Alveolar lipoproteinosis in an acid sphingomyelinase-deficient mouse model of Niemann-Pick disease

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Submitted 2 August 2002; accepted in final form 19 November 2002

Types A and B Niemann-Pick disease (NPD) are lysosomal storage disorders caused by the deficient activity of acid sphingomyelinase (ASM). In humans, NPD is associated with the dysfunction of numerous organs including the lung. Gene targeting of the ASM gene in transgenic mice produced an animal model with features typical of NPD, including pulmonary inflammation. To assess mechanisms by which ASM perturbed lung function, we studied lung morphology, surfactant content, and metabolism in ASM-deficient mice in vivo. Pulmonary inflammation, with increased cellular infiltrates and the accumulation of alveolar material, was associated with alterations in surfactant content. Saturated phosphatidylcholine (SatPC) content was increased twofold, and sphingomyelin content was increased 5.5-fold in lungs of the ASM knockout (ASMKO) mice. Additional sphingomyelin enhanced the sensitivity of surfactant inhibition by plasma proteins. Clearance of SatPC from the lungs of ASMKO mice was decreased. Catabolism of SatPC by alveolar macrophages from the ASMKO mouse was significantly decreased, likely accounting for decreased pulmonary SatPC in vivo. In summary, ASM is required for normal surfactant catabolism by alveolar macrophages in vivo. Alterations in surfactant composition, including increased sphingomyelin content, contributed to the abnormal surfactant function observed in the ASM-deficient mouse.

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Mice. The ASMKO mouse (C57BL/6) was developed (12), and a colony was established from heterozygous breeding pairs in the vivarium at Mount Sinai School of Medicine, according to protocols approved by the Institutional Animal Care and Use Committee. After genotypes were identified by PCR (12), ASMKO mice and their wild-type littermates were

sent to Cincinnati Children’s Hospital Medical Center for study. All studies were performed simultaneously with 8- to 10-wk-old mice. The studies were carried according to protocols approved by the Institutional Animal Care and Use Committee of Cincinnati Children’s Hospital Medical Center.

Lung morphology and type II cell counts. Lungs were inflated fixed at 25 cmH₂O with 4% paraformaldehyde, and type II cells were visualized using paraffin sections (5 μm) immunostained with rabbit polyclonal pro-surfactant protein (SP)-B antisera as described previously (26). Cells on four consecutive 2 × 10⁻⁴ μm² fields were determined for wild-type (n = 3) and ASMKO mice (n = 3). Lung paraffin sections were stained with hematoxylin and eosin to visualize lung morphology. The rabbit anti-SP-D antibody was generated against purified mouse SP-D, and immunohistochemistry was performed.

Saturated phosphatidylcholine pool size, phospholipid composition, and protein. Mice were deeply anesthetized with intraperitoneal pentobarbital sodium, and the distal aorta was cut to exsanguinate. A 20-gauge blunt needle was tied into the trachea, the chest was opened, and 0.9% NaCl was used. The lungs were fully expanded (~1 ml). The fluid was then withdrawn by syringes three times for each aliquot, and the saline BAL was repeated five times. The samples were pooled, and the volume was measured. The lavaged lung tissue was homogenized in 0.9% NaCl. Aliquots of BALF and the lung homogenates were extracted with chloroform-methanol (2:1), and saturated phosphatidylcholine (SatPC) was isolated with 0.04 oxidases using carbon tetrachloride and column chromatography (20). Under the conditions of our assay, isolated SatPC was confirmed to contain only saturated fatty acids, predominantly palmitate, on gas chromatography of the oxidation product (1). SatPC was quantified by measuring phosphorus with the Bartlett assay (2). For phospholipid composition, chloroform-methanol extracts of alveolar lavages, large aggregate surfactant, and lung tissue were used for two-dimensional thin-layer chromatography (18). The spots were visualized with iodine vapor, scraped, and assayed for phosphorus content (18). To isolate large aggregate surfactant, we centrifuged BALF at 40,000 g over 0.8 M sucrose in 0.9% NaCl cushion for 15 min. The large aggregate surfactant then was collected from the interface, diluted with 0.9% NaCl, and centrifuged again at 40,000 g for 15 min. The pellet was suspended in 0.9% NaCl and used as large aggregate surfactant (15, 16) for surface activity and phospholipid composition analyses. Total protein was determined for each sample of BALF (19).

Surfactant protein pool sizes. The surfactant proteins in the BALF were analyzed by Western blot (17). Samples containing 1.35 nmol of SatPC were used for analysis of SP-A and SP-D, and samples containing 2.7 nmol of SatPC were used for analyses of SP-C. Proteins were separated by SDS-PAGE in the presence of β-mercaptoethanol. For SP-B analyses, aliquots containing 0.27 nmol of SatPC were electrophoresed under nonreducing conditions. SP-A and SP-D were separated on 8–16% acrylamide gels with Tris-glycine buffer. SP-B and SP-C samples were separated on 10–20% acrylamide gels with Tricine buffer (Novex, San Diego, CA). After electrophoresis, proteins were transferred to nitrocellulose paper (Schleicher & Schuell, Keene, NH) for SP-A and SP-D or to nitrocellulose into the acrylamide wells (Bio-Rad, Hercules, CA) for SP-B and SP-C. Immunoblot analyses were carried out with the following dilutions of antisera: 1:25,000 for guinea pig anti-rat SP-A (kind gift from Jeffrey Whitsett, Cincinnati Children’s Hospital), 1:10,000 for rabbit anti-bovine SP-B (Chemicon, Temecula, CA), 1:25,000 for rabbit anti-recombinant human SP-C (kind gift from Byk Gulden, Konstanz, Germany), and 1:10,000 rabbit anti-rat SP-D (Chemicon). Appropriate peroxidase-conjugated secondary antibodies were used at 1:10,000 dilution. Immunoreactive bands were detected with enhanced chemiluminescence reagents (Amer sham, Chicago, IL). Protein bands were quantitated by densitometric analyses with Alpha Imager 2000 documentation and ImageQuant analysis software.

Precursor incorporation into SatPC. ASMKO and wild-type mice were given intraperitoneal injections of 10 μl saline/kg body wt containing 0.8 μCi [³H]palmitic acid (16). The palmitic acid was stabilized in solution with 5% human serum albumin. Groups of five or six mice were killed at 5 and 48 h after radiolabeled precursor injection. BALF was recovered from each animal, and lung tissue was homogenized in saline. SatPC was isolated from the BALF and lung homogenate as described in Saturated phosphatidylcholine pool size, phospholipid composition, and protein, and radioactivity was measured. The percent secretion of labeled SatPC was calculated for the 5-h groups as the percentage of radioactivity in alveolar SatPC relative to the total radioactivity in the BALF plus lung tissue.

1,3-Phosphatidyl-N-methyl-choline 1,2-dipalmitinol degradation by alveolar macrophage. BALF was performed with PBS containing 0.5 mM EDTA and centrifuged at 1,000 g for 5 min. Recovered cells were resuspended in DMEM containing 0.1% BSA and were cultured at a density of 1 × 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 (31). Natural surfactant isolated from normal mouse BALF was labeled by mixing with 1,3-phosphatidyl-N-methyl-[³H]choline,1,2-dipalmitoyl ([³H]DPPC; Amersham, Arlington Heights, IL) followed by 30-min incubation on ice. The labeled surfactant made by this method showed uniform association of [³H]-DPPC to natural surfactant (14). The labeled surfactant was resuspended in medium containing 100 μg/ml phospholipid with 10 μCi/ml [³H]DPPC. Recovered cells were incubated for 5 h at 37°C with the [³H]DPPC-labeled surfactant in culture medium. After incubation, the supernatant was collected, the cells were resuspended in medium containing 100 μg/ml Tris-HCl, 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, 0.1% sodium deoxycholate, and 5 mM EDTA). The lipid and aqueous fractions were recovered from combined samples of supernatants and cells with chloroform-methanol (2:1). 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 (13, 31).

DPPC degradation by ASM. To test whether recombinant human ASM can degrade DPPC in vitro, we suspended [³H]DPPC and DPPC in buffer (0.2 M Na-acetate, pH 5.0, 0.6% Triton X-100, and 0.2 mM ZnCl₂). Recombinant human ASM (1, 3, or 10 ng) was added to each suspension containing 3.6 nCi/100 μl [³H]DPPC and DPPC in buffer (0.2 M Na-acetate, pH 5.0, 0.6% Triton X-100, and 0.2 mM ZnCl₂). We have previously shown that this recombinant human ASM has full biological functions (11). We estimated the degradation of [³H]DPPC by ASM using the same lipid extraction methods as the above degradation by macrophage. The radioactive products partitioning in the water-methanol phase and chloroform phase were counted, and background radioactivity in the absence of ASM was subtracted.
Measurement of granulocyte-monocyte colony-stimulating factor. Granulocyte-monocyte colony-stimulating factor (GM-CSF) is one of the factors known to regulate macrophage proliferation. In the GM-CSF-deficient transgenic mouse lung, surfactant accumulates due to the marked decrease in surfactant catabolism by macrophages (16, 31). To access the possible involvement of GM-CSF in surfactant catabolism in the ASMKO mouse lung, we measured GM-CSF levels. Lungs (four mice/group) were homogenized in saline containing a Complete Mini EDTA-free protease inhibitor mixture tablet (Roche Molecular Biochemicals, Indianapolis, IN), and lysates were recovered after low-speed centrifugation. Samples were concentrated 10-fold by ultrafiltration (Centricon 10; Amicon, Bedford, MA). GM-CSF was quantified by mouse ELISA assay kit (Pierce Endogen, Rockford, IL).

Surface activity. Surface activity of large aggregate surfactant was measured with the captive bubble surfactometer (27). The concentration of each sample was adjusted to 3 nmol SatPC/μl. Three microliters of surfactant samples were applied to the air-water interface of the 25-μl bubble by microsyringe. Sensitivity to protein inhibition was measured in the presence of 2 or 10% (vol/vol) sheep plasma protein. Surface tension was measured every 10 s for 300 s, equilibrium surface tension was measured, then bubble pulsation was started. The minimum surface tension after 65% bubble volume reduction was measured at the fifth pulsation.

Statistics. Values are means ± SE. Two-group comparisons were carried out by unpaired two-tailed t-test. Significance was accepted at \( P < 0.05 \).

RESULTS

Lung morphology and type II cell count. To determine whether the absence of ASM caused abnormalities in lung structure or in type II cell numbers, we inflation-fixed lungs and assessed lung morphology by light microscopy. Hematoxylin- and eosin-stained sections from ASMKO mice showed no air space changes compared with wild-type mice (Fig. 1, A and B). Foamy enlarged alveolar macrophages were observed throughout the lungs and filling air spaces (Fig. 1, C and D). Increased intensity of SP-D staining was observed in alveolar space, consistent with finding of pulmonary alveolar proteinosis (Fig. 1, E and F). SP-D staining was detected in alveolar type II cells and in alveolar macrophages.

Type II cells were stained for pro-SP-B. Type II cell numbers were not significantly different in ASMKO control mice. The numbers of type II cells counted per field were 35.8 ± 1.7 for wild-type mice and 35.3 ± 1.9 for ASMKO mice.

Fig. 1. Hematoxylin and eosin (H&E) staining of lung sections from wild-type (A, C) (original magnification ×20) and acid sphingomyelinase knockout (ASMKO) mice (B, D) (original magnification ×40). Representative lung sections from wild-type and ASMKO mice are shown. Large-size macrophages are seen in sections from ASMKO mice, but there are no obvious air space changes in ASMKO mice compared with wild-type mice. Immunohistochemistry was performed with rabbit anti-mouse surfactant protein (SP)-D (E, F). Increased intensity of SP-D staining is seen in the ASMKO mouse lung.
SatPC, phospholipid composition, and total protein.

All studies of the two genotypes were performed simultaneously with 8- to 10-wk-old mice. Surfactant SatPC pool size (µmol/kg) was measured in BALF, lung tissue after lavage, and total lung (Fig. 2). SatPC was twofold higher in BALF, tissue, and total lung of ASMKO mice than in wild-type mice (P < 0.01). A striking difference in phospholipid composition was observed in BALF. Sphingomyelin was increased 5.5-fold in ASMKO mice (P < 0.01) (Fig. 3A). Phospholipid compositions were determined in isolated large aggregate surfactant (Fig. 3B) and homogenized lung tissues (Fig. 3C). The percentage of sphingomyelin was increased fivefold in large aggregate surfactant (P < 0.01) and threefold in lung homogenates (P < 0.01) in ASMKO mice. The percent increase of sphingomyelin in lung homogenates from ASMKO mice was associated with a significantly lower percentage of phosphatidylcholine than in wild-type mice. Total protein in BALF was relatively low in both groups of animals but was significantly higher in ASMKO (22 ± 1 mg/kg) than in wild-type mice (18 ± 1 mg/kg) (P < 0.01).

Surfactant protein pool size. Surfactant proteins in BALF relative to SatPC are shown in Fig. 4A. Alveolar SatPC increased twofold in ASMKO mice, and the net amount of surfactant proteins relative to wild-type mice was calculated (Fig. 4B). The SP-A pool size was not different in ASMKO mice and wild-type mice. There are large variations in SP-B levels between the animals, and SP-B in ASMKO mice was not statistically different (P = 0.07) from wild-type mice. In ASMKO mice, SP-C was threefold higher, and SP-D was 4.5-fold higher relative to SatPC (Fig. 4A). Total SP-C content was sixfold higher, and SP-D was eightfold higher in ASMKO mice compared with wild-type mice (Fig. 4B).

Precursor incorporation. Mice were given body weight-adjusted doses of [3H]palmitic acid, and the amount of labeled SatPC was measured at 5 h. This time point best indicates net incorporation of the precursor into SatPC. Secretion of labeled SatPC into the alveoli was assessed after 5 h. The catabolism of labeled SatPC synthesized by the lung could be evaluated 48 h after precursor injection (Fig. 5). Five hours after precursor injection, no significant differences in incorporation of precursor into SatPC were observed between the two groups. Likewise, percent secretion in alveolar lavage fluid, calculated at 5 h as the percentage of radioactivity in alveolar lavage SatPC relative to the radioactivity in the total lung, was similar in both groups of mice: 9.1 ± 0.7% secretion in wild-type and 7.2 ± 0.8% in ASMKO mice. In contrast, 48 h after precursor injection, the amount of labeled SatPC in total lung and alveolar was 2.4-fold higher in ASMKO mice than in the wild-type mice, consistent with increased SatPC pool sizes and a decreased clearance of the surfactant lipid from the lung of ASMKO mice.

Decreased DPPC degradation by alveolar macrophages. Surfactant lipids are catabolized by both alveolar macrophage and type II cells in approximately equal proportions (10). Because loss of endogenously labeled surfactant evaluated 48 h after radiolabeled palmitic acid injection was significantly slower in ASMKO mice, catabolism of DPPC by isolated alveolar macrophages was studied in vitro. There was a marked decrease in [3H]DPPC catabolism by alveolar macro-
phages from ASMKO mice in vitro (Fig. 6). The reduction of DPPC degradation by alveolar macrophages seen in ASMKO mice was similar to that previously shown in GM-CSF-deficient mice (31).

**Pulmonary GM-CSF concentrations.** To assess whether the defect in DPPC degradation by macrophages was associated with low or deficient GM-CSF, we measured GM-CSF in lung homogenates from ASMKO mice. Lung GM-CSF levels were 10.4 ± 2.4 pg/g body wt for ASMKO mice and 6.8 ± 1.4 pg/g body wt for wild-type mice. There were no significant differences in lung GM-CSF concentrations between the two groups. These results suggest that GM-CSF deficiency does not mediate decreased surfactant catabolism by alveolar macrophages in the ASMKO mice.

ASM does not degrade DPPC. To assess whether the ASM was directly involved in the degradation of SatPC, we incubated [3H]DPPC with three different concentrations of recombinant ASM. After the incubation, 96% ± 2% of radioactivity was recovered in the chloroform phase for all the ASM concentrations studied (three experiments for each concentration). DPPC degradation by ASM was not detected. Thus altered catabolism of surfactant SatPC by macrophage in ASMKO mice does not directly contribute to surfactant abnormalities in the ASMKO mice.

**Altered surface activity caused by increased sphingomyelin.** To evaluate the function of surfactant isolated from ASMKO mice, we measured equilibrium surface tension and minimum surface tensions using large aggregate surfactant in the presence and absence of plasma as an inhibitor of surfactant function. Equilibrium surface tensions and minimum surface tensions for surfactant from ASMKO mice were similar to wild-type mice (Fig. 7). Surfactant from both wild-type mice and ASMKO mice was inhibited by 10% plasma protein as indicated by the increased equilibrium surface tensions and increased minimum surface tensions. The ASMKO surfactant was significantly more sensitive to plasma inhibition compared with surfactant from wild-type mice.

To test whether increased sphingomyelin levels inhibited surface activity, we added sphingomyelin (Sigma, St. Louis, MO) to wild-type large aggregate surfactant from normal mice and measured the minimum surface tension.

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**Fig. 4.** Surfactant proteins (SPs) in ASMKO mice and wild-type mice. A: amounts of SPs in BALF were estimated by Western blot relative to the amount of SatPC. Values for wild-type mice were normalized to a value of 1. SP-C and SP-D were increased in ASMKO mice. B: pool sizes for SP-A, SP-B, SP-C, and SP-D in BALF were normalized to a value of 1 for wild-type mice. SP-A in ASMKO mice was similar to wild-type mice. The mean value for SP-B in ASMKO mice was higher than wild-type mice but not statistically different. SP-C pool size was increased sixfold, and SP-D was increased eightfold in ASMKO mice relative to wild-type mice (n = 6/group); *P < 0.05.

**Fig. 5.** [3H]palmitic acid labeling of SatPC for the total lung (A) and for alveolar (BAL, B) in mouse at 5 and 48 h after injection of radiolabeled precursor. At 5 h, the net incorporation into SatPC and amount of labeled SatPC in BAL were similar for ASMKO mice and wild-type mice. At 48 h, more radiolabeled SatPC was in the lungs of ASMKO mice than in wild-type mice lung (n = 5–6/group); *P < 0.001.

**Fig. 6.** Degradation of 1,3-phosphatidyl-N-methyl-[3H]choline,1,2-dipalmityl ([3H]DPPC) by alveolar macrophages. Alveolar macrophages were isolated from wild-type and ASMKO mice and incubated with a surfactant suspension containing [3H]DPPC for 5 h at 37°C. Degradation of [3H]DPPC was estimated by measuring the radioactivity recovered in the water-methanol phase after chloroform-methanol lipid extraction. Degradation by alveolar macrophages from ASMKO mice was decreased (n = 3/group); *P < 0.0001.

**Fig. 7.** Surfactant proteins (SPs) in ASMKO mice and wild-type mice. A: amounts of SPs in BALF were estimated by Western blot relative to the amount of SatPC. Values for wild-type mice were normalized to a value of 1. SP-C and SP-D were increased in ASMKO mice. B: pool sizes for SP-A, SP-B, SP-C, and SP-D in BALF were normalized to a value of 1 for wild-type mice. SP-A in ASMKO mice was similar to wild-type mice. The mean value for SP-B in ASMKO mice was higher than wild-type mice but not statistically different. SP-C pool size was increased sixfold, and SP-D was increased eightfold in ASMKO mice relative to wild-type mice (n = 6/group); *P < 0.05.

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AJP-Lung Cell Mol Physiol • VOL 284 • MARCH 2003 • www.ajplung.org
LIPID-IN SURFACANT FUNCTION LIKELY CONTRIBUTES TO PULMONARY METABOLISM BY ALCHEO MACROPHAGES

ALTERATIONS IN SURFACANT LIPID AND PROTEINS, AND ABNORMAL SURFACE ACTIVITY IN SURFACANT FROM ASMKO MICE. SURFACANT CATABOLISM WAS DECREASED, MEDIATED AT LEAST IN PART BY DECREASED SURFACANT PHOSPHOLIPID CATABOLISM BY THE ALCHEO MACROPHAGES. SPHINGOMYELIN CONTENT WAS INCREASED IN LARGE AGGREGATE SURFACANT AND LUNG TISSUE HOMOGENATES FROM ASMKO MICE, WHICH ACCOUNTS FOR THE DECREASED SURFACANT SURFACE ACTIVITY IN THE PRESENCE OF PLASMA.

RESPIRATORY INFECTIONS AND PROGRESSIVE PULMONARY DYSFUNCTION ARE A COMMON CAUSE OF MORBIDITY FOR TYPE B NPD PATIENTS (28). THE PRESENCE OF FOAMY CELLS IN THE BALF (30), CHEST RADIOLOGICAL EVIDENCE OF RETICULENDOSULAR INFILTRATES (3, 6–8, 24), HAS BEEN CHARACTERIZED IN TYPE A AND B NPD PATIENTS, BUT LUNG PATHOPHYSIOLOGY OF NPD HAS NOT BEEN STUDIED. THE CLINICAL, BIOCHEMICAL, AND PATHOLOGICAL FEATURES OF THE ASMKO MICE MIMIC HUMAN TYPE A AND B NPD. ASMKO MICE HAVE AN INTERMEDIATE PHENOTYPE THAT RESULTS IN SEVERE ATAXIA AND DEATH BY 6–8 MO OF AGE. SIGNIFICANT STORAGE OF SPHINGOMYELIN IS EVIDENT IN CELLS OF THE RETICULOENDOTHELIAL SYSTEM AND NEURONS IN THIS MOUSE MODEL OF NPD.

DISCUSSION

DEFICIENCY OF ASMKO CAUSES A PULMONARY ALCHEO PROTEINOSIS-LIKE SYNDROME CAUSED BY DEFECTS IN SURFACANT METABOLISM BY ALCHEO MACROPHAGES. ALTERATIONS IN SURFACANT FUNCTION LIKELY CONTRIBUTE TO PULMONARY ABNORMALITIES ASSOCIATED WITH NPD. LIPID-FILLED ALCHEO MACROPHAGES ACCUMULATED IN LUNGS OF ASMKO MICE AND NPD PATIENTS. THE PRESENT STUDIES DEMONSTRATE DECREASED SURFACANT CATABOLISM, ALTERATIONS IN SURFACANT LIPID AND PROTEINS, AND ABNORMAL SURFACE ACTIVITY IN SURFACANT FROM ASMKO MICE. SURFACANT CATABOLISM WAS DECREASED, MEDIATED AT LEAST IN PART BY DECREASED SURFACANT PHOSPHOLIPID CATABOLISM BY THE ALCHEO MACROPHAGES. SPHINGOMYELIN CONTENT WAS INCREASED IN LARGE AGGREGATE SURFACANT AND LUNG TISSUE HOMOGENATES FROM ASMKO MICE, WHICH ACCOUNTS FOR THE DECREASED SURFACANT SURFACE ACTIVITY IN THE PRESENCE OF PLASMA.

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Fig. 7. Equilibrium surface tension (ST) and minimum ST of large aggregate surfactant isolated from ASMKO mice and wild-type mice. Samples were applied to the air-liquid interface of a captive bubble surfactometer. Change in the shape of the static bubble from rounded to discoid was monitored over time and used to calculate surface tension. Measurements were made in the absence and presence of 10% plasma. Surface activity of surfactant isolated from ASMKO mice was similar to wild-type mice surfactant in the absence of plasma (vol/vol). Surfactant from both genotype groups was inhibited by plasma, resulting in higher ST. Equilibrium ST and minimum ST were significantly higher for ASMKO mice surfactant than wild-type mice surfactant in the presence of plasma. Surface activity of surfactant from ASMKO mice was more susceptible to plasma inhibition than surfactant from wild-type mice (n = 4/group), *P < 0.05.

Fig. 8. Effect of SM on surfactant stability. To see whether a higher SM composition would make surfactant more sensitive to plasma inhibition, we added exogenous SM to wild-type surfactant. The SM was increased to the same level as ASMKO mice surfactant 17% of total phospholipid. ST measurements were made with surfactant samples containing 9 nmol SatPC applied to the bubble in the absence and presence of 2% and 10% plasma (vol/vol) in the bubble chamber. A: surfactant containing SP-A (with SP-A); B: surfactant SP-A removed by lipid extraction (no SP-A). In the absence of plasma, all the surfactants show low minimum STs. Increased SM or lack of SP-A did not affect this ST-lowering property of surfactant. Addition of SM made surfactant unstable in the presence of plasma and showed higher minimum ST than wild-type surfactant. Surface activity of both surfactants not containing SP-A was inhibited by 10% plasma, whereas only the surfactant with additional SM was affected by 2% plasma. *P < 0.01 vs. wild-type mice surfactant.
The frequency and severity of pulmonary disease in NPD may be influenced by altered host defense due to a combination of altered macrophage function (4) and abnormal surfactant function. As a result of altered host defense in the lung, type B NPD patients have frequent respiratory infections (21). In infected and/or injured lungs, protein leak occurs from the vascular space to the alveolus. Because the function of surfactant with increased sphingomyelin content is easily inhibited, these abnormalities may enhance susceptibility of NPD patients to respiratory abnormalities following lung infection. The present study showed altered surfactant function by addition of sphingomyelin in surfactants both with and without SP-A in vitro.

Although surfactant protein levels in NPD surfactant were similar or higher than wild-type surfactant, resistance of NPD surfactant against protein inhibition was weak and related to enhanced sphingomyelin content. Susceptibility to infection may be enhanced by the abnormal surfactant composition in NPD patients and ASMKO mice. In addition, surfactant pool size is the abnormal surfactant composition in NPD patients tent. Susceptibility to infection may be enhanced by the abnormal surfactant composition in NPD patients and ASMKO mice. In addition, surfactant pool size is higher than in normal individuals, and pulmonary alveolar lipoproteinosis and proteinosis occur in NPD patients and ASMKO mice. Chest X-rays with diffuse reticular or fine nodular infiltrates seen in type A and B NPD patients may be partially due to pulmonary alveolar lipoproteinosis. In fact, a recent case report of a type B NPD patient showed significant improvement in lung function and chest X-ray after whole lung lavage treatment. Although it does not cure NPD, whole lung lavage, a treatment used for patients with pulmonary alveolar proteinosis (22), was effective in NPD.

The precise mechanisms by which surfactant homeostasis is maintained in normal lung remain unknown. Surfactant lipids are synthesized, catabolized, and recycled in type II epithelial cells. Alveolar macrophages also play an important role in surfactant catabolism. A recent study in the mouse suggest that macrophages and type II cells equally contribute to the catabolism of DPPC and SP-A (10). In vitro degradation of DPPC by alveolar macrophages isolated from ASMKO mice was markedly lower than in normal mice and similar to that found in GM-CSF-deficient mice (13, 31). However, the GM-CSF levels in lungs of ASMKO mice were normal, indicating that GM-CSF deficiency does not mediate decreased surfactant catabolism by alveolar macrophages in this model. The increase in SP-D is consistent with findings in pulmonary alveolar proteinosis. Because SP-D is rapidly degraded by alveolar macrophages (5), the increased SP-D concentration and increased immunostaining seen in ASMKO mice may be related to decreased SP-D catabolism by alveolar macrophages.

Dhami et al. (4) recently showed that the alveolar macrophage was the most dramatically affected cell of all the alveolar cells increased in ASMKO mice lung. The alveolar macrophages in ASMKO mice were increased, enlarged, and often multinucleated. Production of chemokines, including macrophage inflammatory protein-2 and IL-1α, was increased in the lungs of ASMKO mice while superoxide production was decreased.

Although catabolism of DPPC was reduced in alveolar macrophages from ASMKO mice, it is unclear whether ASM is directly involved in catabolism of surfactant components or whether ASM deficiency causes secondary change in alveolar macrophage function. Because DPPC and sphingomyelin have similar structures, it is possible that ASM may catabolize both DPPC and sphingomyelin. However, in the present study, we found that ASM did not degrade DPPC in vivo. Alternatively, it is possible that metabolic defects caused by ASM deficiency secondarily perturb alveolar macrophage function, leading to altered surfactant homeostasis. Because sphingosine and sphingosine phosphate may accumulate in these cells, altered cell signaling related to abnormal lipid metabolism of the accumulation and metabolic products may influence macrophage function to repair surfactant degradation.

In summary, ASM deficiency caused a pulmonary alveolar proteinosis-like syndrome. Surfactant catabolism by alveolar macrophage was decreased, and surfactant function was diminished. Surfactant from ASM mice contained excessive concentrations of sphingomyelin and exhibited decreased resistance against inhibition of surface tension-lowering properties by plasma protein. Because pulmonary complications are a common clinical problem in NPD patients, it is likely that these results explain, at least in part, the mechanism(s) underlying lung disease in individuals with NPD.

We thank Dr. Jeffrey A. Whitsett, Cincinnati Children’s Hospital, for valuable suggestions.

This work was supported by National Institutes of Health Grants HL-61646 (M. Ikegami), HD-11932 (M. Ikegami), and HD-28607 (E. H. Schuchman).

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