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Review

Structure, biologic properties, and expression of surfactant protein D (SP-D)

Erika C. Crouch *

Department of Pathology, Barnes-Jewish Hospital at Washington University Medical Center, 216 S. Kingshighway, St. Louis, MO 63110, USA

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Abstract

Surfactant protein D (SP-D) is a member of the family of collagenous host defense lectins, designated collectins. There is increasing evidence that SP-D, like SP-A, is an important component of the innate immune response to microbial challenge, and that it may participate in other aspects of immune and inflammatory regulation within the lung. SP-D binds to glycoconjugates and/or lipid moieties expressed by a wide variety of microorganisms and certain other organic particles, in vitro. Although binding may facilitate microbial clearance through aggregation or other direct effects on the organism, SP-D also has the capacity to modulate leukocyte function, and in some circumstances, to enhance their killing of microorganisms. © 1998 Elsevier Science B.V. All rights reserved.

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* Corresponding author. Fax: +1 (314) 454-5505; E-mail: crouch@path.wustl.edu

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1. Introduction

Surfactant protein D (SP-D) was initially described as one of several collagenous glycoproteins secreted by freshly isolated rat type II cells [1]. It was subsequently identified in bronchoalveolar lavage (BAL) and in association with crude surfactant [2]. Protein and cDNA sequencing of SP-D led to the identification of a C-type lectin domain [3–5], and prompted studies that demonstrated lectin activity and saccharide-mediated binding to surfactant in vitro [6]. SP-D is now recognized as a member of a family of collagenous carbohydrate binding proteins (collagenous C-type lectins), commonly known as collectins [7,8]. These include SP-A, serum mannose binding protein and two bovine serum lectins, conglutinin and CL-43.

Kuan and coworkers demonstrated that SP-D binds to Gram-negative bacteria via its lectin domain, and that SP-D is a potent agglutinin of some bacterial strains [9]. Numerous subsequent studies have confirmed that SP-D shows specific interactions with various microorganisms and leukocytes in vitro. Recent observations suggest that SP-D is part of a more generalized response of the lung to acute lung injury, and that it may modulate local inflammatory/immune responses, as well as participate in the recognition or clearance of other complex organic materials, such as dust mite allergens [10–13].

2. Molecular structure

SP-D (43 kDa, reduced) is predominantly assembled as dodecamers consisting of four homotrimeric subunits ($4 \times 3 = 12$ chains) with relatively long triple helical arms [14] (Figs. 1 and 2). Each trimeric subunit contains four major domains: a short cysteine-containing NH_2-terminal crosslinking domain (N), a triple helical collagen domain of variable length, a trimeric coiled-coil linking domain or neck (L), and a carboxy-terminal, C-type lectin carbohydrate recognition domain (CRD). Interactions between the amino-terminal domains of SP-D sub-

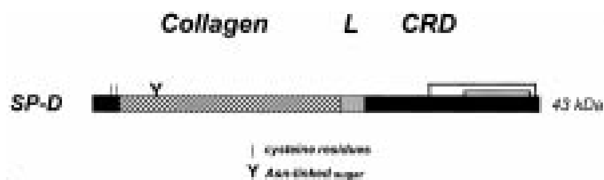

Fig. 1. Domain structure of SP-D. The amino-terminal, collagenous, L peptide, and carbohydrate recognition domains (CRD) are identified. The Asn-linked oligosaccharide in SP-D is located in the collagen domain.

units are stabilized by interchain disulfide bonds [14,15]. In contrast to SP-A, the collagen domain of SP-D contains hydroxylysine and hydroxylysyl glycosides [2] and lacks detectable blood group determinants (W. Longmore, unpublished data). Other possible modifications, including sulfation, acetylation, *O*-glycosylation at sites other than hydroxylysine, have not been specifically excluded.

Although natural and recombinant rat SP-Ds are almost exclusively assembled as dodecamers, preparations of natural human and bovine SP-D can also include trimers [4,16,17]. It is unclear whether the accumulation of these incompletely assembled molecules reflects differences in the efficiency of intracellular multimerization or in the stability of secreted dodecamers. Mason and coworkers have also identified a novel SP-D variant that elutes much later from gel filtration columns than dodecamers [18]. It is present in the lavage from some but not all normal individuals and proteinosis patients. The constituent chains migrate more slowly than the major form of SP-D on SDS-PAGE and show evidence of threonine-linked glycosylation of the pepsin-resistant collagen domain (R. Mason, personal communication).

SP-D dodecamers can self-associate at their amino-termini to form highly ordered, multimers with complex arrays of up to 32 (or more) trimeric CRDs [14,17,19] (Fig. 3). Natural SP-D from human alveolar proteinosis and bovine lavage and recombinant human SP-D contain a high proportion of these multimers. They are not dissociated by EDTA or competing sugars, and are crosslinked by disulfide and non-disulfide bonds. SP-D multimers show higher apparent binding affinity to a variety of ligands and are considerably more potent on a molar or weight basis in mediating microbial aggregation and aggregation-dependent interactions with leukocytes [17,20].

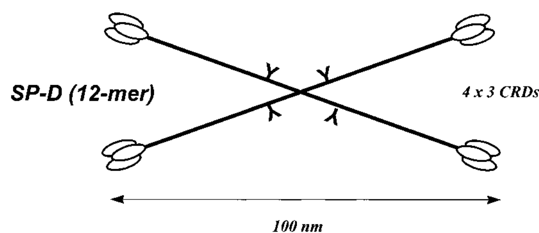

Fig. 2. Molecular organization of SP-D. The predominant molecular form of SP-D is a dodecamer consisting of four trimeric subunits.

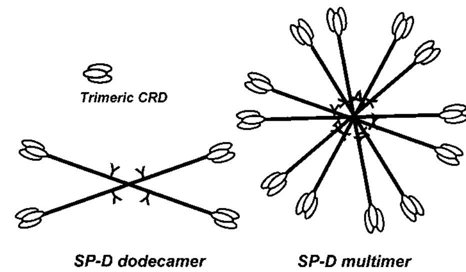

Fig. 3. Alternative molecular forms of SP-D. Trimeric CRDs and SP-D multimers are compared with SP-D dodecamers. The minimum structure required for high affinity binding is the trimeric CRD.

3. Biochemical properties

3.1. Charge

SP-D is secreted as several distinct basic isoforms (p*I* 5–9) that can be resolved by 2-D isoelectric focusing/SDS-PAGE [1]. Because the predicted p*I* of the CRD is < 5, the charge is largely determined by the collagen domain, which is very basic (p$I \approx 10$). Resolution of newly synthesized or natural rat SP-D by DEAE chromatography under non-denaturing conditions also partially resolves species of different monomer size attributable to differences in terminal sialylation [1].

3.2. Binding of divalent cations

The saccharide binding activity of SP-D is abolished with 2 mM EDTA (or EGTA), and can be restored following readdition of excess calcium [6]. Neoglycoprotein binding assays are consistent with the presence of at least one high affinity binding

site (10^{-6} M) [6]. Although SP-D is fully soluble in saline at neutral pH and precipitates in low salt buffers, it can reversibly precipitate in the presence of calcium at physiologic pH and ionic strength. This results from lectin-dependent aggregation, presumably mediated by binding of the CRD to *N*- or *O*-linked glycoconjugates within the collagen domain.

3.3. Disulfide crosslinks

By analogy with other collectins, crosslinking of the cysteines of SP-D (Cys^{261}-Cys^{353} and Cys^{331}-Cys^{345}) is predicted. A variety of observations suggest the *intrachain* bonds are required for calcium-dependent saccharide binding, and confer resistance to thermal denaturation or proteolytic degradation. *Interchain* disulfide bonds are required for the formation of stable trimeric subunits and higher order oligomers. Low concentrations of sulfhydryl reducing agents can liberate trimeric subunits from collectin molecules, sometimes with partial preservation of intratrimeric disulfide bonds depending on the concentration of reducing agent and incubation temperature [14].

4. Interactions with carbohydrate and lipid ligands

4.1. Carbohydrate binding

Purified natural rat, human, and bovine SP-D, as well as recombinant rat and human molecules, preferentially recognize the α-anomeric configuration of nonreducing glucopyranosides [6]. The order of preference of human SP-D in solid phase competition assays using maltosyl-BSA as the ligand is approximately: *maltose, (inositol) > glucose, mannose, fucose > galactose, lactose, glucosamine > N-acetylglucosamine*. This binding specificity is consistent with known interactions of SP-D with the glucose-containing core oligosaccharides of LPS, and with the mannose-rich *N*-linked oligosaccharides of the hemagglutinin of influenza A and the gpA of *Pneumocystis carinii* (see below).

4.2. Lipid binding

Purified SP-D shows high affinity binding to phosphatidylinositol (PI) resolved on thin-layer chromatography (TLC) plates or presented in liposomes [21–23]. PI is also the major surfactant-associated ligand of SP-D. In addition, SP-D binds to glucosyl-ceramide when displayed on TLC plates [24]. The interactions of SP-D with PI and glucosyl-ceramide are calcium-dependent and inhibited by competing sugars [22]. Surface balance studies have demonstrated only limited interactions of recombinant rat SP-D with monomolecular layers of phospholipids [25]. In these experiments, there was no detectable phospholipid head-group preference and interactions of SP-D with the lipid were attributed to hydrophobic interactions. The reason for the apparent discrepancy with previous studies is unknown, but could reflect the comparatively low sensitivity of the physical measurements.

4.3. Structural requirements for ligand binding

As indicated in Section 1 the primary sequence of the carboxy-terminal domains SP-D contains characteristic elements of the mannose-type C-type lectin motif [3–5]. Biochemical and molecular studies have definitively established that these domains are primarily responsible for the carbohydrate binding activity. For example, site-directed mutagenesis of conserved residues of the mannose-type saccharide binding site (Glu^{321} to Gln and Asn^{323} to Asp) reversed the relative carbohydrate binding specificity from maltose, glucose > galactose to galactose > maltose, glucose [26]. Phospholipid binding by SP-D involves interactions with the CRD but also requires the L peptide [26]. However, it is not known whether the L peptide directly participates in binding in the intact protein, or whether hydrophobic interactions between the L peptide and CRD are required to maintain a specific conformation or spatial distribution of the CRDs [27].

The linking peptide in conjunction with the CRD domain (L+CRD) can form trimers, and interactions between hydrophobic sequences on L and the CRD determine the spatial distribution of the three CRDs, thereby generating a single, trimeric, high affinity ligand binding site [27]. The trimeric CRDs show the same saccharide inhibition profile as natural human SP-D [28]. However, the capacity for bridging interactions between spatially separated ligands depends

on an appropriate oligomerization of trimeric subunits, resulting in a characteristic spatial distribution of the trimeric CRDs. A single-arm mutant, RrSP-D-Ser15/20, and bacterially expressed trimeric L+CRDs, do not cause significant bacterial aggregation and viral precipitation [15]. In addition, trimeric CRDs and single-arm forms of the protein can function as competitive inhibitors of SP-D-mediated microbial aggregation. Thus, trimeric SP-D CRDs appear to be functionally univalent with regard to their capacity to participate in bridging interactions between large particulate ligands.

5. Interactions of lung SP-D with microbial ligands

The lining material of the alveoli and distal airways is ideally positioned to participate in the neutralization and clearance of inhaled microorganisms. Because the large serum collectins are presumed to be present at very low concentrations in the extravascular space in uninjured tissues, SP-A and SP-D probably constitute the major collectin defenses of the lung.

5.1. Viral glycoconjugates

The interactions of lung collectins with influenza A viruses (IAVs) have been extensively characterized. IAV attaches to and infects cells by binding through its hemagglutinin to sialic acid-bearing components on the cell surface. The collectins, including SP-D, are potent inhibitors of HA mediated agglutination, and the levels of SP-D in lavage fluids are sufficient to account for a significant fraction of the total HA inhibitory activity of the fluids [29].

HA inhibition by SP-D involves the binding of SP-D through its CRD to glycoconjugates expressed near the sialic acid binding site on the hemagglutinin (or neuraminidase) of specific strains of IAV [20]. Higher degrees of valency or multimerization among the various SP-D preparations are associated with increased HA inhibitory activity [17]. At least with some strains of IAV there is also binding to glycoconjugates associated with the viral neuraminidase, and it has been suggested that collectin binding to the NA can sterically interfere with HA activity [30].

As discussed in greater detail below, an important aspect of the interaction of collectins with IAV is their ability to cause viral aggregation, and to enhance other aggregation-dependent activities [17,20]. Among the collectins, SP-D is the most potent at aggregating IAV particles, and multimers of dodecamers are much more potent than dodecamers.

SP-D is a potent inhibitor of IAV infectivity as measured by the egg inoculation assay, and more potent at inhibiting IAV infectivity than SP-A (or MBL) [17]. Recent studies have also shown that the susceptibility of various IAV strains to neutralization by SP-D in vitro is inversely correlated with pulmonary viral replication in mice following nasal inoculation [31] and directly correlates with specific differences in the number of glycoconjugates expressed on the hemagglutinin. Inoculation of mice in the presence of mannan, a saccharide competitor of SP-D binding to IAV, also increases viral replication in mice consistent with involvement of a C-type lectin.

5.2. Bacterial glycoconjugates

SP-D binds to glycoconjugates expressed by a variety of Gram-negative bacteria including specific strains of such important pulmonary pathogens as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Hemophilus influenzae*, and *Escherichia coli*. Core sugars of LPS (glucose and/or heptose) have been identified have been identified as major ligands for rat or human SP-D on *E. coli* and *Salmonella minnesota* [9]. SP-D also binds to isolated LPS from a variety of other Gram-negative bacteria including *K. pneumoniae* and *P. aeruginosa* [9,28,32]. Purified natural or recombinant dodecamers are potent agglutinins for bacterial strains expressing O-antigen-deficient LPS molecules (e.g. rough strains of *E. coli*), and cause gross aggregation and precipitation of suspended organisms. In contrast to SP-A, SP-D binding to LPS and its effects on bacterial aggregation are blocked by EDTA, competing sugars, LPS, and rough mutant forms of LPS, but not by lipid A [9]. Although SP-D also binds poorly to most smooth (O-antigen-containing) LPS on lectin blots, SP-D can still bind to O-antigen-expressing bacteria, as evidenced by specific labeling and microaggregation in immunofluorescence assays [9]. We have observed that growth conditions (e.g. aeration) can markedly influence the aggregation of specific

Gram-negative bacterial strains by SP-D, and that the extent of macroscopic aggregation inversely correlates with the size and complexity of the terminal O-antigen. Interestingly, SP-D was identified as the major *E. coli* binding protein in cell-free rat BAL [9].

In collaborative studies with Dr. Itzhak Ofek, we observed interactions of SP-D with specific strains of *K. pneumoniae* distinct from those recognized by SP-A. Specifically, SP-D showed lectin-dependent agglutination of two unencapsulated variants of K50 and K21a, and at least one weakly encapsulated strain [32]. The binding of SP-D to the isolated *Klebsiella* LPS was inhibited by EDTA, competing sugars, and LPS from other Gram-negative bacteria, but not by capsular polysaccharides. Although encapsulated strains can apparently bind SP-D, they do not undergo macroscopic aggregation. These observations suggest that SP-D does not recognize the capsular polysaccharides of *K. pneumoniae*, and that the presence of a well-formed capsule limits interactions of SP-D with underlying LPS molecules.

5.3. Fungal glycoconjugates

Recent studies have demonstrated specific lectin-dependent interactions of both rat and human SP-D with gpA, a mannose- and glucose-rich glycoprotein expressed on cysts and trophozoites of *Pneumocystis carinii* (PC) [33–35]. SP-D is associated with PC in vivo, and are present on the surface of freshly isolated organisms. Clusters of organisms in bronchoalveolar lavage can be partially disaggregated with EDTA or competing sugars, and 'stripped' organisms can be agglutinated by purified SP-D, suggesting that SP-D contributes to the clustering of cysts observed in vivo. Interestingly, the production and accumulation of SP-D is increased in rats and immunosuppressed patients with *Pneumocystis* pneumonia [33].

Recombinant rat and human SP-D bind to pathogenic unencapsulated, but not the capsulated, forms of *Cryptococcus neoformans* through a lectin-dependent mechanism [36]. Although the unencapsulated organisms are readily agglutinated by SP-D, there is no significant aggregation by SP-A. More recently human proteinosis SP-D was shown to bind to *Aspergillus fumigatus* conidia in a calcium- and carbohydrate-dependent fashion consistent with binding of the CRD to cell wall glycoconjugates [37]. SP-D efficiently agglutinated the conidia. These interactions likely involve binding to glucans and/or glycoproteins associated with the fungal cell wall.

6. Interactions of SP-D with phagocytes

There are at least three mechanisms by which SP-D can modify the activities of pulmonary leukocytes. These include: opsonization or other effects mediated by the specific interactions of the leukocyte with SP-D-coated organisms, direct effects of SP-D on phagocyte function, and SP-D-mediated alterations in particle presentation secondary to aggregation. The first two mechanisms require binding sites for SP-D on the leukocyte cell surface, while the latter could use pre-existing leukocyte 'receptors' (ligands) for microorganisms. Particle opsonization may theoretically involve: protein receptor-mediated binding of the SP-D to the leukocyte, collectin-mediated bridging interactions between glycoconjugates expressed on the particle and host defense cell, and/or binding of the host cell to glycoconjugates expressed on SP-D. It seems probable that all of these mechanisms may be operative, alone or in combination, in specific microbial-phagocyte interactions.

6.1. SP-D receptors

SP-D does not interact with C1q receptors [38]. However, SP-D has been shown to be recognized by one or more receptors or binding proteins on alveolar macrophages [38]. This binding does not appear to involve the lectin activity of SP-D since binding was performed in the presence of EDTA. It remains unclear to what extent the binding moieties may be related to the non-C1qR SP-A receptors.

Holmskov and coworkers have recently reported the isolation of an SP-D binding protein (GP-340) from human proteinosis lavage [39]. The protein, which is a member of the scavenger receptor superfamily, specifically binds to the L peptide region of SP-D. Antibodies to the protein to the protein show selective binding to alveolar macrophages. The GP-340 gene has been sequenced, and is closely related to a putative tumor-suppressor gene, DMBT1 [40].

6.2. Glycoconjugate 'receptors' on leukocytes

SP-D can interact with leukocytes via lectin-dependent binding to cell surface glycoconjugates [10,41]. Biotinylated SP-D shows significant specific maltose-dependent binding to rat lung macrophages in frozen tissue sections [41]. Binding of FITC-labeled SP-D to freshly isolated aldehyde-fixed rat alveolar macrophages is eliminated after extraction of the fixed cells with low concentrations of Triton X-100. Although the binding sites have not been elucidated, a glycolipid binding site is suggested [42].

7. Collectin-mediated alterations in leukocyte function

7.1. Chemotaxis/haptotaxis

Natural and recombinant rat SP-D and human proteinosis SP-D are potent chemoattractant and haptotactic agents (maximal chemotactic response at 5 ng/ml or $\sim 10^{-11}$ M) for both monocytes and neutrophils [10,37]. This effect may be mediated by attachment of the CRD to phagocyte surface carbohydrate structures, because it is inhibited by competing saccharides and by antibodies directed against the SP-D CRD [10]. Furthermore, trimeric human SP-D CRDs are still very potent ($\sim 10^{-10}$ M) (R. Senior and W. Longmore, unpublished data). Interestingly, SP-D concentrations sufficient to cause chemotaxis were not associated with enhanced oxidative metabolism or phagocytic activity. These results suggest that carbohydrate binding properties of SP-D result in distinctive interactions with phagocytes, and that SP-D may play an important role in recruitment or retention of phagocytic cells and the modulation of inflammatory or immune responses under certain circumstances in vivo.

7.2. Respiratory activity

Rat SP-D has been reported to directly stimulate the respiratory burst response of alveolar macrophages [43]. However, we have not observed significant stimulation of the respiratory response of human neutrophils or peripheral blood monocytes by recombinant rat or human SP-D in the absence of a ligand or contaminating endotoxin [10,29]. The state of protein aggregation, method of purification, and various assay conditions may be important variables.

7.3. Opsonization

There is abundant evidence that lung collectins can bind to microorganisms and enhance their association with leukocytes, and in a few recent studies enhanced internalization and killing of specific organisms has been demonstrated. For example, SP-D can opsonize and enhance the killing of an unencapsulated strain of *K. pneumoniae* by adherent macrophages (Itzhak Ofek, in preparation).

7.4. Non-opsonic enhancement of phagocytosis

There is also some evidence that SP-D can increase the internalization (and sometimes the killing) of a variety of microorganisms by modulating the function of phagocytic cells. For example, the *presence* of rat or human SP-D increases the binding and internalization of *E. coli* by neutrophils in the presence of calcium (Hartshorn, submitted). Significantly, internalization was also increased to some extent by preincubation of the neutrophils with SP-D. As described for IAV, the potency of various molecular forms of SP-D correlated with the degree of subunit multimerization. SP-D opsonized *E. coli* also show enhanced binding (Hartshorn, submitted).

7.5. Effects on T-lymphocyte proliferation

Recombinant rat and human SP-D were found to potently inhibit proliferation under all three conditions of lymphocyte stimulation [12]. This effect did not require long range bridging interactions because trimeric subunits of rat SP-D were more potent than dodecamers. It is not yet known whether these effects involve direct interactions of the collectin with binding sites on the lymphocyte, or are mediated through interactions with a subpopulation of mononuclear phagocytes.

7.6. 'Indirect effects' on leukocyte function

In addition to binding to and activating phagocytes, collectins can indirectly influence phagocyte

function by altering the presentation of pathogens or microbial ligands to the host defense cells.

7.6.1. Viral aggregation

Lectin-dependent binding aggregation of IAV by SP-D enhances the binding of virus to neutrophils, augments the respiratory burst response to bound virus, enhances viral uptake, and decreases IAV-mediated neutrophil deactivation [29]. Prior neuraminidase treatment of the neutrophil abrogates all of these effects, indicating that SP-D mediated viral aggregation enhances the binding of virus to the natural sialic acid 'ligands' on the leukocyte surface. Multimers of SP-D dodecamers are significantly more effective than dodecamers on a protein concentration basis at enhancing IAV binding. Single arms and isolated trimeric CRDs do not increase IAV binding, and actually decrease IAV binding at relatively high concentrations.

7.6.2. Possible effects on LPS signaling pathways

SP-D has been reported to be recovered as a complex with LPS in LPS-induced acute lung injury in rats [13]. Interactions between collectins and LPS suggest that under appropriate conditions these molecules could modulate cellular activation and signaling pathways involving LPS. Such effects could theoretically involve completion for binding by other LPS binding proteins or altered presentation of LPS to inflammatory cells.

8. Tissue and airspace distribution of SP-D

Although SP-D was initially identified in lung there is now considerable evidence for extrapulmonary sites of expression. Our original immunohistochemical studies suggested that in the rat SP-D is produced in rat salivary and lacrimal gland [44]. More recently, SP-D was identified in rat gastric antrum [45] and murine tracheal submucosal glands [46]. Studies by Holmskov and coworkers using monoclonal antibodies to human SP-D demonstrated SP-D in gastric mucosa, parotid gland, lacrimal gland, and mammary and sweat glands (Uffe Holmskov, personal communication). Furthermore, reverse transcriptase-polymerase chain reaction assays identified SP-D mRNA in stomach, parotid gland, breast, and prostate. SP-D accumulates in human and rat amniotic fluid [4,44,47]. Although most of this material may be derived from the lung, local synthesis by amnionic epithelial cells has not been excluded [47].

The majority of the total immunoreactive SP-D remains in the BAL supernatant following high speed centrifugation (50–90% depending on the species) [2,24]. Immunologic studies have shown that the insoluble fraction is associated with amorphous granular material, and that the immunoreactive material can be efficiently solubilized with EDTA or specific saccharides [48]. Early studies suggested that there was much less total SP-D than SP-A in the airspace. However, recent comparative assays by Honda and coworkers gave 3.1 ± 0.4 μg/ml and 1.3 ± 0.2 μg/ml for SP-A and SP-D, respectively, in lavage from healthy non-smokers [49].

Immunoreactive SP-D has also been identified in human serum [50]. The concentration of protein increases dramatically in association with idiopathic pulmonary fibrosis [51,52].

9. Regulation of SP-D expression

9.1. Genomic organization

Human SP-D is encoded by a single gene on chromosome 10 in the region of 10q22.2–23.1 [53]. However, protein, cDNA, and genomic sequencing together suggest the existence of a number of SP-D alleles, some of which are characterized by amino acid substitutions in the coding region [4,53]. The SP-D gene encodes at least one, and probably two, untranslated exons at the 5′ end of the gene, similar to SP-A [53]. Although initial studies identified only a single untranslated exon an additional untranslated exon has recently been identified, the highly homologous bovine conglutinin gene contains an additional untranslated exon [54]. A sequence virtually identical to this exon is present in a cDNA clone of bovine SP-D [55], and a highly homologous sequence has been recently identified in the previously designated first intron of the human gene (Crouch and Rust, unpublished data).

The organization of the human SP-D and SP-A genes suggest a close evolutionary relationship. Pre-

sumably gene duplication and exon shuffling events gave rise to a primordial collagenous lectin [56,57]. Sequence comparisons suggest divergence of the MBP and SP-D genes after divergence of a primitive SP-A gene [58], which later diverged to give the two SP-A genes. In the case of SP-D, insertion of tandem duplications of an additional collagenous cassette (117 bp) (exons 3–6) resulted in a longer collagen domain than either SP-A or MBP [53]. Both conglutinin and CL-43 may have evolved from SP-D [58].

9.2. Biosynthesis and cellular metabolism of SP-D

SP-D is synthesized and secreted by alveolar type II and non-ciliated bronchiolar epithelial cells [44,59,60]. Although there is evidence for cell-to-cell variation in the relative production or accumulation of SP-D by these cells in rat lung [61], populations of these cells show similar levels of SP-D production in vitro (Longmore and coworkers, unpublished data). Consistent with previous studies which showed the accumulation of SP-D in Clara cell granules, the secretion of SP-D by Clara cells, but not type II cells, is stimulated by terbutaline. Studies using human fetal lung explants have demonstrated stimulatory effects of glucocorticoids, but showed no effect of γ-interferon, PMA, LPS, or TNF-α under conditions leading to altered expression of SP-A [62].

9.3. Developmental regulation

The accumulation of SP-D in the rat lung increases abruptly in late gestation [44,52,63]. Unlike SP-A, SP-D mRNA and protein levels continue to increase during the early postnatal period, and reach their highest levels in the adult lung. The increase during the late fetal and early post-natal period reflects increased cellular expression but is also temporally correlated with increased numbers of SP-D expressing epithelial cells [64,65]. The administration of dexamethasone in utero accelerates the appearance of SP-D producing cells and increases the cellular level of SP-D mRNA [63–65]. In the human, SP-D mRNA is first detected at low levels (4–13% of adult) in the second trimester and message levels rise steadily during late fetal lung and postnatal lung development [62]. The level of immunoreactive SP-D protein roughly parallels the level of mRNA consistent with predominant regulation at the level of transcription.

9.4. Alterations in SP-D production associated with lung injury

Immunohistochemical and in situ hybridization studies strongly suggest that the production of SP-D is increased in association with acute injury and epithelial activation [48,61,66]. For example, acute hyperoxia in rats is associated with differential, time-dependent alterations in expression of SP-D by type II and Clara cells [67].The production and accumulation of both SP-D is increased within several hours following intratracheal instillation of LPS [68].

9.5. SP-D gene regulation

Sequences upstream from the start site of SP-D transcription contain numerous potential *cis*-regulatory elements. These include a conserved canonical AP-1 sequence, several AP-1 and CRE-like sequences, as well as sequences similar to those identified in conglutinin and other acute phase proteins (e.g., NF-IL6). The conserved AP-1 element at −109 binds to AP-1 proteins (e.g., *fra-1* and *junD*) in H441 cell nuclear extracts. In addition, site-directed mutagenesis of the consensus markedly decreases basal and dexamethasone-stimulated promoter activity in transient transfection assays using H441 cells. Although dexamethasone increases SP-D promoter activity [69], these effects are indirect and possibly involve interactions of glucocorticoid receptor with other transcription factors. SP-D appears to be encoded by a single major transcript in adult rat and human lung, developmental or pharmacologically induced changes in RNA processing have not been excluded.

9.6. Cellular pathway of assembly and secretion

Recent studies of recombinant SP-D assembly by CHO-K1 cells indicate that folding of the CRD, trimerization of monomers, triple helix formation, the amino-terminal association of trimeric subunits, and the formation of interchain disulfide crosslinks occur in the rough endoplasmic reticulum, and that oligosaccharide maturation occurs in the Golgi immedi-

ately prior to secretion [70]. Reid and coworkers have shown that the linking peptide (L) of bovine SP-D and conglutinin can spontaneously form stable trimers consistent with a trimeric coiled-coil [71]. Thus, within the context of the full length molecule the L peptide domain may serve to register the three polypeptide chains of SP-D prior to folding of the collagen helix, and the subsequent formation of interchain disulfide bonds.

9.7. Degradation of SP-D

Relatively little is known about the degradation or turnover of SP-D. SP-D is found in both endosomal vesicles and lysosomal granules of alveolar macrophages indicating that these cells can also internalize SP-D [42]. Furthermore, it has recently been shown that alveolar macrophages rapidly degrade radiolabeled SP-D by cell-associated proteases in vitro [72]. Interestingly, purified SP-D dodecamers are not degraded by elastase or a variety of secreted mammalian neutral proteinases at 37°C in the presence of physiologic calcium concentrations [15].

10. Summary

There is growing evidence that SP-D modulates important interactions between the host and inhaled microorganisms and participate in aspects of pulmonary immune and inflammatory regulation. SP-D shows calcium-dependent lectin-mediated interactions with glycoconjugates expressed on a wide variety of microorganisms (and certain complex organic particulates) in vitro. In addition, the collectins can influence the activity of phagocytes through lectin-dependent and -independent interactions. Trimeric subunits of SP-D molecules show specific and high affinity binding to various saccharide ligands. However, the oligomerization of trimeric subunits is essential for agglutination and other long-range bridging interactions.

There are several biochemical differences between SP-A and SP-D that likely confer distinctive functional properties in vivo. The most important of these include: (1) differences in solubility at physiologic ionic strength which may influence compartmentalization and availability in the airspace lining material, (2) differences in the length of the collagen domain which determine their capacities to participate in long-range bridging interactions, (3) differences in CRD specificity or valency, and (4) differences in the localization of Asn-linked sugars.

The distinct yet overlapping activities actions of SP-A and SP-D in vitro suggest that the proteins may be functionally complementary in vivo. The molecules appear to occupy different airspace compartments, and sometimes recognize different ligands or ligand domains. The biologic importance of binding to surfactant lipids is unknown, however these interactions could primarily serve to orient the molecules in relation to the alveolar lining material or to modify the presentation of other bound materials to pulmonary cells.

The possibility that acquired or genetically determined differences in SP-D activity may in part account for differences in the susceptibility of individuals to microbial challenge, particularly in the setting of inadequate or impaired specific immunity, or contribute to the pathogenesis of certain immunologically mediated lung disorders, such as allergic asthma, requires further investigation.

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