Complementation of Pulmonary Abnormalities in SP-D(−/−) Mice with an SP-D/Conglutinin Fusion Protein*

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Surfactant protein D (SP-D) and serum conglutinin are closely related members of the collectin family of host defense lectins. Although normally synthesized at different anatomic sites, both proteins participate in the innate immune response to microbial challenge. To discern the roles of specific domains in the function of SP-D in vivo, a fusion protein (SP-D/Conglycon) consisting of the NH2-terminal and collagenous domains of rat SP-D (rSP-D) and the neck and carbohydrate recognition domains (CRDs) of bovine conglutinin (Cong) was expressed in the respiratory epithelium of SP-D gene-targeted (SP-D(−/−)) mice. While SP-D/Conglycon fusion protein did not affect lung morphology and surfactant phospholipid levels in the lungs of wild type mice, the chimeric protein substantially corrected the increased lung phospholipids in SP-D(−/−) mice. The SP-D/Conglycon fusion protein also completely corrected defects in influenza A clearance and inhibited the exaggerated inflammatory response that occurs following viral infection. However, the chimeric protein did not ameliorate the ongoing lung inflammation, enhanced metalloproteinase expression, and alveolar destruction that characterize this model of SP-D deficiency. By contrast, a single arm mutant (RrSP-D) expressed in vivo partially restored antiviral activity but otherwise failed to rescue the deficient phenotype. Our findings directly implicate the CRDs of both SP-D and conglutinin in host defense in vivo. Our findings also strongly suggest that the molecular mechanisms underlying impaired pulmonary host defense and abnormal lipid metabolism are distinct from those that promote ongoing inflammation and the development of emphysema.

Surfactant protein D (SP-D)3 is a member of the collectin family of C-type lectins (1, 2). These proteins contribute to innate immunity, at least in part, by recognizing carbohydrates and lipids expressed on the surface of viral, bacterial, or fungal pathogens and enhancing their presentation to host defense cells. The collectins, which include SP-A, SP-D, conglutinin, CL-43, and the mannose-binding protein (MBP), share well conserved carboxyl-terminal carbohydrate recognition domains (CRDs) and possess collagenous amino-terminal regions that associate to the larger oligomerized formation of the proteins found in the airspace or bodily fluids. SP-D and conglutinin are unique in that they are both preferentially assembled as dodecamers of homotrimeric subunits.

SP-D is highly expressed by epithelial cells lining the lung, but it is also synthesized and secreted by various other tissues (3–6). By contrast, conglutinin is synthesized by the liver and accumulates in the serum. Conglutinin has no known role in lung physiology (7). In vitro studies support the role of both proteins in innate defense against a variety of pathogens including influenza A (8, 9). SP-D and conglutinin bind to N-linked oligosaccharides on the hemagglutinin and neuraminidase of influenza virus via their CRDs (10–12). SP-D recognizes various carbohydrate and lipid moieties and binds monosaccharides and complex oligosaccharides with affinities distinct from those of conglutinin (13). While SP-D binds N-acetyl glucosamine (GlcNAc) and high mannose oligosaccharides with relatively low affinity, conglutinin displays high affinities for both of these saccharide types. Previous in vitro findings demonstrated that the CRD of conglutinin binds to many strains of influenza A more avidly than that of SP-D and displayed considerably greater antiviral activity than SP-D in vitro (14). By contrast SP-D is a more effective agglutinin and can more potently enhance certain aggregation-dependent activities such as neutrophil binding and oxidant responses (12). Studies of an SP-D conglutinin chimera (SP-D/Conglycon) which fused the amino-terminal and collagen domains of SP-D with the neck and CRD of conglutinin, combined attributes of both proteins (15). Thus, in vitro findings suggest that the CRD of conglutinin shares antiviral activities with SP-D and that the amino-terminal and collagen domains contribute to antiviral activity.

Gene targeting in transgenic mice revealed a number of unexpected functions for SP-D in pulmonary homeostasis. While SP-D(−/−) mice survived after birth, the mice developed pulmonary emphysema and accumulations of both tissue and airspace phospholipids (16). Alveolar macrophages from SP-D(−/−) mice were lipid-laden and constitutively expressed matrix metalloproteinases MMP-2, MMP-9, and MMP-12 (17). In

N-acetyl glucosamine; MBL, mannose binding lectin; RSV, respiratory syncytial virus; Cong, conglutinin.
addition, SP-D(-/-) macrophages expressed high levels of H2O2, demonstrating that SP-D plays a critical role in the constitutive regulation of oxidant signaling in alveolar macrophages (18). Increased metalloproteinase expression was at least in part mediated by the spontaneous activation of NF-κB in alveolar macrophages occurring in the absence of SP-D (19). All of these abnormalities were corrected with a genetic rescue in which expression of rat SP-D was targeted to lung epithelial cells (20). However, trimeric SP-D subunits (RrSP-D(ΔSer15,20)) failed to correct the lipodisosis, emphysema, and inflammation in SP-D(-/-) mice in vivo (21), strongly suggesting that the oligomerization of trimeric subunits contribute to the biological activity of SP-D.

SP-D-deficient mice were more susceptible to pulmonary infection and inflammation after exposure to influenza A or respiratory syncytial virus in vivo (18). Viral killing was defective, and lung inflammatory responses were enhanced in the absence of SP-D. It is known that the interactions of SP-D with influenza virus involve binding of the CRDs to N-linked oligosaccharides on viral coat proteins (2). For example, recombinant trimeric neck and CRD domains bind to respiratory syncytial virus (RSV), inhibit infectivity in vitro, and increase viral clearance when administered to RSV-infected mice in vivo (22). However, the precise domains of SP-D required to ameliorate the various metabolic and structural abnormalities seen in the absence of SP-D remain unclear.

In the present studies the SP-D and conglutinin fusion protein SP-D/Congneck+CRD was expressed under control of the lung epithelial-specific promoter element derived from the SP-C promoter in lungs of wild type and SP-D(-/-) mice. We hypothesized that the CRD of conglutinin would restore host defense activities and possibly exert an anti-inflammatory effect while failing to correct surfactant lipid homeostasis. Although the SP-D/Congneck+CRD protein corrected defects in the clearance of influenza virus, it also substantively corrected the abnormal lipid homeostasis. On the other hand, the chimera did not influence alveolar macrophage activation, metalloproteinase expression, or the development of emphysema. These findings strongly suggest that the domains of SP-D regulating oxidant injury and lung remodeling are specific for the neck and CRD of SP-D and are not complementable by the corresponding domains of conglutinin.

**EXPERIMENTAL PROCEDURES**

**Animal Husbandry**—Mice that are described under “Experimental Procedures” were handled in accordance with approved protocols through the Institutional Animal Care and Use Committee at Cincinnati Children's Hospital Medical Center. All mice had been maintained in the vivarium in barrier containment facilities. Sentinel mice in the colony were serologically negative for common murine pathogens.

**Generation of Transgenic Mice**—The 1.3-kb hybrid cDNA encoding SP-D/Congneck+CRD was described previously (15). The cDNA was inserted into the EcoRI site of the 3.7SP-C/SV40 expression vector (23) (Fig. 1). Restriction enzyme digestion confirmed the orientation of the insert. The vector contained the 3.7-kb human SP-C promoter, which drives expression in bronchiolar and alveolar epithelial cells (24) in a pattern similar to that of the endogenous SP-D gene. The transgene was microinjected into fertilized FVB/N oocytes by the Cincinnati Children's Hospital Transgenic Core facility, and founders were identified by Southern blot analysis using a 32p-labeled probe that recognized the SP-D/Congneck+CRD hybrid cDNA. Transgene-specific PCR using the upstream primer 5′-ATA GGA CCC CAA GGC AAA CCA G-3′ and the downstream primer 5′-AGG TTC AGG GGG TGG TGT GG-3′ was also used. Transgenic animals were crossed with SP-D-null mice (16) to generate heterozygous transgenic mice. Heterozygous mice were bred to generate SP-D/Congneck+CRD transgenic mice either in wild type (SP-D/Congneck+CRD +/-), SP-D(-/-) or SP-D-null backgrounds (SP-D/Congneck+CRD -/-).

**FIG. 1. Schematic representation of the SP-D/Congneck+CRD transgene.** The construct was generated by inserting the 1.3-kb SP-D/Congneck+CRD hybrid cDNA into the EcoRI site of the 3.7SP-C/SV40 expression vector. The entire 1.3-kb cDNA fragment was radiolabeled with [α-32p]dCTP and used as a probe for Southern blot analysis. A transgene-specific 1.2-kb PCR product confirmed the genotype using an upstream primer specific for the rSP-D sequence and a downstream primer located in the SV40 intron poly(A) sequence.
against phosphate-buffered saline (PBS), divided into aliquots, and stored at \(-70{\degree}C\).

**Intranasal Instillation of Influenza A Virus—**Six-week-old wild type, SP-D(\(--\)), line 81 and 85 (SP-D/\(\text{Congneck}^{\text{CRD}}\)+), SP-D(\(--\)) mice and and lines 52 and 75 (R2SP-D/H11001) mice and lines 15, 19, 20 (R2SP-D/H11002), SP-D(\(--\)) mice were anesthetized with 3% isofluorane. IAV \(5 \times 10^6\) ffu in 50 \(\mu\)l of PBS was dripped into the nostril for inhalation into lungs. Three days after viral instillation the mice were killed by an overdose of pentobarbital, and their lungs were weighed and homogenized. After centrifugation aliquots of the supernatants were analyzed for IAV titer and cytokine concentrations. IL-6, TNF-\(\alpha\), and IFN-\(\gamma\) concentrations were determined using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN). Statistical differences between mouse lines were evaluated by ANOVA Fisher analysis. Differences of \(p < 0.05\) were considered significant.

**Measurement of Influenza Viral Titer—**For quantitative viral titers of mouse lung homogenates, the entire lung was removed, homogenized in 2 ml of sterile PBS, quick-frozen, weighed, and then stored at \(-80{\degree}C\). Madin-Darby canine kidney monolayers were prepared in 96-well plates for the viral focus assay as previously described (15). The layers were incubated with lung homogenates diluted in PBS containing 2\% calcium for 45 min at 37 \(\text{C}\). The monolayers were washed three times in virus-free Dulbecco’s modified Eagle’s medium containing 1\% penicillin and streptomycin. The monolayers were incubated for 7 h at 37 \(\text{C}\) in Dulbecco’s modified Eagle’s medium and repeatedly washed. The cells were fixed with 80\% (v/v) acetone for 10 min at \(-20{\degree}C\). The monolayers were then incubated with monoclonal antibody directed against IAV nucleoprotein (monoclonal antibody A-3) and then with rhodamine-labeled goat anti-mouse IgG. Fluorescent foci were counted directly under fluorescence microscopy. The resulting titer was divided by the lung weight and reported as fluorescent foci (ffu)/gram of lung.

**Saccharide Binding Specificity of SP-D/Congneck CRD**—We performed the saccharide inhibition ELISAs (13). Microtiter plates were coated with 10 \(\mu\)g/ml yeast mannan in 5 \(\text{mM}\) Na\(_2\)CO\(_3\), 35 \(\text{mM}\) NaHCO\(_3\) at 4 \(\text{C}\) overnight. The plates were washed three times with TBS/NTC (20 \(\text{mM}\) Tris, 140 \(\text{mM}\) NaCl, 5 \(\text{mM}\) CaCl\(_2\), 0.05\% Tween-20) after each subsequent step. After coating, the plates were blocked with 1\% bovine serum albumin in the same buffer for 1 h at room temperature. BALF from wild type, line 81 and 85 SP-D/\(\text{Congneck}^{\text{CRD}}\)(+), SP-D(\(--\)) mice was incubated with increasing concentrations of maltose or GlcNAc, rabbit anti-SP-D antibody (1:1,000 dilution), and peroxidase-conjugated goat anti-rabbit IgG antibody (1:1,000 dilution). After washing, o-phenylenediamine (Sigma) was added to each well, and \(A_{492}\) was read in an ELISA plate reader. The concentration of sugar that inhibited 50\% binding was defined as IC\(_{50}\).

**Lung Morphology—**Lungs were fixed at 25 cm of water pressure with 10\% formaldehyde for 24 h. The lungs were trimmed, weighed, and stored at \(-20{\degree}C\). Lung Morphology—Lungs were trimmed, weighed, and stored at \(-20{\degree}C\). The lung weights were placed and homogenized. Saturated phosphatidylcholine (Sat PC) was measured as previously described (25). Lung phospholipid levels were determined (\(n = 13-28\)) in each genotype. Differences between each genotype were analyzed by ANOVA Fisher. Differences of \(p < 0.05\) were considered significant.

**Lung Morphology—**Lungs were fixed at 25 cm of water pressure with 4\% paraformaldehyde in PBS and processed into paraffin blocks. 7-\(\mu\)m sections from each lobe were stained with hematoxylin and eosin.

**Metalloproteinase Activity Measurement—**Alveolar macrophages (5 \(\times\) 10\(^6\)) were isolated by centrifuging BALF from wild type, SP-D(\(--\)), line 81 and 85 (SP-D/\(\text{Congneck}^{\text{CRD}}\)+), SP-D(\(--\)) mice and cultured for 24 h in 1\% Nutridoma/RPMI 1640 (Roche, Indianapolis, IN). Proteinases in the conditioned media were detected in BALF from both lines. SP-D/\(\text{Congneck}^{\text{CRD}}\) protein was detected in BALF from both lines.

**Expression of SP-D/\(\text{Congneck}^{\text{CRD}}\) Protein—**The calculated molecular mass of SP-D/\(\text{Congneck}^{\text{CRD}}\) fusion protein is virtually identical to that of the SP-D protein (43-kDa monomer). Under reducing conditions, a rabbit anti-mouse SP-D antibody was used to detect the 43-kDa fusion protein in BALF from SP-D/\(\text{Congneck}^{\text{CRD}}\)(+), SP-D(\(--\)) mice in both transgenic mouse lines (Fig. 2A, lower panel, lanes 4 and 8). Because of interchain disulfide bond linkages, SP-D protein in wild type mouse BALF was detected as an oligomer migrating more slowly than the 200-kDa standard (Fig. 2A, upper panel, lanes 1 and 5). The SP-D/\(\text{Congneck}^{\text{CRD}}\) fusion protein from SP-D/\(\text{Congneck}^{\text{CRD}}\)(+), SP-D(\(--\)) mice migrated at the same position when separated by non-reducing SDS-PAGE (Fig. 2A, upper panel, lanes 4 and 8), demonstrating that the SP-D/\(\text{Congneck}^{\text{CRD}}\) fusion protein formed disulfide cross-linked oligomers similar to those formed by SP-D. To assess partitioning of SP-D and the mutant protein in lipids and supernatant, Western blot analysis of the surfactant lipid pellet and supernatant was performed. While mouse SP-D was associated with both the supernatant and lipid phases. SP-D/\(\text{Congneck}^{\text{CRD}}\) fusion protein was not detected in the lipid fraction, but was readily detected in the supernatant (Fig. 2B).

**Saccharide Binding Specificity of SP-D/\(\text{Congneck}^{\text{CRD}}\) Fusion Protein—**To determine the saccharide binding specificity of the secreted SP-D/\(\text{Congneck}^{\text{CRD}}\) fusion protein, inhibition ELISAs were performed using BALF from controls and mice expressing the chimera. The binding of SP-D or SP-D/\(\text{Congneck}^{\text{CRD}}\) to yeast mannan was competitively inhibited with increasing concentrations of maltose or GlcNAc. The IC\(_{50}\) of maltose for binding to BALF from wild type mice was in the range of 2 to 4 \(\mu\)M, consistent with that previously observed with the SP-D protein (13) (Table 1). The IC\(_{50}\) of maltose for BALF from SP-D/\(\text{Congneck}^{\text{CRD}}\)(+), SP-D(\(--\)) mice was 23 \(\mu\)M for line 81 and greater than 50 \(\mu\)M for line 85 (Table 1), demonstrating that the SP-D/\(\text{Congneck}^{\text{CRD}}\) fusion protein had less affinity for maltose, consistent with the saccharide preferences of conglutinin. The IC\(_{50}\) for GlcNAc binding to BALF protein from SP-D/\(\text{Congneck}^{\text{CRD}}\)(+), SP-D(\(--\)) mice was 2–3 \(\mu\)M for lines 81 and 85, but greater than 50 \(\mu\)M for wild type BALF.
Since SP-D has higher affinity for maltose than GlcNAc, and conglutinin has a higher affinity for GlcNAc than maltose (26), the secreted SP-D/Congneck CRD fusion protein binds saccharides with a selectivity similar to that of conglutinin.

**Correction of Lung Phospholipids by SP-D/Congneck CRD Fusion Protein**—Expression of SP-D/Congneck CRD transgene in the wild type background did not perturb alveolar, tissue, or total Sat PC levels (Fig. 3, panels A and C). However, expression of SP-D/Congneck CRD fusion protein in SP-D(−/−) background did not correct the emphysema and foamy macrophage accumulation typical of SP-D(−/−). These findings are distinct from previous studies in which the SP-C promoter was used to express the wild type rat SP-D protein in SP-D(−/−) mice (20). Emphysema, enlarged, and foamy macrophages (arrowheads) and peribronchiolar lymphocyte aggregates (arrowheads) were detected in both transgenic mouse lines in SP-D(−/−) background (Fig. 4, panels E and F). Findings identical to those in SP-D(−/−) mice (Fig. 4, panel D).

**MMP-9 and MMP-2 Activity in Alveolar Macrophages**—Protease activity gels were used to assess the level of production of MMP-9 and MMP-2 by alveolar macrophages isolated from wild type, SP-D(−/−) and SP-D/Congneck CRD(+/−), SP-D(−/−) mice. While MMP-9 and MMP-2 production was barely detected in conditioned media from wild type mice (Fig. 5, lane 1), metalloproteinase activities were markedly increased in both lines of SP-D(−/−) mice expressing the SP-D/Congneck CRD protein (Fig. 5, lanes 3 and 5).

**Correction of Influenza A Infection by the SP-D/Congneck CRD**—In vitro studies previously demonstrated that the SP-D/Congneck CRD fusion protein had enhanced anti-influenza activity compared with SP-D or conglutinin (15). To evaluate anti-influenza A viral activity of the SP-D/Congneck CRD fusion protein in vivo, IAV (5 × 10³ ffu) was administered into mouse lungs intranasally, and viral titers were measured in lung homogenates three days later. While the viral titer from SP-D(−/−) mouse lung homogenates was 16,052 ± 2,326 ffu/g of tissue (mean ± S.E., n = 10), no detectable IAV was recovered from lung homogenates from wild type or from SP-D/Congneck CRD(+/−), SP-D(−/−) mice for either line 81 or line 85, demonstrating complete correction of viral clearance. Significantly increased IL-6, TNF-α, and IFN-γ were observed in lung homogenates from SP-D(−/−) compared with wild type mice. In contrast, cytokine concentrations in SP-D/Congneck CRD(+/−) (both line 81 and line 85) mice were not different from those from wild type mice following infection (Fig. 6), demonstrating complete correction of both viral clearance and inflammatory responses by the chimeric protein.

To further determine the role of CRD and oligomerization in viral clearance we tested whether another mutant SP-D protein, RrSP-DSer15,20, which consists of functional trimeric CRDs but cannot form disulfide cross-linked oligomers mediated by the NH₂-terminal Cys residues (21), restored the clearance of IAV in vivo. Two lines of mice expressing the mutant protein in the SP-D(−/−) background were tested. In the RrSP-DSer15,20, SP-D(−/−) mice (line 52) in which mutant protein is expressed at levels similar to those of wild type mice, viral clearance was not corrected, and cytokine expression was not inhibited following IAV administration (Fig. 7, lane 3). In line 75, which expressed the RrSP-DSer15,20 at levels greater than in wild type mice, viral clearance and cytokine production were substantially but not completely corrected (Fig. 7, lane 2).
DISCUSSION

SP-D plays multiple roles in pulmonary homeostasis including regulating phospholipid metabolism, innate host defense, inflammation, and airspace remodeling (16, 17, 27, 28). In the present study we determined whether a chimeric protein consisting of the neck and CRD of conglutinin and the collagenous and amino-terminal domains of SP-D complemented specific functions of SP-D in the regulation of pulmonary homeostasis in vivo. The chimeric protein formed SP-D-like oligomers in vitro, bound saccharides in the manner of conglutinin, and was secreted into the airspace. Interestingly, unlike native SP-D the chimera did not bind surfactant lipids, partitioning in the BALF supernatant. SP-D/ConCRD completely restored antiviral activity and substantially reduced surfactant phospholipid levels in the lungs of SP-D(-/-) mice. In contrast, the chimeric protein did not influence the lymphocytic infiltration, metalloproteinase activation, or emphysema caused by the absence of SP-D. Thus, the regions in the SP-D CRD required for lipid binding, complementation of emphysema, and metalloproteinase activation are distinct from those mediating viral clearance and phospholipid homeostasis. In addition, the presence and severity of emphysema was not influenced by reduction of lung phospholipid concentrations.

While the structures of various members of the collectin family are relatively well conserved, differences in the tissue distribution of expression, affinities for carbohydrates, and the distinct interactions with ligands underlies the collectin family's distinct physiologic roles (1, 29). Despite the conservation of the predicted tertiary structures of the SP-A and SP-D CRDs, in vivo studies demonstrate that the lung collectins play unique roles in the organization of lipids, innate defense, and pulmonary homeostasis, suggesting that each CRD mediates distinct activities and that the two proteins are not interchangeable. Table II. Gene targeting studies demonstrated distinct alterations in pulmonary function in SP-A as compared with SP-D knockout mice. While tubular myelin was absent and host defense against viral, bacterial, and fungal pathogens were deficient in SP-A(-/-) mice, no abnormalities in lung structure, function, or phospholipid metabolism were observed in vivo (25). In contrast, SP-D-deficient mice spontaneously
developed pulmonary lipidosis and emphysema in association with activation of alveolar macrophages and increased metalloproteinase production (16, 17). Complementation studies demonstrated that pulmonary abnormalities in SP-D knockout mice were completely corrected by the expression of the appropriate recombinant wild type protein in vivo. Increased levels of SP-A and SP-D did not perturb lung function or homeostasis in vivo (20, 30).

In recent studies we found that a chimeric protein containing the collagen domain, neck, and CRD of SP-A and the NH₂-terminal domain of SP-D did not rescue pulmonary lipid homeostasis or emphysema in SP-D(−/−) mice. This suggests that the alveolar macrophage activation, increased metalloproteinase production, and development of emphysema are rescued by domains specific for the SP-D CRD and that are not complemented by the conglutinin CRD. Furthermore, phospholipid binding is not required for correction of the pulmonary lipidosis in SP-D(−/−) mice.

In the present study, the SP-D/Cong neck was expressed under control of the SP-C promoter. By SDS-PAGE the amount of mutant protein was similar to that seen in wild type SP-D(+/+) mice; however, the precise levels were not directly determined since we utilized a cross-reacting anti-SP-D antibody for Western blot analysis. This promoter expresses transgenes in respiratory epithelial cells in bronchiolar and alveolar regions of the lung (24) at sites similar to those in which SP-D is normally expressed (32, 33).

The functions of SP-D were correlated with outcomes of studies comparing wild type SP-D and SP-D(−/−) mice in which the 3.7-kb SP-C promoter was used to replace the mutant protein in the SP-D(−/−) mice. The size and oligomeric structure of the SP-D(−/−) mouse in vivo (21). Notably, in the current studies we found that the same SP-D mutant, when expressed at high concentrations in vivo, significantly improved viral clearance and blunted the inflammatory response to virus.

These findings provide further support for the concept that distinct molecular structures are required for various functions of SP-D. While interactions with saccharide ligands and viral particles do not require oligomerization mediated by the NH₂-terminal domain, the correction of phospholipid homeostasis, metalloproteinase activation, and emphysema require the oligomerization of trimeric subunits and specific structural features of the neck and CRD domains (Table II). In particular, the conglutinin neck and CRD could substitute for the corresponding domains of SP-D with respect to viral clearance and phospholipid accumulation but could not bind phospholipid or prevent metalloproteinase activation and the development of emphysema in SP-D(−/−) mice. Thus, the alveolar macrophage activation, augmented metalloproteinase production, and development of emphysema occur by distinct mechanisms that are rescued by domains specific for the SP-D CRD and that are not complemented by the conglutinin CRD. Furthermore, phospholipid binding is not required for correction of the pulmonary lipidosis in SP-D(−/−) mice.

In addition, a single arm mutant SP-D, in which the two amino-terminal cysteine residues were replaced with serine (RrSP-DSer15,20), inhibited the formation of larger SP-D oligomers in normal mice but failed to correct the increased phospholipid levels or other abnormalities seen in the SP-D(−/−) mouse.

TABLE II
Correlation of SP-D structure to SP-D function

| Function                  | SP-D⁻¹ | SP-D/Cong | SP-A/SP-D⁻¹ | RrSP-D⁻¹<sup>Ser15,20</sup> |
|---------------------------|--------|-----------|-------------|-----------------------------|
| Sugar binding             | ++     | +         | +           | ++                          |
| Phospholipid              | ++     | +         | -           | ++                          |
| IAV binding or clearance  | +      | +         | ND<sup>a</sup> | +                           |
| Emphysema                 | +      | +         | -           | -                           |
| Macrophage activation     | +      | +         | -           | -                           |
| Metalloproteinase production | +     | -         | -           | -                           |
| Oligomerization           | 12-mer | 12-mer    | 8-mer       | 3-mer                       |

<sup>a</sup> Ref. 20.  
<sup>b</sup> Footnote 2.  
<sup>c</sup> Complete rescue of the abnormalities seen in SP-D(−/−) mice.  
<sup>d</sup> Partial rescue of the abnormalities seen in SP-D(−/−) mice.  
<sup>e</sup> No complementation.  
<sup>f</sup> Not determined.
SP-D. The migration of the chimeric protein suggests comparable glycosylation, and N-glycanase treatment similarly increased the mobility of SP-D and SP-D/Cong_neck+CRD. In addition, the secreted protein in BALF showed a relative carbohydrate selectivity typical of conglutinin. Given the known in vitro antiviral activities (15), the complete correction of IAV clearance (and substantial correction of phospholipid homeostasis) strongly suggests that assembly, secretion, and function of the chimera is retained in vivo. Thus, the failure to ameliorate emphysema, correct the accumulation of foamy macrophages, and inhibit metalloproteinase activation further suggest that specific structural features of the SP-D neck + CRD are required for these functions. Thus, the SP-D CRD mediates viral clearance and phospholipid metabolism through processes distinct from those regulating emphysema and macrophage activation.

The present findings suggest that structural differences in the SP-D and conglutinin neck+CRD domains differentially influence abnormalities that characterize the complex SP-D-null phenotype. These findings further suggest that the processes leading to abnormal lipid homeostasis and the inflammatory and structural changes in the absence of SP-D involve distinct pathways and signaling mechanisms. Because the emphysema, lymphocytic and macrophage infiltration, and increased metalloproteinase production persist in the presence of the chimera despite a substantial correction of phospholipids, we also conclude that these processes are largely independent of the lipid accumulation.

The correction of antiviral deficits by the conglutinin CRD was anticipated because of the known potent effects of conglutinin on influenza A and certain respiratory viruses in vitro (14). Similarly, the failure to normalize the inflammatory milieu in the lungs is readily rationalized given that SP-D and conglutinin are different, albeit genetically related, proteins (14). Similarly, the failure to normalize the inflammatory milieu in the lungs is readily rationalized given that SP-D and conglutinin are different, albeit genetically related, proteins (14).

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