Surfactant protein-D regulates the postnatal maturation of pulmonary surfactant lipid pool sizes

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Ikegami M, Grant S, Korchagna T, Scheule RK, Whitsett JA. Surfactant protein-D regulates the postnatal maturation of pulmonary surfactant lipid pool sizes. J Appl Physiol 106: 1545–1552, 2009—Surfactant protein (SP)-D plays an important role in host defense and pulmonary surfactant homeostasis. In SP-D-deficient (Sftpd−/−) mice, the abnormal large surfactant forms seen at the ultrastructural level are taken up inefficiently by type II cells, resulting in an over threefold increase in the surfactant pool size. The mechanisms by which SP-D influences surfactant ultrastructure are unknown. We hypothesized that SP-D binds to surfactant immediately after being secreted and influences surfactant ultrastructure conversion. In newborn and adult sheep lungs, immunogold-labeled SP-D was associated with both lamellated membranous lipid structures of newly secreted surfactant and with small aggregate surfactant but not with tubular myelin. Since SP-D preferentially binds to phosphatidylinositol (PI) in vitro, the postnatal changes in PI were assessed. PI content in the bronchoalveolar lavage fluid increased after birth and peaked at 2–5 days of age, a time of rapid conversion of surfactant forms that is associated with the peak of surfactant lipid pool size. SP-D selectively interacted with PI-rich liposomes in vitro, causing their lysis. Similarly, the abnormal surfactant ultrastructure in Sftpd−/− mice was corrected by the addition of SP-D or melitin, and both peptides caused lysis of lipid vesicles. The normal conversion of surfactant ultrastructure requires SP-D that preferentially interacts with PI-rich, newly secreted surfactant, causing lysis of surfactant lipid membranes, converting the lipid forms into smaller surfactant lamellated structures that are critical for surfactant uptake by type II cells and normal surfactant homeostasis. SP-D regulates the dramatic decreases in the surfactant pool size that occurs in the newborn period.

postnatal lung development; surfactant protein D−/− mice; surfactant ultrastructure; phosphatidylinositol

Surfactant protein (SP)-D is a member of the collectin family of C-type lectins. In addition to its important role in the innate defense of the lung (6, 26), SP-D regulates surfactant lipid pool sizes in adult mice (15) and is likely required for the dramatic postnatal decrease in surfactant pool size that occur in normal newborn periods. Uptake of particles by alveolar type II cells is selective and affected by charge and size (39). Ultrastructure conversion of surfactant to a smaller size after being secreted is critical for the normal uptake of surfactant by type II cells. In SP-D-deficient (Sftpd−/−) mice, the lamellated structure of surfactant lipids present in bronchoalveolar lavage fluid (BALF) was large, and the normal ultrastructure was disrupted. Surfactant uptake by alveolar type II cells was decreased (15) in Sftpd−/− mice and associated with a three- to fivefold increase in surfactant pool size (3, 21). Surfactant ultrastructure and pool size change rapidly during normal newborn periods, peaking at 2–5 days of age (4, 9). In Sftpd−/− mice, neither surfactant ultrastructure nor surfactant pool sizes show these normal postnatal developmental changes but are rapidly normalized by replacement of SP-D (8, 15, 44). Despite these findings, the mechanisms by which SP-D influences surfactant ultrastructure are unknown. We hypothesized that SP-D binds to surfactant lipids immediately after being secreted, thereby lysing surfactant lipid membranes, and converting its form to smaller surfactant structures that are actively taken up by type II cells. To test this hypothesis, we used the newborn lamb, in which BALF, rich in newly secreted surfactant, can be recovered for experiments.

Surfactant in the airways exists as multiple structural forms that can be separated into large aggregate (LA) and small aggregate (SA) surfactant fractions from BALF by centrifugation (25). LA consist of lamellar body and tubular myelin, is surface active, and contains SP-A, SP-B, and SP-C, whereas SA is rich in small lipid vesicles that do not contain SP-A, SP-B, and SP-C and is not surface active. Surfactant lipids are secreted as lamellated membranous sheets that are found in LA surfactant and are converted to SA over time (9, 40). Most SP-D in BALF is soluble (nonlipid associated), present in SA-containing fractions, and not readily detectable in LA by Western blot analysis (15, 23), yet SP-D is required for the formation of normal LA structures in the alveolus. The phospholipid composition of LA is the same as SA in the adult lung (41), supporting the concept that changes in phospholipid composition alone do not account for surfactant form conversion. In vitro, SP-D is merely associated with surfactant lipid, preferentially binds to phosphatidylinositol (PI; a minor surfactant phospholipid), and does not bind to saturated phosphatidylcholine (Sat PC). Whether the PI composition in surfactant changes after birth and influences interactions with SP-D are presently unknown (23, 31).

In this study, we demonstrated that 1) postnatal changes in surfactant PI content accompany the association of SP-D with surfactant lipids; 2) SP-D is selectively associated with newly secreted surfactant lipids in newborn and adult lungs; and 3) SP-D influences surfactant structure, at least in part, by its PI-dependent lytic activity on phospholipid membranes.

MATERIALS AND METHODS

Sheep. Term newborn lambs at the ages of 1, 2, 3, 5, and 10 days and adult sheep (n = 4 animals/group) were used for the experiments. Protocols were approved by the Animal Care and Use Committee of the Cincinnati Children’s Research Foundation. This large animal model enables multiple measurements on the same animal. Intravascular blood coagulation was inhibited by an intravenous injection of heparin, and an overdose of pentobarbital (100 mg/kg) was given intravenously followed by exsanguination. The lungs were carefully
excised from the chest cavity and weighed. The right upper lobe was slowly inflated to 25 cmH2O as the total lung capacity and then reduced and maintained at 5 cmH2O during perfusion fixation with 2.5% glutaraldehyde (Electron Microscopy Sciences, Washington, PA) in PBS. BALF was recovered from the left lung by filling airways with 0.9% NaCl at 4°C until the lung was visually distended. The bronchoalveolar lavage was repeated three times and then pooled. An aliquot of BALF was used for analyses of Sat PC (2, 27), SP-D content by ELISA (11), and phospholipid composition by TLC (12).

Electron microscopy and immunogold labeling. Fixed tissues were divided into six portions, cut into 1- to 2-mm³ pieces for postfixation in 2% osmium tetroxide in 0.1 M sodium cacodylate buffer with 1.5% potassium ferrocyanide, 4% uranylacetate solution, and then dehydrated by a series of ethanol solution and propylene oxide. Two pieces of fixed tissues were blindly selected from each portion and embedded in resin (Embed 812, Electron Microscope Sciences). Ultrathin (<90 nm) sections were prepared by an ultramicrotome (Ultra Cut E, Reichert-Jung, Vienna, Austria) and examined using an electron microscope (H7600, Hitachi High-Techologies America, Pleasanton, CA). Lamellar body sizes were quantified in four randomly selected areas on a 200-mesh grid (total: 0.04 nm²) as previously described (15). SP-D was labeled with 10-nm immunogold (Nanoprobes, Yaphank, NY) as previously described (29) using a polyclonal antibody against ovine SP-D (1:100) previously developed in our laboratory.

Association of recombinant human SP-D with surfactant phospholipids. Recombinant human (rh)SP-D was synthesized as previously described (11) by the transfection of Chinese hamster ovary cells with cDNA encoding full-length hSP-D. The biological activities of rhSP-D in host defense have been previously demonstrated (11, 16). To determine whether postnatal changes in PI composition in surfactant influence the association of SP-D, rhSP-D (0.1% of total phospholipid) was added to phospholipid liposomes containing various amounts of PI. The mixture of dipalmitoyl-L-α-phosphatidylcholine (DPPC, Sigma, St. Louis, MO) and 1-palmitoyl-2-oleoyl-phosphatidylglycerol (PG; Avanti, Birmingham, AL) (7:3), which contained 0% and 2% (adult level) and 7% (newborn level) 1-α-PI (Avanti) or DPPC alone in chloroform, were dried under nitrogen, and liposomes were made by two sonications for 20 s in a buffer containing 10 mM Tris·HCl, 2.5 mM CaCl₂, 1 mM MgSO₄, 0.1 mM EDTA, and 15 mM NaCl (pH 7.0). After a 10-min incubation with rhSP-D at 37°C, phospholipid liposomes were isolated as a pellet by 15 min of centrifugation at 40,000 g, and the percent recovery of rhSP-D in the pellet relative to the amount of rhSP-D added to the phospholipid mixtures was calculated.

Lytic activity of SP-D on surfactant phospholipid membranes. Melittin is the principal component of the venom of the honeybee and has strong lytic activity on phospholipid membranes (28). To test whether the abnormal surfactant ultrastructure in Sfpid⁻/⁻ mice can be corrected by lysis of phospholipid layers, melittin (Sigma, 1.5% of total phospholipid) was added to pooled BALF from two Sfpid⁻/⁻ mice containing 1.2 mg phospholipid and incubated at 37°C for 2 h with slow rotation. LAs were isolated by centrifugation, and the ultrastructure of the LA was determined as previously described (15, 18). Briefly, isolated LAs were fixed and stained with osmium tetroxide, potassium ferrocyanide, and uranyl acetate. Fixed tissues were dehydrated, embedded in Embed 812 resin, and cut into ultrathin (90 nm) sections from duplicated epon blocks from each group. Sections were transferred to 200-mesh copper grids. Five to ten electron micrographs were randomly taken at 1 section/block, and the ultrastructure was evaluated. The Tris buffer containing 2.5 mM CaCl₂ used for bronchoalveolar lavage of Sfpid⁻/⁻ mice was the same as described above for the creation of liposomes. Surfactant ultrastructure after the addition of rhSP-D (1.5% of total phospholipid or 18 μg) to pooled BALF from two Sfpid⁻/⁻ mice was studied and compared with that of melittin. The same dose of rhSP-D as that of rat SP-D, which corrected the surfactant ultrastructure of Sfpid⁻/⁻ mouse BALF (15), was used for this study.

The lysis of surfactant phospholipid membranes by rhSP-D was measured as previously described for SP-B (34). Briefly, liposomes containing a total of 2 mg DPPC-PG (7:3), with or without 7% PI and 2 mg DPPC-PI (7:3), were generated in the presence of 12.5 mM 1-aminonaphthalene-3,6,8-trisulfonic acid (ANTS), 45 mM NaN₃, (p-phenylendimethylene)-(bis-(pyridinium bromide) (DPX; Invitrogen, Carlsbad, CA) by two sonication for 20 s. Encapsulated probes were separated from free probes by 10 min of centrifugation (2,000 g) repeated three times. Membrane disruption causes the deletion of the encapsulated ANTS probe from the DPX quencher, resulting in an increase in fluorescence. rhSP-D [6%: a dose similar to that in normal newborn lamb BALF (15)] or melittin [30 μg: a dose previously demonstrated to have strong lytic activity (34)] was introduced to the liposomes 20 s after the fluorescence intensity recording had begun. The excitation of ANTS was measured at 360 nm and emission was recorded at 520 nm using a RatioMaster fluorometer (Photon Technology, South Brunswick, NJ) for 10 min. Fluorescence intensity at 10 min for melittin was used as the maximum (100%) lytic activity. Fluorescence intensity relative to the maximum lytic activity of melittin is presented.

Statistics. Results are presented as means ± SE. Comparisons among groups were made by ANOVA with Tukey’s tests used for post hoc analyses. Significance was accepted at \( P < 0.05 \).

RESULTS

Postnatal changes in Sat PC, SP-D pool sizes, and lamellar body sizes in BALF. The synthesis and storage of pulmonary surfactant by type II cells increase dramatically at the end of gestation as the fetus prepares for the transition to air breathing. In the neonatal lamb, surfactant is secreted immediately at birth, and the Sat PC pool size in BALF increases postnatally, peaking at 3–5 days of age, and then gradually decreases to a lower adult level (Fig. 1A). SP-D in BALF increased after birth (Fig. 1B) in a pattern similar to that of Sat PC, but the increase in SP-D was proportionately greater than that of Sat PC. Therefore, SP-D relative to Sat PC (Fig. 1D) from 1 to 3 or 5 days of age was increased 10-fold. Lamellar body sizes in the alveoli were larger in 1- to 3-day-old newborn lambs and gradually decreased with age (Fig. 1C). Thus, surfactant lipids are secreted as large, loosely packed lipid particles that progressively decrease in size during a period in which SP-D concentrations are relatively high. Percentages of Sat PC in LA relative to total Sat PC (LA + SA) were 88.9 ± 2.1% at 1 day of age, 73.2 ± 9.5% at 2 days of age, and gradually decreased to the adult sheep level of 47.0 ± 0.1%. The pattern of change with age in SP-D in LAs (Fig. 1E) was similar to that in BALF (Fig. 1B). Interestingly, the percentage of SP-D in LA was highest at 1 day of age and gradually decreased to the level seen in the adult by 10 days of age (Fig. 1F). Unlike the newborn lung, LA from the mature lung consisted of not only newly secreted large lamellated forms of surfactant but also included smaller lamellar bodies and tubular myelin. SP-D in LAs in 10-day-old and adult sheep BALF was <10% of total SP-D. Taken together, these experiments demonstrated the selective association of SP-D with newly secreted surfactant.

Association of SP-D with surfactant lipid membranes. The association of SP-D with surfactant lipid membranes was demonstrated in alveoli using immunogold labeling. In the newborn lamb, newly secreted surfactant consisted primarily of loose lamellated forms. In contrast, SA surfactant and tubular myelin were more abundant in the adult than in new-
borns. The pattern of association of SP-D with various surfactant forms, including newly secreted loose lamellated forms, lamellar bodies, tubular myelin, and SA lipid vesicles, was similar at all ages studied: 1, 2, 3, 5, and 10 days and adults. Representative electron micrographs for each of the surfactant forms are shown in Figs. 2 (5-day-old lamb) and 3 (adult sheep). SP-D was closely associated with newly secreted surfactant, consisting of primarily of loosely packed lipid layers (Fig. 2, A and B, arrowheads). In the adult sheep lung, immunogold-labeled SP-D was detected primarily along the surface of lipid membranes of lamellar bodies (Fig. 3, A and C, arrowheads). SP-A was located at each corner of tubular myelin as described in a previous immunogold-labeling study (37) for SP-A. In contrast to the location of SP-A, little or no SP-D was associated with tubular myelin (Fig. 3, A and B, arrows). SP-D was closely associated with SA forms of surfactant (Fig. 3C, arrows). The mechanisms that determine the selective association of SP-D with SA surfactant rather than with tubular myelin are currently unknown.

Association of rhSP-D with specific surfactant phospholipids. The composition of phospholipids in BALF as well as LA and SA isolated from BALF were studied in the newborn lamb and adult sheep (Fig. 4). PI increased 2–5 days after birth and gradually decreased to the normal adult level in BALF, LA, and SA (Fig. 4, A, C, and D). The overall pattern of percent PI changes in LA and SA after birth was similar to that in BALF. In contrast, PG was higher in adult sheep than newborns, and there were no differences in PG content from birth to 10 days of age (Fig. 4B). Most of the surfactant lipids were LA in 1-day-old lamb BALF and the amount of SA sample was not sufficient to study surfactant phospholipid composition (Fig. 4D, not available). There were no other significant differences in postnatal surfactant phospholipid composition in BALF, LA, and SA, including phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin (data not shown). The mechanisms as to why SP-D does not associate with tubular myelin are currently unknown.

The association of rhSP-D with phospholipid liposomes containing DPPC, PG, and PI (7% in the newborn lung and 2% in the adult) was studied (Fig. 5). The association of rhSP-D with phospholipid liposomes increased as the PI content in the phospholipid mixtures increased. Since most surfactant in newborn BALF consisted of newly secreted surfactant that was rich in PI, the preferential association of SP-D with newly secreted surfactant in the newborn is likely related to its selective interaction with the PI-rich lipids in these forms. SP-D did not bind to DPPC vesicles and bound weakly to DPPC-PG-containing vesicles compared with its binding to PI-rich liposomes.

Lytic activity of SP-D on surfactant phospholipid membranes. The abnormally large LA forms in BALF from SftpD−/− mice were corrected by incubation with rhSP-D in vitro (Fig. 6), demonstrating the biological activity of rhSP-D for surfactant ultrastructure conversion. To test whether a protein with known membrane lytic activity could induce the surfactant ultrastructure conversion, BALF from SftpD−/− mice (Fig. 7A) was incubated with melittin, a protein with high lytic activity. The large lamellar bodies characteristic of SftpD−/− mice were corrected by the addition of melittin (Fig. 7, B and C). To test whether SP-D has lytic activity, the disruption of liposome
membranes by rhSP-D was monitored by recording the release of the fluorescent probe ANTS and its quencher DPX from encapsulated liposomes of DPPC-PG with or without PI and DPPC-PI without PG. The changes in fluorescent intensity from 40 s to 10 min were similar between DPPC-PG-PI (25.8 ± 1.0) and DPPC-PG (25.6 ± 1.2), whereas changes in DPPC-PI-containing vesicles were only 2.2 ± 0.4. As demonstrated in Fig. 5, SP-D weakly binds to PG, and this lipid likely contributes to the continuous lysis of phospholipid membranes mediated by SP-D.

**DISCUSSION**

Surfactant pool size is maintained at defined levels by regulating its synthesis, secretion, surfactant structure conversion, uptake by type II cells for catabolism and recycling, and uptake by alveolar macrophages for catabolism (10, 13). The importance of surfactant structural conversion in surfactant metabolism has been recognized for more than two decades (25, 40), whereas the role of SP-D in surfactant ultrastructure was unknown until recently (15), when SftpΔ−/− and conditional SftpΔ mice were developed (3, 21). Phagocytosis of particles by alveolar type II cells is selective and affected by charge and size (39). Uptake of the larger particles by type II cells was lower than that of smaller particles. The influence of SP-D on surfactant ultrastructure size is critical for the normal continuous increase in lysis seen throughout the course of the incubation was detected in PG-containing liposomes of DPPC-PG-PI and DPPC-PG. The changes in fluorescent intensity from 40 s to 10 min were similar between DPPC-PG-PI (25.8 ± 1.0) and DPPC-PG (25.6 ± 1.2), whereas changes in DPPC-PI-containing vesicles were only 2.2 ± 0.4. As demonstrated in Fig. 5, SP-D weakly binds to PG, and this lipid likely contributes to the continuous lysis of phospholipid membranes mediated by SP-D.

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uptake of surfactant by type II cells. A previous study (15) with adult Sftpδ−/− mice and conditional Sftpδ mice demonstrated that the presence of SP-D decreased the size of alveolar LA and SA surfactant forms at the ultrastructural level, which, in turn, increased surfactant uptake by type II cells. The mechanisms by which SP-D influence surfactant structure were unknown.

Almost all the SP-D in BALF remained in the supernatant during the isolation of functionally active LA surfactant by centrifugation (23). SP-D was not detectable in LAs by Western blot analysis (15). In the present study, SP-D content in BALF and LAs was analyzed by ELISA. Approximately 8% of SP-D in BALF was associated with LA surfactant in the adult lung. While the content of various surfactant structural forms changes with age, the pattern of association of immunogold-labeled SP-D with various surfactant forms, including newly secreted loose lamellated forms, lamellar bodies, tubular myelin, and SA lipid vesicles, was similar at all ages. Taken together, SP-D likely associates with newly secreted surfactant in the lung at all ages. PI was increased in BALF and isolated LAs and SAs in 2- to 5-day-old newborns, the ages at which surfactant levels were maximal. The high levels of PI in newborn lamb BALF and LAs were similar to that of intracellular lamellar bodies isolated from adult rabbit lung tissues (7.7%) (32), supporting the concept that newly secreted lamellar bodies contain higher levels of PI at all ages. We found that SP-D preferentially associated with liposomes composed of phospholipid mixtures containing high levels of PI, characteristic of newly secreted surfactant. Taken together, we propose

![Fig. 4. Changes in phosphatidylinositol (PI) and 1-palmitoyl-2-oleoyl-phosphatidylglycerol (PG) content in BALF, LA, and SA with age. The phospholipid composition in BALF was analyzed by TLC followed by a phosphorus assay. A, C, and D: percentages of PI increased up to 3–5 days of age and then gradually decreased to normal adult levels in BALF (A), LA (C), and SA (D). Most surfactant consisted of newly secreted LAs at 1 day of age, and SA surfactant concentrations were not adequate to perform phospholipid composition analyses. NA, not available. B: percentages of PG were low in 1- to 5-day-old newborn lambs and then increased to that of adult sheep. The composition of other phospholipids was similar in the groups. n = 4 animals/group. *P < 0.05 vs. adult sheep.](Figure 4)

![Fig. 5. Association of SP-D with surfactant phospholipid depends on the PI content. Recombinant human (rh)SP-D was added to dipalmitoyl-L-phosphatidylcholine (DPPC)-PG-containing liposomes containing 0%, 2%, or 7% PI. The percent recovery of rhSP-D in isolated phospholipid liposomes is shown. The association of rhSP-D with phospholipid liposomes increased as the PI content increased. There were small but significant differences in the binding of SP-D to liposomes containing DPPC alone and DPPC + PG, suggesting that there is some association of rhSP-D with PG. The association with PG was significantly less than with PI. n = 3 animals/group. *P < 0.05 vs. 0% PI; †P < 0.05 vs. 2% PI.](Figure 5)

![Fig. 6. rhSP-D corrects the abnormal ultrastructure of surfactant from SP-D-deficient (Sftpδ−/−) mice. The addition of rhSP-D to BALF isolated from Sftpδ−/− mice (Sftpδ−/− + rhSP-D) modified the ultrastructure of LA, changing it to a structure similar to that of normal (Sftpδ+/+) mice. A: representative surfactant ultrastructures for each group are shown. B: relative LB sizes in BALF from Sftpδ−/− mice (value given as 1) were corrected by the addition of rhSP-D, which converted LB sizes to sizes similar to those in Sftpδ+/+ mice. n = 3 experiments/group with 200 LBs/group. *P < 0.05, Sftpδ−/− mice vs. other groups.](Figure 6)
a model in which SP-D selectively associates with newly secreted surfactant that is PI enriched. In contrast to PI, PG increased with age to a mature lung level of 5.5% of phospholipid content. PI and PG are acidic phospholipids, and the content fatty acid species are comparable. Both PI and PG are likely to be synthesized de novo from cytidinediphosphate diacylglycerol in lung microsomes. Functionally, PI and PG can replace each other as a component of surfactant (1, 33).

While the association of SP-D with DPPC-PG-containing liposomes with 7% PI were higher, SP-D also weakly associated with DPPC-PG-containing liposomes with adult levels of PI (2%) and DPPC-PG without PI. Furthermore, a continuous increase in vesicle lysis caused by rhSP-D was observed in liposomes containing PG. Binding and lysis of surfactant lipid membranes by SP-D are most active on surfactant containing high newborn levels of PI and are less active on surfactant containing lower, mature levels PI and higher level PG characteristic of the adult lung. SP-D lyases or permeabilizes surfactant lipid layers to influence the ultrastructure of newly secreted lamellated forms, converting them to smaller lamellar bodies. Over 30% of SP-D in BALF associates with newly secreted LAs and plays a role in the dramatic decrease of surfactant pool sizes occurring after birth. While only a small portion of the SP-D in BALF associates with LA in the mature lung, SP-D is likely associated with newly secreted surfactant, where it plays a critical role in surfactant homeostasis. The majority of SP-D in the SA fraction in the mature lung likely plays an important role in pulmonary innate defense. The importance of SP-D in the neonatal period may be selectively influenced by the remarkably large surfactant pool size when regulation is dependent on the lipid flux ratio in multiple cellular and extracellular compartments. The lack of SP-D exaggerates the already expanded pool of newly secreted surfactant in the neonate.

The structure of SP-D is critical for its function. Mouse SP-D cDNA encodes a primary translation product consisting of 335 amino acids with a distinct NH2-terminal collagenous domain that contains Gly X-Y repeats that are linked to a...
COOH-terminal carbohydrate recognition domain (CRD) (7). SP-D forms larger-molecular-weight oligomers that form trimers that are stabilized by disulfide bonds and subsequently higher-ordered multimers, producing a wheel-like dodecameric structure that is stabilized by interchain sulfdryl bonds. The structure of rhSP-D is similar to that of native SP-D (11). Similar to native SP-D, synthesized full-length rhSP-D migrated as a trimer on SDS-PAGE gels under nonreducing conditions, and multimeric forms of rhSP-D have been demonstrated by size-exclusion chromatography (11) and atomic force microscopy (16). Our previous SP-D structure-function studies (8, 18, 30, 43–45) in vivo using transgenic mice expressing mutant SP-D proteins in Sftpdp<sup>−/−</sup> mice demonstrated that 1) the effects of specific SP-D structures on surfactant homeostasis can be dissociated from SP-D’s effects on macrophage activation and emphysema, 2) oligomerization is required for the optimal function of SP-D in surfactant homeostasis, and 3) the influence of SP-D on surfactant structure and surfactant metabolism is not mediated by CRD alone. CRD is known to mediate binding of SP-D to pathogens. The cleft between the three CRDs presents an extended positively charged surface that facilitates binding to negatively charged structures, such as LPS (19, 22, 24). While specific binding regions in CRD to PI have been previously demonstrated (5, 35, 36), the CRD region of SP-D alone did not influence surfactant ultrastructure. The expression of a chimeric SP-D mutant, namely, SP-A (NH<sub>2</sub>-terminus, collagenous domain) + SP-D (neck, CRD), as well as deletion of the collagen domain of SP-D, both of which contain the neck and CRD of SP-D, failed to correct the increased surfactant pool sizes characteristics of Sftpdp<sup>−/−</sup> mice (18, 45). Likewise, incubation of BALF from Sftpdp<sup>−/−</sup> mice with human and mouse neck and CRD of SP-D did not influence the ultrastructure of surfactant from Sftpdp<sup>−/−</sup> mice (data not shown). In contrast, SP-D polypeptide, consisting of the NH<sub>2</sub>-terminus and collagenous domain of SP-D, partially corrected surfactant homeostasis in Sftpdp<sup>−/−</sup> mice (42). Thus, neither the neck nor CRD alone were sufficient for full interactions with surfactant lipids to influence surfactant ultrastructure. rhSP-D used in the present in vitro study is full-length SP-D, which forms oligomers and corrects the abnormal surfactant ultrastructure in Sftpdp<sup>−/−</sup> mice. In mature surfactant, tubular myelin is one of the LA structures, and continuous transition from lamellar body surfactant lipid layers to tubular myelin has been previously demonstrated in the presence of SP-A (38). SP-A is located at each corner of tubular myelin (37), and surfactant in SP-A deficient (Sftp<sup>−/−</sup> ) mice lacks tubular myelin (20). The present study demonstrated distinct sites of association of SP-A and SP-D with secreted surfactant lipids.

In summary, the present study demonstrated that SP-D preferentially associates with and lyses newly secreted surfactant lipid membranes that are rich in PI, which, in turn, converts surfactant to smaller forms. The size of surfactant forms determines their uptake by type II epithelial cells. SP-D is required for establishing phospholipid pool sizes and surfactant ultrastructure as the lung matures after birth and is required for normal surfactant homeostasis in the mature lung.

**REFERENCES**


