Characteristics of surfactant from SP-A-deficient mice

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Characteristics of surfactant from SP-A-deficient mice. Am. J. Physiol. 275 (Lung Cell. Mol. Physiol. 19): L247–L254, 1998.—Mice that are surfactant protein (SP) A deficient [SP-A(−/−)] have no apparent abnormalities in lung function. To understand the contributions of SP-A to surfactant, the biophysical properties and functional characteristics of surfactant from normal [SP-A(+/+)] and SP-A(−/−) mice were evaluated. SP-A-deficient surfactant had a lower buoyant density, a lower percentage of large-aggregate forms, an increased rate of conversion from large-aggregate to small-aggregate forms with surface area cycling, increased sensitivity to inhibition of minimum surface tension by plasma protein, and no tubular myelin by electron microscopy. Nevertheless, large-aggregate surfactants from SP-A(−/−) and SP-A(+/+) mice had similar adsorption rates and improved the lung volume of surfactant-deficient pretterm rabbits similarly. Pulmonary edema and death caused by N-nitroso-N-methylurethane-induced lung injury were not different in SP-A(−/−) and SP-A(+/+) mice. The clearance of 125I-labeled SP-A from lungs of SP-A(−/−) mice was slightly slower than from SP-A(+/+) mice. Although the absence of SP-A changed the structure and in vitro properties of surfactant, the in vivo function of surfactant in SP-A(−/−) mice was not changed under the conditions of these experiments.

surfactant protein A; transgenic mice; tubular myelin; surface tension; adsorption rate; surfactant treatment

PULMONARY SURFACTANT is a highly conserved mixture of phospholipids, neutral lipids, and proteins that is essential for normal lung function in all mammals. The generally accepted sequence of the biogenesis of these complexes begins with the cocrystallization of surfactant lipid and the lipophilic surfactant protein (SP) B and SP-C into the alveolus in the form of lamellar bodies (31). De novo synthesized SP-A is secreted via separate pathways by type II cells and perhaps by Clara cells (13). In the hypophase of air spaces, lamellar bodies unravel, and SP-A associates with the lipoprotein complexes to form tubular myelin and loose membranous arrays (22, 27). Tubular myelin forms of surfactant are highly surface active and contribute to the pool in the hypophase from which the surface film is generated and maintained. Loss of material from the surface film likely occurs by formation of small vesicles containing lipids of the same phospholipid composition as tubular myelin and lipid arrays but that is depleted of SP-A, SP-B, and SP-C (1, 32). Several functions of SP-A thought to be important for the maintenance of surfactant homeostasis were identified by in vitro studies. SP-A was essential for tubular myelin formation (22, 27), and SP-A acted cooperatively with SP-B and SP-C to increase the rate of surface adsorption of the lipids (7, 24, 35). The resistance of surfactant lipid mixtures to inactivation by proteins in edema fluid was also increased by SP-A (4, 30). These effects of SP-A were identified in experiments where SP-A was added to mixtures of lipids and the other surfactant proteins. The recently described SP-A-deficient [SP-A(−/−)] mouse made possible the evaluation of the properties of a native surfactant that is SP-A deficient, avoiding potential artifacts resulting from in vitro recombination of purified lipids and proteins (16). The SP-A(−/−) mouse reproduces normally and has normal lung anatomy and function. Normal tubular myelin was not seen within alveoli of SP-A(−/−) mice, and at physiologically relevant concentrations, surfactant from alveolar washes of SP-A(−/−) mice had minimal surface tension values comparable to surfactant from normal [SP-A(+/+) ] mice. In the present study, we have further characterized the surfactant from SP-A(−/−) mice by assessing the physical, biophysical, and functional properties in comparison to surfactant from SP-A(+/+) mice.

METHODS

Mice. SP-A knockout mice were generated from embryonic stem cells in which the mouse SP-A gene was disrupted by homologous recombination (16). SP-A(−/−) mice have been maintained for >3 yr and are carried as breeding colonies in the vivarium at Children's Hospital (Cincinnati, OH) according to protocols approved by the Institutional Animal Use and Care Committee. The SP-A(−/−) mice were bred into the Black Swiss genetic background for nine generations and have been maintained in filtered cages under strict isolation conditions. SP-A(+/+) mice were from National Institutes of Health Black Swiss stock. Genotyping was performed by Southern blot of total DNA as previously described (16).

Surfactant isolation. For surfactant studies, ~130 mice from each genotype [SP-A(−/−) and SP-A(+/+) ] were injected intraperitoneally with pentobarbital sodium to achieve deep anesthesia. The distal aorta was cut to exsanguinate each animal. The chest of the animal was opened, a 20-gauge blunt needle was tied into the proximal trachea, five aliquots of 0.15 M NaCl were infused into the lungs until they were inflated, and then the aliquots were withdrawn by syringe three times for each aliquot (12). Large-aggregate surfactant was isolated from the lavage fluid by centrifugation (12). Either pooled alveolar washes or alveolar washes from a single mouse were centrifuged at 40,000 g over a 0.8 M sucrose in 0.15 M NaCl cushion for 15 min. The large-aggregate surfactant then was collected from the interface, diluted with 0.15 M NaCl, and centrifuged again at 40,000 g for 15 min. The pellet was suspended in normal saline and used as large-aggregate surfactant.

The supernatant from the first 40,000-g centrifugation that contained small-aggregate surfactant was concentrated at 4°C by ultrafiltration with a 300,000 molecular-weight retention filter (Amicon, Beverly, MA) (34). The small-aggregate surfactant was diluted three times with 50 ml of 0.15 M NaCl and ultrafiltered three times in a stirred cell with an XM300 membrane (Amicon, Beverly, MA) to remove
soluble proteins. Surfactant fractions were stored at −20°C until used. In separate measurements, alveolar washes were recovered for measurement of the number and distribution of cell types in the lavage fluid.

Surfactant function in preterm rabbits. A surfactant-deficient premature rabbit model was used to test the functional properties of the large- and small-aggregate surfactant fractions (34). Preterm rabbits at 27 days ± 2 h gestational age were sequentially delivered, weighed, and anesthetized with an intraperitoneal injection of 10 mg/kg of ketamine and 0.1 mg/kg of acepromazine. The trachea of each rabbit was cannulated, and 7.5 µmol saturated phosphatidylcholine (Sat PC)/kg body weight of large-aggregate or small-aggregate surfactant from SP-A(−/−) or SP-A(+/+) mice was randomly given via the tracheal tube. A control group was untreated.

The rabbits were ventilated in a series of 37°C temperature-controlled plethysmographs for 15 min with 100% oxygen at a rate of 30 breaths/min, with an inspiratory time of 1 s and a 2 s expiratory time. Inspiratory pressures were individually regulated to adjust the tidal volume to −8 ml/kg. Dynamic compliance was calculated by dividing tidal volume per kilogram body weight by (peak inspiratory pressure + baseline positive end-expiratory pressure). After 15 min of ventilation, the endotracheal tube was plugged for 5 min to allow absorption atelectasis to occur. Pressure-volume curves at 37°C were measured by inflating the lungs in 5-cmH₂O increments to 35 cmH₂O, with the volume measured after 30 s at each pressure. The lungs were then deflated with the same 5-cmH₂O increments, with the volumes recorded. The lung volume was corrected for the compression volume of the system and is expressed as milliliters per kilogram body weight.

Adsorption rate of surfactant. Isolated large-aggregate surfactants from pooled alveolar washes from SP-A(−/−) and SP-A(+/-) mouse lungs (n = 3 for each genotype group) were mixed with saline such that the final concentration of Sat PC was 5 µmol/ml. The large-aggregate surfactant (1.2 µmol) was injected into the subphase of 40 ml of 0.15 M NaCl that was being continuously stirred at 37°C in a 5-cm diameter and 4-cm deep Teflon well. The time from the addition of surfactant to the establishment of an equilibrium surface tension was recorded. Surfactant was mixed with a platinum dipping plate connected to a force transducer (15).

Sensitivity of surfactant to plasma protein inhibition. Minimum surface tensions were measured with a Wilhelmy balance using a platinum dipping plate, with 3-min area cycling from 64 to 12.8 cm² at a temperature of 37°C (11). Adult sheep plasma was added to a surfactant suspension containing 0.025 µmol/ml of Sat PC in 35 ml of 0.15 M NaCl to achieve each plasma protein concentration, and the solutions were mixed well by stirring with a glass rod followed by immediate surface tension measurements. The surface tension-area loops overlapped by the third or fourth compression were measured after 30 s at each pressure. The lungs were then deflated with the same 5-cmH₂O increments, with the volumes recorded. The lung volume was corrected for the compression volume of the system and is expressed as milliliters per kilogram body weight.

Conversion of large-aggregate surfactant. Large-aggregate surfactant (0.1 µmol Sat PC) was mixed with 2 ml of Tris buffer in a capped 12 × 75-mm polystyrene tube, and surface-area cycling was then performed as previously described (6, 8, 12). Briefly, the tubes were attached to the disk of a Rototorque rotator (Cole-Parmer Instruments, Chicago, IL) in an incubator at 37°C, and four tubes were rotated for 6 h at 40 rotations/min to change the surface area from 1.1 to 9.0 cm² two times per cycle. Two other tubes were incubated at 37°C for 6 h without rotation. The large- and small-aggregate surfactants were isolated by centrifugation at 40,000 g for 15 min. The quantity of large-aggregate surfactant in the pellets and the residual small-aggregate surfactant in the supernatants were measured by phosphorus assay.

Electron microscopy. Fresh isolates of large-aggregate surfactant from three separate pooled alveolar washes from SP-A(−/−) and SP-A(+/-) mice, each containing lavages from seven mice for each pool, were pelleted and fixed overnight at 4°C with 2.5% glutaraldehyde-2% paraformaldehyde in 0.1 M sodium cacodylate buffer at pH 7.3. The pellets were then washed in the buffer and postfixed in 1% potassium ferrocyanide-reduced osmium tetroxide in 0.1 M sodium cacodylate buffer for 1 h at 4°C. The aggregates were washed in buffer, resuspended, pelleted in 1% low-temperature-gelling agarose (Sigma, St. Louis, MO), and allowed to solidify overnight in the cold. The agarose blocks were then rinsed in 70% ethanol, stained en bloc with 0.5% uranyl acetate at 70°C ethanol for 10 min at room temperature, dehydrated through 70% and 100% ethanol, and embedded in Eponate 12 resin (Ted Pella, Redding, CA). Ultrathin sections from each pool were stained with lead citrate and uranyl acetate and examined in triplicate with a JEM 100CX electron microscope.

Lung injury with N-nitroso-N-methylurethane. Acute lung injury was induced in SP-A(−/−) and SP-A(+/-) mice with a subcutaneous injection of N-nitroso-N-methylurethane (NMU; Kings Laboratory, Greenville, SC) at a dose of 12 mg/kg. Previous studies (17, 18) using this dose in rabbits, rats, and dogs demonstrated a progressive, primarily epithelial lung injury over several days. Measurement of protein and percentage of large-aggregate surfactant in alveolar washes were performed 1 and 2 days after NMU injection. The protein permeability of the lung was evaluated with 125I-labeled albumin 2 days after NMU treatment. Mice were given 10 µCi of 125I-albumin by intraperitoneal injection, and the recovery of 125I from the alveolar washes was measured 2 h later. The 125I-albumin was made from monomer standard bovine serum albumin and carrier-free 125I (ICN, Irvine, CA) using chloramine T. Labeled albumin was extensively dialyzed before use, and tetrachloroacetic acid precipitation verified that >97% of the label was associated with albumin (17).

Clearance of SP-A. SP-A was isolated from the alveolar washes from granulocyte-macrophage colony-stimulating factor-deficient transgenic mice that have elevated alveolar pools of surfactant lipids and proteins (12). The SP-A was purified with octyl glucopyranoside according to Hawgood et al. (7) and iodinated with Bolton-Hunter reagent (Amersham, Arlington Heights, IL) as before (12). The iodinated SP-A was mixed with liposomes of dipalmitylphosphatidylcholine (DPC) to yield a final suspension containing 0.01 µmol DPC/ml in 0.15 M NaCl. SP-A(+/-) or SP-A(−/−) mice (7–9 wk old) were randomized to groups of four to six animals for measurements of the clearance of 125I-SP-A. Each mouse was given 50 µl of saline containing 0.1 µCi of 125I-SP-A by intratracheal injection. For the tracheal injection, mice were sedated with intraperitoneal ketamine (50 mg/kg). The trachea was exposed through a 0.5-cm midline skin incision in
the neck, and the isotope mixture was injected with a 30-gauge needle. Alveolar washes were performed as described in Surfactant isolation, and the five washes for each lung were pooled (10). The recovered $^{125}$I radioactivity was measured from the alveolar wash and the lung tissue after alveolar wash.

Analytic techniques. Lipids were extracted with chloroform-methanol (2:1 vol/vol), and Sat PC was recovered from the lipid extracts after exposure to osmium tetroxide by neutral alumina column chromatography (20). Sat PC was quantified by phosphorus assay (2). Protein quantification in alveolar washes was by the method of Lowry et al. (19) with a bovine serum albumin standard.

Data analysis. All values are given as means ± SE. Differences between two groups were tested by two-tailed Student’s t-test. The between-group comparisons were made by analysis of variance followed by the Student-Newman-Keuls multiple comparison procedure.

RESULTS

Surfactant function in preterm rabbits. Preterm rabbits at 27 days gestational age were treated with ~50 mg/kg of the large- and small-aggregate fractions of surfactant from the alveolar washes of the SP-A(+/-) and SP-A(-/-) mice. Body weights of the rabbits were similar, and the rabbits were ventilated with a mean tidal volume of 7.9 ml/kg (Table 1). Ventilatory pressures were significantly lower for the rabbits treated with large-aggregate surfactant from both SP-A(-/-) and SP-A(+/-) mice compared with the untreated control rabbits. Total thoracic compliance increased, with large-aggregate surfactant recovered from SP-A(+/-) mice (Fig. 1A). Although large-aggregate surfactant from SP-A(+/-) mice tended to result in higher compliances in the premature rabbit lungs, the differences between surfactant from SP-A(-/-) and SP-A(+/-) mice were not significant. Maximal lung volumes measured at 30 cmH$_2$O for surfactant from SP-A(-/-) and SP-A(+/-) mice were not different ($P = 0.09$; Fig. 1B). Lung volumes measured at 30 and 10 cmH$_2$O pressure were increased by large-aggregate surfactants from both SP-A(+/-) and SP-A(-/-) mice relative to the untreated group (Fig. 1, B and C). The small-aggregate surfactant fractions from either genotype did not improve lung function.

Adsorption rate. There were no differences in adsorption rate for the surfactants from SP-A(+/-) and SP-A(-/-) mice. Even at high dilutions, the surfactants rapidly adsorbed to the surface, and equilibrium surface tensions were achieved in 48 ± 3 s for SP-A(+/-) mice and in 30 ± 8 s for SP-A(-/-) mice. There were no differences in the shape of the adsorption curves for the surfactants from the two genotypes.

Sensitivity to plasma protein inhibition. Three large-aggregate surfactant pools isolated from 10 mice for each pool from each genotype were used for these measurements. The large-aggregate surfactants from both SP-A(+/-) and SP-A(-/-) mice had low minimum surface tensions when measured at 0.025 µmol Sat PC/ml in the absence of plasma (Fig. 2). The surfactant from SP-A(-/-) mice was more sensitive to inactivation by plasma than was the surfactant from SP-A(+/-) mice at protein concentrations > 0.6 mg/ml ($P < 0.01$).

**Table 1. Preterm rabbit used for testing surfactant function in vivo**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Body Weight, g</th>
<th>Tidal Volume, ml/kg</th>
<th>PIP – PEEP, cmH$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>9</td>
<td>32 ± 5</td>
<td>8.1 ± 0.9</td>
<td>16.7 ± 0.7</td>
</tr>
<tr>
<td>Large aggregate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP-A(+/-)</td>
<td>11</td>
<td>30 ± 1</td>
<td>7.7 ± 0.3</td>
<td>13.6 ± 1.8*</td>
</tr>
<tr>
<td>SP-A(-/-)</td>
<td>12</td>
<td>29 ± 1</td>
<td>7.9 ± 0.5</td>
<td>10.5 ± 1.3*</td>
</tr>
<tr>
<td>Small aggregate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP-A(+/-)</td>
<td>5</td>
<td>29 ± 2</td>
<td>7.8 ± 0.3</td>
<td>15.5 ± 1.0</td>
</tr>
<tr>
<td>SP-A(-/-)</td>
<td>8</td>
<td>27 ± 2</td>
<td>8.0 ± 0.1</td>
<td>15.3 ± 1.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rabbits. PIP, positive inspiratory pressure; PEEP, positive end-expiratory pressure; SP-A(-/-), surfactant protein A deficient; SP-A(+/-), normal. *Significantly different from untreated, $P < 0.05$.

Fig. 1. Function of surfactant fractions from normal [surfactant protein (SP) A(+/-)] and SP-A-deficient [SP-A(-/-)] mice tested in ventilated preterm rabbits. Large-aggregate surfactant (LA) fractions from SP-A(+/-) treatment increased total lung compliance relative to untreated rabbits (A). SA, small-aggregate surfactant. There were no differences in compliance of premature rabbit lungs between LA from SP-A(+/-) and SP-A(-/-) mice. Lung volumes at 30 (B) and 10 (C) cmH$_2$O pressure from deflation limbs of pressure-volume curves were increased by LA from both SP-A(+/-) and SP-A(-/-) mice. *$P < 0.05$ vs. untreated rabbits.
Surfactant density and form conversion. Two samples of pooled large-aggregate surfactants from eight mice of both genotypes had similar densities on the linear sucrose density gradients (Fig. 3A). The peak sucrose density of large-aggregate surfactant from SP-A(−/−) mice was lower (1.042 and 1.044) than that of the surfactant from SP-A(+/+) mice (1.059 and 1.060). The percentages of small-aggregate surfactant derived from large-aggregate surfactant after 6 h of surface area cycling or no cycling are shown in Fig. 3B. The conversion was low for both genotypes without surface area cycling. After 6 h of cycling, the conversion from large aggregates to small aggregates was 72.9 ± 5.2% for surfactant from SP-A(−/−) mice and 37.0 ± 2.6% for surfactant from SP-A(+/+) mice (P < 0.001). The percentages of the aggregate forms isolated from the alveolar washes of the mice also were different. In lavages from SP-A(+/+) mice, 48 ± 1% of Sat PC was in the large-aggregate fraction, and only 15 ± 2% of lavage Sat PC from SP-A(−/−) mice was in the large-aggregate fraction (P < 0.01).

Electron microscopy of surfactant. Tubular myelin was easily identified in sections from pellets of large-aggregate surfactant from SP-A(+/+) mice (Fig. 4A). In contrast, no tubular myelin was seen in multiple sections of the large-aggregate surfactant from SP-A(−/−) mice (Fig. 4B). Although mixtures of densely packed and loose lipid arrays were observed in both genotypes, the lipid arrays were larger in samples from SP-A(+/+) mice (Fig. 4, C and D).

Lung injury by NMU. NMU caused a severe injury in the mice, resulting in the death of four of eight SP-A(+/+) mice and two of eight SP-A(−/−) mice on day 3. Total protein in the alveolar washes increased 1 day after NMU treatment and increased further 2 days after NMU treatment, and the increases were similar in both genotypes (Fig. 5A). The recovery of 125I-albumin also was increased on day 2 after NMU treatment, and there were no differences between the two genotypes (Fig. 5B). NMU did not alter the percentage of large-aggregate forms in the SP-A(+/+) and SP-A(−/−) mice, although the percentages were different for the two genotypes (Fig. 5C).

Clearance of SP-A. The curves for the clearance of 125I-SP-A from the air spaces of SP-A(+/+) and SP-A(−/−) mice were similar (Fig. 6). The loss of 125I-SP-A from the lung was best fit by semilog curves, with half-life values of 10.2 h for the SP-A(+/+) mice (r = 0.95) and 14.5 h for the SP-A(−/−) mice (r = 0.94). Although these values were not very different, the recoveries of SP-A at 24 and 40 h were significantly different (P < 0.05).

Cell content of alveolar washes. The total cells recovered by alveolar wash from the lungs of three 1-mo-old and three 2-mo-old SP-A(+/+) mice were 9.7 ± 0.3 × 10⁶ and 7.9 ± 1.1 × 10⁵ cells, respectively. Parallel measurements in SP-A(−/−) mice yielded 7.4 ± 0.2 × 10⁶ and 6.8 ± 0.9 × 10⁵ cells in alveolar washes at 1 and 2 mo of age, respectively. More than 96% of the cells in all samples were macrophages, with the residual cells being a small number of lymphocytes and a few granulocytes.

DISCUSSION

Although pulmonary function in SP-A(−/−) mice is unaltered under normal conditions, distinct structural and functional properties of SP-A-deficient surfactant
were noted in vitro. SP-A-deficient surfactant lacks tubular myelin, is more easily inhibited by plasma protein, and forms less dense lipid aggregates. Despite these structural and functional differences, SP-A-deficient surfactant is as effective as normal surfactant at restoring lung volume in surfactant-deficient premature rabbits, and the lack of SP-A did not increase the lung injury caused by NMU. Therefore, the effects of SP-A on surfactant structure, form, and some biophysical properties do not result in abnormalities in surfactant function in vivo that are sufficient to interfere with lung function.

The transgenic mice with an absolute SP-A deficiency have surfactant that differs only in this major surfactant glycoprotein. There are no differences in SP-B mRNA, SP-C mRNA, and surfactant phospholipid composition in SP-A(−/−) mice compared with SP-A(+/+) mice (16). In a clean environment, SP-A(−/−) mice grow, reproduce normally, and maintain surfactant function and lung function (16). Therefore, SP-A is not essential for survival. This result is in contrast to SP-B deficiency, which results in lethal respiratory failure shortly after birth in mice and humans (3). SP-A also does not regulate the metabolism of Sat PC or SP-B in SP-A(−/−) mice (11). Surfactant pool sizes were similar in SP-A(−/−) mice and SP-A(+/+) mice, as were precursor incorporation rates into Sat PC, secretion of Sat PC, and clearances of Sat PC and SP-B. In the present study, there were small but significant decreases in alveolar and total lung clearances of SP-A from SP-A(−/−) mice compared with SP-A(+/+) mice. SP-A is known to be cleared and catabolized by both macrophages and type II cells (28, 33). The explanation for this small difference in SP-A clearance may be a change in the number of SP-A receptors on type II cells and macrophages. The effects of SP-A deficiency on overall surfactant metabolism is minimal in the SP-A(−/−) mouse (10).

The biophysical function of the SP-A-deficient surfactant was very similar to that of normal surfactant. Minimal surface tensions were previously reported to be similar for SP-A-deficient and normal surfactants except at high dilution in the absence of Ca²⁺ (16). Adsorption rates also were similar in SP-A-deficient and normal surfactants. This result contrasts with previous findings (7, 24, 35) that SP-A enhanced the adsorption rates of lipid extract surfactants containing SP-B and SP-C. This discrepancy may result from the organic solvent extraction steps used for reconstruction experiments that remove SP-A and disrupt the associations of SP-B and SP-C with phospholipids. Surfactant that contains no tubular myelin can have adsorption rates and minimal surface tension values similar to values for normal surfactant when measured at high dilutions. However, in vivo alveolar surfactant is at a much higher concentration. The Sat PC pool size is ∼10 µmol/kg in a 30-g mouse (12), and if it is assumed that alveolar fluid volume in the mouse is similar to estimates for other mammals (26), the alveolar surfactant concentration should be ∼25 mg/ml. This concentration is three orders of magnitude higher than the concentrations generally used for biophysical studies of surfactant function (4, 35). Therefore, under normal conditions, the biophysical properties of the large-aggregate surfactant in the SP-A(−/−) mouse should result in normal lung function. Tubular myelin is not needed for normal lung function, a result consistent with the good physiological responses achieved after the treatment of...
infants with respiratory distress syndrome with surfactants that do not contain SP-A or tubular myelin (14). However, the interpretation of the normal function of surfactant from SP-A(-/-) mice when used to treat preterm rabbits must be tempered in that the function was tested at a dose known to give maximal treatment responses (25), and differences between normal and SP-A-deficient surfactant might be apparent at lower treatment doses. The preterm rabbit lung could contribute some endogenous SP-A to the SP-A-deficient surfactant, although SP-A mRNA is low in preterm rabbit lungs at 27 days gestational age (5, 21).

Although the alveolar pool size of Sat PC was similar for SP-A(+/-) and SP-A(-/-) mice (10), the amount of large-aggregate surfactant was decreased in alveolar washes from SP-A(-/-) mice to 15% of the pool, in contrast to the value of 48% large-aggregate forms for the SP-A(+/-) mice. Korfhagen et al. (16) previously reported that there were no differences in the amount of large-aggregate forms from SP-A(+/-) and SP-A(-/-) mice. For the measurements reported here, a sucrose step gradient was used to separate large-aggregate forms from alveolar cells and other debris (12), although no differences in cell number or cell type were found. Our measurement of a decrease in large-aggregate forms in SP-A(-/-) mice was repeated multiple times for this report, and the result is consistent with the increased rate of conversion of large-aggregate surfactant to small-aggregate surfactant with surface area cycling and with previous reports (8, 29) that SP-A decreases the rate of form conversion.

SP-A-deficient surfactant was more sensitive to inhibition by plasma, a result consistent with the increased resistance of surfactant mixtures to protein inactivation when SP-A is added (4, 30). The increased inactivation of SP-A-deficient surfactant by plasma protein suggests that the surfactant of the SP-A(-/-) mouse might be more sensitive to inactivation with injury. Yukitake et al. (36) demonstrated that SP-A could modify the inhibitory effects of plasma proteins in surfactant-treated preterm rabbits. The decreased percentage of large-aggregate surfactant in the alveolar pool and the increased rate of conversion to small-

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**Fig. 5.** Lung injury by N-nitroso-N-methylurethane (NMU). A: total protein in alveolar wash (AW) was increased in NMU-treated mice. There were no differences in protein amount between the 2 genotypes on days 1 or 2 after NMU treatment (n = 8 mice/group). *P < 0.05 vs. without NMU (NO NMU). B: protein permeability of lung on day 2 after NMU treatment. There were no differences between SP-A(+/-) and SP-A(-/-) mice without NMU. *Percent recovery of 125I-albumin in AW increased after NMU exposure, P < 0.05 vs. NO NMU. C: percentage of LA was decreased in SP-A(-/-) mice compared with SP-A(+/-) mice. †P < 0.01 vs. SP-A(+/-) mice. Percentage of LA did not change after NMU injection.

**Fig. 6.** Clearance of 125I-SP-A from AW (A), lung tissue after lavage (B), and total lung (C) after intratracheal instillation in 4 mice/group at each time point. Clearance of SP-A was not very different 7 h after instillation and was slightly slower at later times. *P < 0.05 vs. SP-A(+/-) mice.
aggregate surfactant for surfactant from SP-A(−/−) mice might also be expected to compromise surfactant function with lung injury (8, 17). In lung injuries such as acute respiratory distress syndrome, the amount of large-aggregate surfactant is decreased, and respiratory dysfunction correlates with decreased large-aggregate pools in animal models of lung injury. In previous studies (17, 18), NMU caused a slowly progressive but severe pulmonary edema in rabbits, rats, and dogs. Therefore, we used NMU-induced lung injury to test the surfactant system in the SP-A(−/−) mouse. Although NMU caused pulmonary edema in both SP-A(+/+) and SP-A(−/−) mice, the death rates after NMU treatment were similar. Inhibition of surfactant by edema depends on the absolute concentration of surfactant more than on the amount of protein inhibitors (9). At high surfactant concentrations, plasma proteins do not inhibit surfactant very effectively. The SP-A(−/−) mouse may not be more sensitive to NMU-induced lung injury than the SP-A(+/+) mouse because of the high concentrations of surfactant that are present in vivo or because of adaptations to the injury. The unique characteristic of the injury caused by NMU is the progression of the injury to death over 3–4 days. Perhaps a more acute injury with endotoxin or tumor necrosis factor-α would identify the effects of SP-A deficiency.

These results demonstrate that the surfactant from the SP-A(−/−) mouse is different in structure, density, and several other properties from normal surfactant. However, these differences do not result in abnormal lung function in SP-A(−/−) mice or in the treatment responses of surfactant-deficient preterm rabbits. It must be stressed that although surfactant function was evaluated with NMU-induced injury, other injuries to the surfactant system may reveal abnormalities of surfactant function. Compensatory mechanisms such as the adaptation of SP-D for some SP-A functions in SP-A(−/−) mice also need to be evaluated.

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