal transducers and activators of transcription
TARC	Thymus and activation-regulated chemokine
TLR	Toll-like receptor
TTF	Thyroid transcription factor

ADIPONECTIN

Adiponectin decreases T_H2 inflammation in mouse models of asthma and decreases insulin resistance in mouse models of diabetes. Treatment with adiponectin increases IL-10 production from macrophages. Pulmonary tissue expresses 3 adiponectin receptors (T-cadherin, AdipoR1, R2).

C1q

The first component of the classic complement cascade, C1 is composed of a trimeric complex of C1q, r, and s. C1q is the C1 subunit that binds aggregated immunoglobulin, resulting in the activation of C1r (a serine protease). Free C1q binds the C1q receptor, which increases phagocytic capacity of neutrophils and macrophages. C1q deficiency, inherited in an autosomal-recessive pattern, is

CD69, CD25

associated with a lupus-like rash, fever, arthritis, and glomerulonephritis.

CD69 is a marker of activated T lymphocytes, B cells, eosinophils, neutrophils, and platelets. CD25 is the α -chain of the IL-2 receptor and is found on activated T cells as well as regulatory T cells, eosinophils, and B cells.

CODON USAGE BIAS, TRANSLATIONAL STABILITY, TRANSCRIPTIONAL CONTROL, SYNONYMOUS/NONSYNONYMOUS MUTATIONS

Codon usage bias refers to the differences in the frequency of usage for a certain base triplet for a given amino acid among organisms. This phenomenon can play a role in molecular evolution because codon usage bias will influence the available tRNA pool and thus the translational efficiency of any given nucleotide triplet for the same amino acid, theoretically making some proteins more efficiently translated than others. *Translational stability* refers to the stability of the mRNA before its translation into protein, whereas *transcriptional control* occurs via promoter and enhancer sequences to alter the rate of gene transcription. Codon usage bias, translational stability, and transcriptional control all serve as mechanisms for ultimately affecting protein levels. *Synonymous mutations* are base pair changes in an exon that do not change the amino acid code in the protein. *Nonsynonymous mutations* are base pair changes that alter an existing amino acid codon, resulting in a change in the protein sequence.

EXON DUPLICATION AND SHUFFLING

Mechanisms for genomic diversity and evolution include exon duplication and shuffling. The theory of exon shuffling explains the assembly/formation of new genes that can occur by the assembly of exon fragments, made possible because of the genomic organization of alternating exons and introns (coding and noncoding regions) and alternate splicing with recombination. Exon duplication can be considered one form of exon shuffling.

GALACTOSYLCERAMIDE

A glycolipid antigen that is presented by CD1d to invariant natural killer T (NKT) cells. NKT cells are detected using tetramers loaded with α -galactosylceramide. Presentation of α -galactosylceramide to NKT cells leads to the

IL-10, IL-12, IFN- γ

production of IL-12 by antigen-presenting cells and of IL-4, IL-13, IL-10, and TGF- β by NKT cells.

IL-10, IL-12, and IFN- γ are all cytokines that dampen the T_H2 response. IL-12 is a dimer of p40/35 that induces IFN- γ production and a shift to T_H1 phenotype. IL-12p40 and IL-12 receptor deficiency leads to severe nontuberculous mycobacterial infections. IL-10 is produced by regulatory T cells and increases in therapies that reduce inflammation, such as specific immunotherapy. IL-10-deficient mice develop spontaneous colitis similar to human Crohn disease.

LPS

LPS is a component of the outer membrane of gram-negative bacteria. Smooth LPS has O-polysaccharides, whereas rough LPS lacks O-polysaccharides. Smooth strains of bacteria are more virulent, elicit an antibody response, and are more resistant to clearance.

MANNOSE-BINDING LECTIN (MBL)

The lectin pathway for complement activation uses MBL binding to mannans on bacterial surface resulting in activation of the MBL associated serine proteases 1 and 2, analogous to activated C1. MBL binds a number of monosaccharides including mannose, N-acetylglucosamine, and L-fucose. MBL associated serine proteases cleave C4 and C2 with subsequent cleavage of C3 and activation of the terminal complement cascade. MBL deficiency may be related to increased infectious susceptibility, including in the respiratory tract.

NEGATIVE GLUCOCORTICOID RESPONSE ELEMENT

Glucocorticoid receptors are nuclear steroid receptors that dimerize and bind to the glucocorticoid response elements to activate or repress transcription. Decreased gene transcription can occur when glucocorticoid receptors bind to a negative glucocorticoid response element consensus sequence (ATYACnnTnTGATCn).

SIGNAL TRANSDUCERS AND ACTIVATORS OF TRANSCRIPTION (STAT), AP-1, NUCLEAR FACTOR OF ACTIVATED T CELLS (NFAT)

AP-1: Heteromeric complexes of jun and fos that activate transcription of the IL-2, IL-4, IL-5, TNF, and GM-CSF promoters. *NFAT*: A Rel transcription family member. Calcineurin-induced dephosphorylation of NFAT is required for nuclear localization. NFAT is modulated by cyclosporine and tacrolimus, which both bind to immunophilins and

THYMUS AND ACTIVATION-REGULATED CHEMOKINE (TARC)

block the phosphatase activity of calcineurin. *STAT*: STAT act as phosphorylated dimers that bind to palindromic DNA elements in response to Janus-activated kinase pathways. STAT1, STAT2, STAT3, STAT4, STAT5, and STAT6 are downstream of IFN, IL-2, IL-6, IL-10, IL-12, IL-2, and IL-4, IL-13 signaling.

Also known as chemokine ligand 17 (CCL17), TARC binds to the chemokine receptor 4 (CCR4), which is increased on T_H2 cells and activated dendritic cells, promoting trafficking to sites of inflammation in asthma and atopic dermatitis.

TOLL-LIKE RECEPTOR (TLR)-4, CD14

TLRs are transmembrane receptors in the innate immune system that activate antigen-presenting cells. TLR ligands are microorganism/pathogen associated molecular patterns. TLR-2 binds to lipotechoic acid on gram-positive bacteria; TLR-4 binds lipotechoic acid as well as LPS on gram-negative bacteria. Endogenous ligands for TLR-4 include fibronectin and β -defensin-2. TLR-4 uses CD14 to activate the nuclear factor- κ B (NF- κ B) pathway and production of IL-6, IL-12, and TNF- α . Mutations of TLR-mediated immunity can result in primary immunodeficiency made up of recurrent pyogenic infections with poor polysaccharide antibody response (IL-1 receptor-associated kinase 4 [IRAK-4]) and herpes simplex virus encephalitis (TLR-3).

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Author: Angela Haczku, MD, PhD

Activity Objectives

1. To understand the unique structure of soluble pattern recognition receptors (collectins), their localization, and their functional versatility.
2. To understand the role of the lung collectins in pathogen recognition, airway defenses, and airway inflammation.

Recognition of Commercial Support

This CME activity is supported by an educational grant from Merck & Co., Inc.

Disclosure of Significant Relationships with Relevant Commercial Companies/Organizations

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FIG. 1.

Lung collectins are produced by epithelial cells of the distal and proximal air spaces. **A**, Photomicrograph of a type II alveolar epithelial cell. Although the lung collectins have been localized to type II alveolar epithelial cells, immunocytochemistry studies indicate that they do not colocalize with lamellar bodies, the subcellular organelles responsible for storage and release of surfactant. Lamellar bodies were labeled with a monoclonal anti-ATP-binding cassette, subfamily A, member 3 antibody, 3C9 (Texas red, *left panel*). SP-D was labeled by a rabbit polyclonal antimouse SP-D (fluorescein isothiocyanate, *second from left*). The merged magnified image (*right panel*) illustrates that SP-D is scattered in the cytoplasm in a diffuse, reticular pattern and is localized outside the lamellar bodies. **B**, SP-A and SP-D are released onto mucosal surfaces to fulfill innate immune regulatory functions. Alveolar type II epithelial cells and Clara cells constitutively release SP-A and SP-D onto the luminal surface, where lung collectins associate with alveolar macrophages. **C**, SP-D expression is prominent in both the alveoli and proximal airway epithelial cells. Collectins can be detected not only in the distal but also in the proximal airways, and their expression can be shifted during the inflammatory airway response to the site of inflammation involving Clara cells and goblet cells. The photomicrograph shows epithelial cells of a proximal airway and type

II alveolar epithelial cells both positively labeled with a rabbit polyclonal anti-SP-D (fluorescein isothiocyanate).

FIG. 2.

The SP-A and SP-D genes are under differential inflammatory regulation. **A**, Allergic sensitization and challenge with *A. fumigatus* induced increased SP-D levels in the BAL fluid. Levels of SP-A did not change. SP-B and SP-C levels decreased by ~50%. Western blot analysis of the small (SP-A, SP-D) and large (SP-B, SP-C) aggregate surfactant fractions. **B**, Murine recombinant IL-4 (1.5 µg) induced a time-dependent increase in SP-D mRNA and protein expression in sensitized BALB/c mice *in vivo*. **C**, IL-13 (but not IFN-γ [not shown]) induced intracellular SP-D protein increase in isolated type II alveolar

epithelial cells *in vitro*. **D**, Schematic drawing of the murine SP-D promoter illustrates proximal location of C/EBP, NFAT, AP-1, and STAT binding sites. The proximal SP-D promoter shows an almost perfect alignment between mice and rats and with the exception of the STAT binding site, a close homology with the human promoter.

FIG. 3.

The collectin structure. **A**, The collectin triple helix. The collectins are assembled as trimeric subunits of a CRD, neck, and collagen region. **B**, SP-A, MBL and C1q have relatively short collagen domains, with a configuration resembling a bouquet of tulips. **C**, SP-D and conglutinin have a characteristic cruciform structure, assembled as tetramers of 3 identical polypeptides.

FIG. 4.

The “head or tail hypothesis”¹⁸⁵: collectins are capable of differential binding through either their CRD or their collagen domain to cell membrane receptors and eliciting respective anti-inflammatory or proinflammatory signaling pathways. **A**, In the absence of pathogens, collectins bind to the innate immune cells with their head and deliver inhibitory signals. **B**, Collectin binding to pathogens via CRD and presenting the collagen tail to phagocytes induce proinflammatory cytokine release. *DC*, Dendritic cell.

FIG. 5.

Lung collectins play an immunoprotective role during resolution of the inflammatory airway response at multiple levels. **A**, Collectins are capable of inhibiting dendritic cell maturation and activation. **B**, Collectins also inhibit T cells and the consequent activation of the adaptive immune response, immunoglobulin production by B cells and influx of granulocytes. Collectins inhibit mediator release directly from inflammatory cells. **C**, Collectins facilitate removal of apoptotic cells during resolution of the inflammatory response.

TABLE I

Lung collectin receptor binding

Receptor	Cells	Ligands	Binding site	Proinflammatory and anti-inflammatory function	References
CD35 (CR1)	B cells, monocytes, neutrophils	SP-A, C1q, MBL	Collagen tail	Phagocytosis	Tenner et al, 1989 ⁹ Tenner et al, 1995 ²⁶⁸ Ghiran et al, 2000 ²⁶⁹
CD93 (C1q Rpr)	Endothelial cells, platelets, neutrophils, microglial cells, monocytes	C1q	Collagen tail	Phagocytosis	McGreal et al, 2002 ²⁷⁰
CD91/calreticulin complex	Macrophages, neutrophils, RAW 264.7 cells	SP-A, SP-D, MBL, conglutinin, CL-43, C1q	Collagen tail	Apoptotic cell clearance (all), proinflammatory cytokine release, p38 and NF- κ B activation (SP-A, SP-D)	Ogden et al, 2001 ¹² Vandivier et al, 2002 ¹³⁷ Gardai et al, 2003 ¹⁸⁵
Gp340	Macrophages	SP-A, SP-D	Calcium-dependent but CRD independent	Phagocytosis	Holmskov et al, 1997 ¹⁸⁸
SP-R210	Macrophages, T-cell type II cells	SP-A	CRD	Phagocytosis of BCG, nitric oxide and TNF- α production, $\downarrow$ T-cell proliferation	Tino et al, 1999 ²¹⁵ Chronos et al, 1996 ¹⁸⁶ Weikert et al, 1997 ¹⁴⁷ Weikert et al, 2000 ¹⁸⁷ Borron et al, 1998 ²³⁰
CD14	Myeloid lineage cells	SP-D, SP-A, MBL	SP-D: CRD SP-A: neck MBL; CRD independent	$\downarrow$ LPS induced cytokine production	Sano et al, 2000 ¹⁹⁴ Chiba et al, 2001 ¹⁹⁵
TLR-2	U937 cells, rat alveolar macrophages	SP-A, SP-D	CRD	$\downarrow$ Peptidoglycan-induced TNF- α production	Murakami et al, 2002 ¹⁹⁶ Ohya et al, 2006 ¹⁹⁷
TLR-4	Macrophages, TLR-4 ^{+/+} Chinese hamster ovary cells	SP-A, SP-D	CRD	$\uparrow$ -I κ B, NF- κ B activation	Guillot et al, 2002 ¹⁹⁸ Ohya et al, 2006 ¹⁹⁷
SIRP- α	Macrophages	SP-A, SP-D	CRD	$\downarrow$ LPS induced cytokine production	Gardai et al, 2003 ¹⁸⁵

TABLE II

Lung collectin genetic polymorphisms and association with disease

Polymorphism	Clinical association			References
SP-A1 allele	Val19Ala	Val50Leu	Arg219Trp	
6A	Ala	Leu	Arg	
6A2	Val	Val	Arg	Lower incidence of otitis media in childhood Ramet et al, 2001 ⁶³
				Increased risk of RDS in preterm infants Haataja et al, 2002 ²⁵³
6A3	Val	Leu	Arg	Reduced risk of RDS in preterm infants Haataja et al, 2002 ²⁵³
6A4	Val	Leu	Trp	Increased incidence of otitis media in childhood Ramet et al, 2001 ⁶³
				Increased susceptibility to tuberculosis Haataja et al, 2002 ²⁵³
				Increased levels in patients with idiopathic pulmonary fibrosis Selman et al, 2003 ²⁵⁹
SP-A2 allele	Asn9Thr	Ala91Pro	Gln223Lys	
1A	Thr	Pro	Gln	Increased incidence of ABPA Saxena et al, 2003 ¹⁷⁴
				Increased susceptibility to tuberculosis Madan et al, 2002 ²⁵⁸
				Reduced severity of RSV infection in infants Lofgren et al, 2002 ²⁵⁶
1A0	Asn	Ala	Gln	Reduced risk of otitis media in childhood Ramet et al, 2001 ⁶³
				Increased risk or RDS in preterm infants Haataja et al, 2002 ²⁵³
1A1	Thr	Ala	Lys	Increased severity of RSV infection in infants Lofgren et al, 2002 ²⁵⁶
				Increased susceptibility to N meningitides Jack et al, 2006 ²⁵⁷
1A2	Thr	Ala	Gln	
1A3	Asn	Ala	Lys	Increased susceptibility to tuberculosis Haataja et al, 2002 ²⁵³

Polymorphism	Clinical association	References
	Increased severity of RSV infection	Haataja et al, 2002 ²⁵³
SP-D single nucleotide polymorphism		
Met	Higher SP-D levels	Leth-Larsen et al, 2005 ²⁶¹
	Increased incidence of RSV infection	Lahti et al, 2002 ²⁶⁰
	Increased incidence of allergic sensitization	Brandt et al, 2008 ²³⁵
Thr	Lower SP-D levels; monomers only	Leth-Larsen et al, 2005 ²⁶¹
	Lower incidence of RSV infection	Lahti et al, 2002 ²⁶⁰
	Increased susceptibility to tuberculosis	Floros et al, 2000 ²⁶²
	Decreased incidence of allergic sensitization	Brandt et al, 2008 ²³⁵

RDS, Respiratory distress syndrome.