SP-D and GM-CSF regulate surfactant homeostasis via distinct mechanisms

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Ikegami, Machiko, William M. Hull, Mitsuhiro Yoshida, Susan E. Wert, and Jeffrey A. Whitsett. SP-D and GM-CSF regulate surfactant homeostasis via distinct mechanisms. Am J Physiol Lung Cell Mol Physiol 281: L697–L703, 2001.—Both surfactant protein (SP) D and granulocyte-macrophage colony-stimulating factor (GM-CSF) influence pulmonary surfactant homeostasis, with the deficiency of either protein causing marked accumulation of surfactant phospholipids in lung tissues and in the alveoli. To assess whether the effects of each gene were mediated by distinct or shared mechanisms, surfactant homeostasis and lung morphology were assessed in 1) double-transgenic mice in which both SP-D and GM-CSF genes were ablated [SP-D(−/−) GM(−/−)] and 2) transgenic mice deficient in both SP-D and GM-CSF in which the expression of GM-CSF was increased in the lung. Saturated phosphatidylcholine (Sat PC) pool sizes were markedly increased in SP-D(−/−) GM(−/−) mice, with the effects of each gene deletion on surfactant Sat PC pool sizes being approximately additive. Expression of GM-CSF in lungs of SP-D(−/−) GM(−/−) mice corrected GM-CSF-dependent abnormalities in surfactant catabolism but did not correct lung pathology characteristic of SP-D deletion. In contrast to findings in GM(−/−) mice, degradation of [3H]dipalmitoylphosphatidylcholine by alveolar macrophages from the SP-D(−/−) mice was normal. The emphysema and foamy macrophage infiltrates characteristic of SP-D(−/−) mice were similar in the presence or absence of GM-CSF. Taken together, these findings demonstrate the distinct roles of SP-D and GM-CSF in the regulation of surfactant homeostasis and lung structure.

phosphatidylcholine; surfactant protein A; pulmonary alveolar proteinosis; emphysema; surfactant protein D; granulocyte-macrophage colony-stimulating factor

IN THE HEALTHY LUNG, alveolar and tissue surfactant pool sizes are tightly regulated. However, the precise mechanisms mediating surfactant homeostasis remain poorly defined. Surfactant homeostasis is maintained by net contributions from synthesis, secretion, uptake, and catabolism by type II cells and alveolar macrophages (23). The metabolism of surfactant components is influenced in complex ways by both surfactant protein (SP) D and granulocyte-macrophage colony-stimulating factor (GM-CSF). Surfactant phospholipids accumulate in lung tissue and airspaces in both GM-CSF (5)- and SP-D-deficient (3, 13) mice. Whether SP-D and GM-CSF regulate distinct or shared pathways to regulate surfactant homeostasis is unknown.

SP-D is a member of the collectin family of calcium-dependent lectins (4). Although SP-D binds relatively weakly to surfactant phospholipids, null mutations in the SP-D gene [SP-D(−/−)] caused marked increases in tissue and alveolar surfactant saturated phosphatidylcholine (Sat PC) in vivo (3, 13). A number of distinct changes in surfactant homeostasis are characteristic of SP-D(−/−) mice, including increases in surfactant phospholipids, markedly decreased SP-A and SP-C relative to Sat PC, rapid conversion from large-aggregate to small-aggregate surfactant forms, and decreased catabolism of endogenously synthesized Sat PC (11). Inactivation of the SP-D gene in mice also caused emphysema and increased numbers of lipid-laden, foamy alveolar macrophages (22). Surfactant abnormalities were corrected by genetic replacement of SP-D in the respiratory epithelium of the SP-D(−/−) mice (6). Thus SP-D deficiency caused abnormalities in lung structure and surfactant metabolism.

Targeted disruption of the GM-CSF gene (5, 10) or the GM-CSF receptor common β-chain genes in mice (18, 20) caused pulmonary alveolar proteinosis associated with a marked increase in tissue and alveolar Sat PC pool sizes. The increase in surfactant phospholipids associated with deficiencies in GM-CSF signaling were similar in extent to those observed in the SP-D(−/−) mice. However, unlike the SP-D(−/−) mice, SPs were also markedly increased in the GM-CSF or GM-CSF receptor β-chain-deficient mice (20). In contrast to findings in SP-D(−/−) mice, catabolism of both surfactant lipid and proteins by alveolar macrophages was markedly decreased in the absence of GM-CSF signaling in vitro (24) and in vivo (10). Local replacement of GM-CSF in lungs of GM-CSF-deficient [GM(−/−)] mice corrected alveolar proteinosis (9, 19) and host defense abnormalities typical of GM-CSF deficiency (15).

Although both genes are important determinants of surfactant phospholipid homeostasis, the precise mechanisms by which GM-CSF and SP-D influence...
surfactant pools remain unclear. The present studies were undertaken to identify potential relationships or interactions between GM-CSF- and SP-D-dependent pathways in the regulation of surfactant homeostasis. Surfactant metabolism was studied in SP-D and GM-CSF null mutant [SP-D(-/-),GM(-/-)] mice and SP-D(-/-),GM(-/-) mice in which GM-CSF expression was increased in the respiratory epithelium by using SP-C as a promoter [SP-D(-/-),GM(-/-),SP-C/GM(+/+) mice]. Data presented support the concept that SP-D and GM-CSF regulate surfactant phospholipid homeostasis by distinct mechanisms.

METHODS

**Mice.** Generation of SP-D(-/-) (13), GM(-/-) (5), and GM(-/-),SP-C/GM(+/+) (8) mice has been described previously. GM(-/-) and SP-C/GM(+/+) mice were mated to produce heterozygous offspring that were mated to produce GM(-/-),SP-C/GM(+/+) mice in the C57BL/6 background. GM-CSF was selectively expressed in the lungs of SP-D/ GM(+/+) mice and absent from other tissues in the GM(-/-) background. Subsequent matings produced litters of mice with the SP-C/GM(+/+) genotype, confirming homozygosity of the offspring. These mice were mated to SP-D(-/-) mice, the latter in the Black Swiss background. Siblings were mated through at least three generations to produce double-knockout mice [SP-D(-/-),GM(-/-)] that were also heterozygous or homozygous for the SP-C/GM transgene. Comparisons were made among control mice (wild type) that were F2 litters of Black Swiss C57BL/6 crosses and compared with identical crosses between SP-D(+/+) and SP-C/GM(+/+) mice to control for potential strain-dependent influences. Genotyping was performed on tail DNA by PCR and/or Southern blot analysis as previously described (5, 8, 13).

All of the studies were performed with 7- to 9-wk-old mice. Mice were maintained in cages containing high-efficiency particulate-filtered air in a barrier facility. There was no evidence of pathogens in sentinel mice comaintained in the vivarium. All procedures were conducted under Institutional Animal Care and Use Committee-approved methods.

**Sat PC pool size.** Sat PC pools (calculated as μmol/kg body wt) were measured in alveolar lavage fluid and lung tissue after alveolar lavage as previously described (11) in 22 wild-type mice; 40 SP-D(-/-),GM(-/-) mice; and 36 SP-D(-/-),GM(-/-),SP-C/GM(+/+) mice. Each mouse was deeply anesthetized with intraperitoneal pentobarbital sodium, and the distal aorta was cut to exsanguinate the animal. After the chest was opened, a 20-gauge blunt needle was tied to the trachea, and 0.9% NaCl at 4°C was flushed in the airway until the lungs were fully expanded. The fluid was withdrawn by syringe three times for each aliquot. The saline lavage was repeated five times, and the samples were pooled. The lavaged lung tissue was homogenized in 0.9% NaCl. Aliquots of alveolar lavage fluid and the lung homogenates were extracted with chloroform-methanol (2:1; see Ref. 2), and Sat PC pool size was determined by 10.220.33.2 on September 20, 2017 http://ajplung.physiology.org/ Downloaded from

**SP-A content.** The SP-A in alveolar lavage fluid was analyzed by Western blot in six mice from each genotype (11, 20). Samples containing 1 μg of Sat PC were used for analyses of SP-A. Proteins were separated by SDS-PAGE in the presence of β-mercaptoethanol. After electrophoresis, SP-A was transferred to nitrocellulose paper (Schleicher and Schnell, Keene, NH), and immunoblot analysis was carried out with dilution of 1:5,000 guinea pig anti-mouse SP-A. Appropriate peroxidase-conjugated secondary antibodies were used at 1:5,000 dilutions. Immunoreactive bands were detected with enhanced chemiluminescence reagents (Amersham, Chicago, IL). Protein bands were quantitated by densitometric analyses with Alpha Imager 2000 documentation and analysis software (Alpha Innotech, San Leandro, CA). Linearity of the assay was confirmed for the range of 10–200 ng of mouse SP-A (R² = 0.95).

**Clearance of dipalmitoylphosphatidylcholine.** Eight mice from each group were anesthetized with methoxyflurane and orally intubated with a 25-gauge animal-feeding needle. Each mouse received 60 μl of saline containing 0.5 μCi of [3H]choline-labeled dipalmitoylphosphatidylcholine (DPPC; American Radiolabeled Chemicals, St. Louis, MO), 1.5 μg of DPPC, and 3.3 μg of lipid-extracted mouse surfactant suspended by the use of glass beads (11). The phospholipids given by intratracheal injection were 2% of the alveolar pool size for wild-type mice. After injection (40 h), mice were killed. Radioactivity was then measured in Sat PC isolated from alveolar lavage fluid and lung homogenate.

**Precursor incorporation in Sat PC.** Mice were given intraperitoneal injections of 10 μl saline/g body wt containing 0.5 μCi [3H]palmitic acid/g body wt (American Radiolabeled Chemicals; see Ref. 10). The palmitic acid was stabilized in solution with 5% human serum albumin. Groups of eight mice were killed at 2, 16, and 48 h after precursor injection, and alveolar lavage fluid was recovered from each animal. Lung tissue was homogenized in 0.9% NaCl. Sat PC was isolated from alveolar lavage fluids and lung homogenates as described for Sat PC pool sizes, and radioactivity was measured.

**DPPC degradation by alveolar macrophage.** Alveolar macrophages were isolated from the alveolar lavage fluid, and the degradation of radiolabeled DPPC was studied as described previously (24). Alveolar macrophages from wild-type mice, SP-D(-/-) mice, SP-D(-/-),GM(-/-) mice, and SP-D(-/-),GM(-/-),SP-C/GM(+/+) mice were studied. Alveolar lavage was performed with PBS containing 0.5 mM EDTA. Lavage fluid was centrifuged at 1,000 g for 5 min. Recovered cells were then resuspended in DMEM containing 0.1% BSA and were cultured at a density of 1 x 10⁶ cells/well in flat-bottom, 96-well tissue culture plates. Cells were allowed to adhere for 1 h at 37°C. Nonadherent cells were removed, and the adherent alveolar macrophages were washed three times with DMEM containing 0.1% BSA. With this method, >90% of cells recovered were macrophages.

To assess DPPC degradation, natural surfactant isolated from normal mouse lung lavage fluid was labeled by mixing with 1,3-dipalmitoyl-N-methyl-[3H]choline,1,2-dipalmitoyl ([3H]DPPC; Amersham, Arlington Heights, IL). The labeled surfactant was resuspended in medium containing 100 μg/ml phospholipid with 10 μCi/ml [3H]DPPC. To determine degradation of [3H]DPPC, cells were incubated for 5 h at 37°C with the [3H]DPPC-labeled surfactant in culture medium. After incubation, the supernatant was collected, and the cells were lysed with lysis buffer (50 mM Tris-HCl (pH 8), 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.1% sodium deoxycholate, and 5 mM EDTA). The lipid and aqueous fractions were extracted from both supernatants and cells according to Bligh and Dyer (2). The degradation of [3H]DPPC by alveolar macrophages was estimated by measuring the generation of radioactive products partitioning in the water-methanol phase during the extraction. Background radioactivity in the absence of the cells was subtracted.
RESULTS

Sat PC and SP-A pool size. Surfactant Sat PC (μmol/kg) was determined in alveolar lavage fluid, lung tissue

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Fig. 1. Increased saturated phosphatidylcholine (Sat PC) in double-transgenic surfactant protein (SP) D-deficient (SP-D(-/-)) and granulocyte-macrophage colony-stimulating factor (GM-CSF)-deficient (GM(-/-)) mice. A: Sat PC pool sizes were determined in wild-type, SP-D(-/-), GM(-/-), and SP-D(-/-), GM(-/-), SP-C/GM(+/-) mice in alveolar lavage fluid (alveolar) and lung tissue after lavage (tissue). The sum of alveolar and tissue fractions (total) is also shown. Sat PC was increased markedly in double-transgenic SP-D(-/-), GM(-/-) mice. Repletion of GM-CSF in SP-D(-/-), GM(-/-), SP-C/GM(+/-) mice partially corrected the Sat PC content seen in double-transgenic mice but not to wild-type levels. B: total Sat PC in lungs of the transgenic mice was normalized to total Sat PC in wild-type mice (given the value of 1.0). Relative Sat PC contents in lungs from SP-D(-/-), GM(-/-), SP-D(-/-), GM(-/-), SP-C/GM(+/-); and SP-D(-/-), GM(+/-), SP-C/GM(+/-) mice are shown. Lung Sat PC in SP-D(-/-) mice and GM(-/-) mice are included for comparison and were published previously (10, 11). Total lung Sat PC content in lungs from SP-D(-/-), GM(-/-) mice was significantly higher than in wild-type mice, likely reflecting the deficiency of SP-A catabolism characteristic of GM-CSF mice. Furthermore, repletion of GM-CSF did not correct the decreased SP-A pool size in SP-D(-/-), GM(-/-) mice. Thus the marked reduction in SP-A content relative to Sat PC in SPC/GM-deficient mice was eightfold higher than in wild-type mice, likely reflecting the deficiency of SP-A catabolism characteristic of GM-CSF mice. Moreover, repletion of GM-CSF did not correct the decreased SP-A pool size in SP-D(-/-), GM(-/-) mice. Thus the marked reduction in SP-A content relative to Sat PC in SP-C/GM-deficient mice was eightfold higher than in wild-type mice, likely reflecting the deficiency of SP-A catabolism characteristic of GM-CSF mice. Therefore, SP-D and GM-CSF on surfactant homeostasis related to GM-CSF deficiency but did not correct SP-D-dependent differences.

SP pool sizes. The content of alveolar SP-A relative to Sat PC was estimated by Western blot analysis and normalized to the quantity of SP-A in wild-type mice, which were given the value of one. The concentration of SP-A in both SP-D(-/-) and GM(-/-) mice relative to the wild type was also included for comparison (Fig. 2A). In GM(-/-), SP-D(-/-) mice, the SP-A content was 0.4 of that in wild-type mice, and in SP-D(-/-), GM(-/-), SP-C/GM(+/-) mice, SP-A was further decreased to 0.2 of the normal value. In previous studies, SP-A content relative to Sat PC was unchanged in GM(-/-) mice, whereas SP-A was decreased to 0.1 of the normal value in SP-D(-/-) mice. SP-A content in SP-D(-/-), GM(-/-), SP-C/GM(+/-) mice was similar to that in SP-D(-/-) mice, whereas SP-D(-/-), GM(-/-) mice had a SP-A content of an intermediate value between that of SP-D(-/-) mice and GM(-/-) mice. Because alveolar Sat PC was increased in the SP-D(-/-), GM(-/-) mice, net alveolar SP-A pool size per kilogram of body weight normalized to that in wild-type mice was also expressed in Fig. 2B. Total lung SP-A content in SP-D(-/-), GM(-/-) mice was eightfold higher than in wild-type mice, likely reflecting the deficiency of SP-A catabolism characteristic of GM-CSF mice. Furthermore, repletion of GM-CSF did not correct the decreased SP-A pool size in SP-D(-/-), GM(-/-) mice. Thus the marked reduction in SP-A content relative to Sat PC in SP-D(-/-) mice was independent of GM-CSF, and the lack of GM-CSF increased SP-A levels independently of the SP-D mutation.

Clearance of DPPC. [3H]DPPC was given by intratracheal injection in mice of each genotype. Labeled DPPC recovered in the Sat PC pool decreased exponentially from the alveolar lavage fluids of the wild-type mice and SP-D(-/-), GM(-/-), SP-C/GM(+/-) mice.
prove [3H]DPPC clearance in the lungs of GM(2/2) mice. Thus SP-D deficiency appears to influence surfactant catabolism characteristics of SP-D(−/−) mice, reflecting decreased clearance.

GM(2/2) mice was similar to that in wild-type mice. SP-A was decreased in SP-D(−/−),GM(−/−) mice to 0.4 of wild-type mice. Relative SP-A content was 0.2 in SP-D(−/−),GM(−/−),SP-C/GM(+/+) mice. SP-A pool size/body wt in alveolar lavage fluid. SP-A pool size in wild-type mice was normalized to a value of 1.0. SP-D(−/−),GM(−/−) mice had 8 times more SP-A than wild-type mice. SP-A pool size decreased to 0.15 in SP-D(−/−),GM(−/−),SP-C/GM(+/+) mice, which is similar to SP-D(−/−) mice. *Data from Ref. 11. Data from Ref. 10. *P < 0.001 vs. wild-type mice. **P < 0.001 vs. SP-D(−/−),GM(−/−) mice.

40 h after injection (Fig. 3). Thus the expression of GM-CSF in the lung corrected the defect in surfactant catabolism characteristics of SP-D(−/−),GM(−/−) mice. Percent recovery of [3H]DPPC in SP-D(−/−),GM(−/−) double-knockout mice was increased sevenfold compared with that in wild-type and SP-D(−/−),GM(−/−),SP-C/GM(+/+) mice, reflecting a marked decrease in DPPC clearance in the SP-D(−/−),GM(−/−) mice. After administration (40 h), percent recovery of labeled DPPC in lungs from wild-type mice was ~10 and 20% in SP-D(−/−) mice, reflecting rapid clearance of DPPC. Labeled DPPC recovered in SP-D(−/−),GM(−/−) mice was ~41% lower than that observed in previous studies in GM(−/−) mice (10), reflecting a modest increase in clearance in the double-knockout SP-D(−/−),GM(−/−) mice compared with the GM(−/−) mice. Thus SP-D deficiency appears to improve [3H]DPPC clearance in the lungs of GM(−/−) mice and may have an effect on surfactant clearance that is independent of degradation by the alveolar macrophage.

Precursor incorporation. Mice were given weight-adjusted doses of [3H]palmitic acid to measure net incorporation in Sat PC at 2 h, secretion of the labeled Sat PC to the alveoli at 16 h, and loss of labeled Sat PC from the lung 48 h after injection (Fig. 4). After injection (2 h), no significant differences in initial incorporation were observed among the three groups of mice. Likewise, percent secretion in alveolar lavage...
The marked decrease in [3H]DPPC catabolism by alveolar macrophages from SP-D(--/-),GM(--/-) mice was similar to that from wild-type mice (Fig. 5). The marked decrease in [3H]DPPC catabolism by alveolar macrophages from GM(--/-) mice was similar to that from wild-type mice (Fig. 5). The marked decrease in [3H]DPPC catabolism by alveolar macrophages from SP-D(--/-),GM(--/-),SP-C/GM(+-/) mice was similar to that from wild-type mice, consistent with the increased precursor incorporation seen at later times after precursor injection typical of SP-D(--/-) mice, as previously described (11).

DPPC degradation by alveolar macrophages. In vitro surfactant catabolism by alveolar macrophages from SP-D(--/-) and SP-D(--/-),GM(--/-),SP-C/GM(+-/) mice was similar to that from wild-type mice (Fig. 5). The marked decrease in [3H]DPPC catabolism by alveolar macrophages from GM(--/-) mice was demonstrated previously (Fig. 5; see Ref. 24). Degradation of [3H]DPPC by alveolar macrophages was markedly and similarly decreased in GM(--/-) and SP-D(--/-),GM(--/-) mice, reflecting the known GM-CSF dependency for DPPC degradation by alveolar macrophages.

Lung histology. Lung histology was assessed by light microscopy in wild-type, SP-D(--/-),GM(--/-), and SP-D(--/-),GM(--/-),SP-C/GM(+-/) mice. Abnormalities in the SP-D(--/-),GM(--/-) mice consisted of heterogeneous airspace enlargement, increased perivascular lymphocytic infiltrates, enlarged foamy macrophages, and alveolar proteinosis, consistent with the histopathology seen in both SP-D(--/-) and GM(--/-) mice (Fig. 6; see Refs. 5 and 13). Repletion of GM-CSF with the SP-C/GM(+-/) transgene corrected the alveolar proteinosis in SP-D(--/-),GM(--/-) mice but did not correct the airspace abnormalities, foamy macrophages, or perivascular lymphocytic infiltrates characteristic of the SP-D(--/-) mice. Thus repletion of GM-CSF corrected the alveolar proteinosis typical of the GM(--/-) mice but did not correct the structural abnormalities typical of the SP-D(--/-) mice.

**DISCUSSION**

Surfactant metabolism in SP-D(--/-),GM(--/-) mouse lung was disrupted in a nearly additive way, with the double-knockout mice sharing characteristics of both GM(--/-) and SP-D(--/-) mice. Expression of excess GM-CSF under control of human SP-C in the lung of SP-D(--/-),GM(--/-) mice decreased Sat PC pool size, enhanced the clearance of surfactant from the lung, and corrected the histological abnormalities typical of alveolar proteinosis, demonstrating that local expression of GM-CSF corrected the GM-CSF-dependent effects or surfactant homeostasis. However, repletion of GM-CSF did not correct emphysema and inflammatory changes typical of SP-D deficiency. These results support the concept that SP-D and GM-CSF influence surfactant homeostasis by distinct pathways.

The precise mechanisms by which surfactant homeostasis is maintained in normal lung remain unknown. Surfactant lipids are synthesized, catabolized, or recycled (12) by type II epithelial cells. Alveolar macrophages play an important role in surfactant catabolism (21). Precise contribution of the respiratory epithelium and alveolar macrophages in catabolism of surfactant remains unclear. Recent studies in the mouse suggest that ~50% of DPPC is catabolized by alveolar- and tissue-associated macrophages, and ~50% is catabolized by type II epithelial cells (7). Deficits in surfactant catabolism account for the increased surfactant pool sizes characteristic of GM(--/-) mice (10, 20, 24). In contrast, tissue and alveolar pool sizes are increased in SP-D(--/-) mice without associated abnormalities in surfactant clearance (11).

Multiple abnormalities in surfactant structure and surfactant homeostasis were caused by targeted disruption of the mouse SP-D gene. However, the mechanisms by which lung surfactant homeostasis is altered in SP-D(--/-) mice are complex and not related to defects in surfactant lipid clearance. Although the increases in surfactant pool sizes are similar in both GM(--/-) and SP-D(--/-) mice, clearance of surfactant by the alveolar macrophage is deficient in GM(--/-) but not in SP-D(--/-) mice. Foamy macrophages accumulate in the lungs of SP-D(--/-) mice; however, the present in vitro studies demonstrate that alveolar macrophages from SP-D(--/-) mice degrade phospholipids normally. Increasing the surfactant pool size by exogenous surfactant also induces a transient foamy macrophage population but does not alter surfactant catabolism in mice (14). Thus the abnormalities in surfactant homeostasis seen in SP-D(--/-) mice do not

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**Fig. 5.** Degradation of [3H]DPPC by alveolar macrophages in vitro. Alveolar macrophages (1 x 10^5/well) were isolated from wild-type, SP-D(--/-),GM(--/-); SP-D(--/-),GM(--/-),SP-C/GM(+-/); and SP-D(--/-) mice and incubated with a surfactant suspension containing [3H]DPPC mouse surfactant for 5 h at 37°C. Degradation of [3H]DPPC by alveolar macrophages was estimated by measuring the radioactivity recovered in the water-methanol phase after Folch extraction. Degradation of [3H]DPPC by alveolar macrophages from transgenic mice was normalized to that from wild-type mice. Identical experiments with alveolar macrophages from GM(--/-) mice are shown for comparison (17). DPPC degradation by alveolar macrophages from SP-D(--/-),GM(--/-) mice was rapid, whereas degradation by alveolar macrophages from SP-D(--/-),GM(--/-) mice was decreased and similar to that in GM(--/-) mice (17). Expression of GM-CSF corrected surfactant catabolism by alveolar macrophages from the SP-D(--/-),GM(--/-),SP-C/GM(+-/) mice. *P < 0.01 vs. wild type.
appear to be caused by foam cell production or ablation of alveolar macrophage function. In contrast, catabolism of surfactant by the foamy macrophages from GM(−/−) mice is markedly decreased, demonstrating that SP-D and GM-CSF deficiency have distinct effects in surfactant clearance by the alveolar macrophages. Deletion of the SP-D gene in the GM(−/−) mice did not improve defective DPPC degradation characteristic of GM(−/−) mice, resulting in a clearance rate typical of SP-D deficiency. Conversely, increased GM-CSF did not correct the abnormalities in macrophage morphology and emphysema typical of the SP-D(−/−) mice.

The present study demonstrates that SP-D and GM-CSF play distinct roles in the maintenance of pulmonary morphology. Although emphysema was not restored by increased expression of GM-CSF in the SP-D(−/−) mice, the increased surfactant pool size seen in double-knockout SP-D(−/−),GM(−/−) mice was substantially corrected, consistent with the requirement of GM-CSF for SP clearance. This result may reflect enhanced macrophage function associated with increased GM-CSF that improves surfactant catabolism in the SP-D(−/−),GM(−/−) mice.

Findings from kinetic experiments assessing surfactant clearance also support the findings at steady state wherein Sat PC pool sizes were increased in SP-D(−/−),GM(−/−) mice in an approximately additive way. The percent recovery of DPPC in SP-D(−/−),GM(−/−),SP-C/GM(+/+) mice at 40 h after intratracheal injection of [3H]DPPC was similar to that in wild-type mice and to that in SP-D(−/−) mice (11), reflecting restoration of clearance of catabolic activity by local production of GM-CSF. In contrast, in SP-D(−/−),GM(−/−) mice, significantly higher concentrations of [3H]DPPC were recovered, reflecting decreased clearance typical of GM-CSF deficiency. However, because alveolar Sat PC pool sizes were 4-fold higher in SP-D(−/−),GM(−/−),SP-C/GM(+/+) mice and 14-fold higher in SP-D(−/−),GM(−/−) mice, the net recovery of [3H]DPPC was increased markedly in lungs of both of these transgenic mice compared with that in wild-type mice. Rapid catabolism of endogenously labeled surfactant was absent in GM-CSF-repleted SP-D(−/−),GM(−/−),SP-C/GM(+/+) mice, and both were similar to that in SP-D(−/−) mice, consistent with the activity of GM-CSF. In contrast, palmitic acid-labeled...
Sat PC continued to accumulate in alveolar lavage fluid in SP-D(−/−), GM(−/−), SP-C/GM(+/+) and SP-D(−/−) mice, consistent with altered surfactant pools seen previously in SP-D(−/−) mice (11). In the present study, we compared surfactant metabolism under identical experimental conditions in mice with distinct genotypes. However, the methodology used to estimate surfactant metabolism requires a number of assumptions, such as similar Sat PC precursor pools in all genotypes. The times chosen for kinetic studies were based on our previous studies of Sat PC synthesis secretion and clearance but are not complete enough to accurately determine turnover time of Sat PC. Likewise, we assumed that catabolism of DPPC in the lung was minimal and that labeled DPPC was uniformly distributed in the alveoli after intratracheal administration.

In vitro degradation of DPPC by alveolar macrophages isolated from GM(−/−) mice was slow and was not influenced by the lack of SP-D. Thus effects of GM-CSF on surfactant homeostasis are related primarily to its effects on surfactant catabolism dependent on alveolar macrophage function and are independent of SP-D. The altered surfactant homeostasis seen in SP-D(−/−) mice is not directly related to surfactant metabolism by type II epithelial cells and the altered surfactant Sat PC pool in both alveolar and cellular components of the lung. Effects of SP-D deficiency are not mediated primarily by effects on surfactant catabolism by alveolar macrophages and were mediated relatively independently of GM-CSF.

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