Deficiency of SP-B reveals protective role of SP-C during oxygen lung injury

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PULMONARY SURFACTANT is a complex mixture of lipids and proteins that reduces surface tension at the air-liquid interface in the alveolus. Surfactant proteins play important roles in surfactant homeostasis (14), host defense (17), and surface activity (33) in the lung. Surfactant protein (SP)-B and SP-C are small, hydrophobic proteins that contribute to the surface activity of surfactant phospholipids, enhancing the adsorption, spreading, and stability at the air-liquid interface to reduce alveolar surface tension. Recent studies with the captive bubble surfactometer demonstrated that both SP-B and SP-C enhanced lipid adsorption and lowered minimum surface tension. SP-C was more effective in promoting lipid adsorption whereas SP-B was more effective in lowering surface tension (8, 28). These results and the unstable microbubbles described for surfactant isolated from SP-C-deficient mice (8) are consistent with the hypothesis that SP-C helps maintain a surfactant reservoir beneath the surface film and facilitates transfer of surfactant lipid to the monolayer. Addition of SP-B and/or SP-C to phospholipid mixtures enhances their surface properties in vitro (1, 7, 24, 28, 34) and improves lung function in surfactant-deficient animals in vivo (6, 20, 21, 25). Although both SP-B and SP-C are active in vitro, the potential unique contributions of either protein to surfactant function have not been clarified in vivo. Knockout mice have not provided clear insight into the distinct actions of SP-B (4, 5) and SP-C (8). SP-B deficiency in mice and humans caused lethal respiratory failure that was accompanied by failure to fully process pro-SP-C to its functional mature peptide form (5, 22, 31); in contrast, mature SP-B was produced normally in SP-C−/− mice, and pulmonary function was only subtly altered (8).

Heterozygous SP-B mice (SP-B+/−) survive with no apparent abnormalities in surfactant function (4). However, strikingly decreased survival and altered pressure-volume curves were observed in SP-B−/− mice after 3 days of O2 exposure. These abnormalities were reversed by pretreatment with a surfactant containing SP-B (30). To discern potential contributions of SP-C to lung function in vivo, heterozygous (SP-B+/−, SP-C−/−) mice were interbred to generate offspring with reduced levels of both SP-B and SP-C. These mice were exposed to 95% O2 for 3 days. Lung inflammation, mechanics, and the surface activity of surfactant were assessed to identify changes in lung function linked to altered SP-C levels.

METHODS

Mice. Heterozygous SP-B+/− mice (FVB) (4) were bred to heterozygous SP-C-deficient mice SP-C−/− (Swiss Black) (8).
to produce heterozygous (SP-B+/−SP-C+/−) mice. SP-B+/−SP-C+/− mice were mated to heterozygous SP-C-deficient littermates (SP-B−/−SP-C+/−) to produce progeny with the following genotypes: wild-type mice (SP-B+/−SP-C+/+), SP-C null mice (SP-B+/−SP-C−/−), heterozygous SP-B-deficient mice (SP-B−/−SP-C+/−), heterozygous SP-B and SP-C-deficient mice (SP-B−/−SP-C−/−), and heterozygous SP-B-deficient and null SP-C mice (SP-B−/−SP-C−/−). Mice were identified by specific PCR of tail DNA with primers that amplified endogenous SP-B (upstream primer 5′-ATCCCTCCTCTTCTGCTCTCC-3′ and downstream primer 5′-TGGTLTTATTGCGTCTGTTGG-3′) and endogenous SP-C (upstream primer 5′-CAGGATTCTCGCAGGTCC-3′ and downstream primer 5′-GTCTGTTGCTCTTCTTGCC-3′). The neomycin resistance gene inserted into the SP-B and SP-C loci was amplified by PCR of tail DNA using SP-B or SP-C upstream primer (5′-CACTTGTGTAGCAGTCAGTGCC-3′) and downstream primer 5′-H11032. Genotype was further confirmed by Western blot analysis for SP-B and SP-C in alveolar lavage samples (14). Mice were maintained in hepa-filtered cages in a barrier facility. There was no evidence of pathogens in sentinel mice co-maintained in the vivarium. All of the studies were performed with 7- to 8-wk-old mice. All procedures were conducted under Institutional Animal Care and Use Committee approved methods.

Content of SP-B and SP-C in bronchoalveolar lavage fluid (BALF) (14) was analyzed by Western blot in six mice from each of (SP-B+/+, SP-C+/+), (SP-B−/−, SP-C+/−), (SP-B+/−, SP-C−/−), and (SP-B+/−, SP-C+/−) groups. For SP-B analysis, aliquots containing 0.27 nmol of saturated phosphatidycholine (Sat PC) were electrophoresed under nonreducing conditions. For SP-C, samples containing 2.7 nmol of Sat PC were electrophoresed in the presence of β-mercaptoethanol. SP-B and SP-C samples were separated on 10–20% SDS-polyacrylamide gels with tricine buffer (Novex, San Diego, CA). After electrophoresis, proteins were transferred to polyvinylidene difluoride paper (Bio-Rad, Hercules, CA). Immunoblot analysis was carried out with rabbit anti-bovine SP-B, 1:10,000, and rabbit anti-recombinant human SP-C 1:25,000. Appropriate peroxidase-conjugated secondary antibodies were used at 1:10,000 dilutions. 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 analysis software (Alpha Innotech, San Leandro, CA).

Hyperoxia. Eight transgenic mice from each group were placed in a Plexiglas chamber in which the O2 concentration was maintained at 95% at 1 atmosphere by mixing 100% O2 and air for 72 h. These exposure conditions were similar to those previously reported for SP-A- and SP-B-deficient mice (13, 30). The stability of O2 percentage in the chamber was monitored two times a day and did not require any readjustment of the O2 or airflow.

Pressure-volume curve. Pressure-volume curves were measured after 3 days of exposure to hyperoxia (13). Mice were sedated with pentobarbitonal sodium (100 mg/kg ip) and placed in a box containing 100% O2 to ensure complete collapse of the alveoli by O2 absorption after spontaneous breathing had stopped. The mice were killed by exsanguination, and the trachea was cannulated and connected by a syringe to a pressure sensor (mouse pulmonary testing system, TSS, Cincinnati, OH) via a three-way connector. After the diaphragm was opened, lungs were inflated in 75-ml increments every 10 s to 360 ± 0.4 cmH2O pressure and were similarly deflated.

Sat PC, protein, and cytokine analysis. One milliliter of 0.9% NaCl at 4°C was flushed into the airway until the lungs were fully expanded. The fluid was withdrawn and infused by syringe three times for each lavage. Lavage was repeated five times, and the samples were pooled. After lavage, lung tissue was homogenized in 0.9% NaCl and adjusted to the same volume for all the samples. Aliquots of BALF and lung homogenate were extracted with chloroform-methanol (2:1), and Sat PC was isolated by the technique of Mason et al. (18). The amount of Sat PC was measured by phosphorus assay (11). To measure the increase in protein leak into the alveolar space caused by hyperoxic lung injury, the protein in an aliquot of BALF was assessed by the method of Lowry et al. (16). Protein in total BALF volume was normalized to body weight of mice in air or before hyperoxia because body weight is proportionate to lung weight and surface area (23). Total protein in lung homogenate after lavage was measured (16) and used to normalize lung air volumes in pressure-volume measurements. IL-6, IL-1β, macrophage inflammatory protein-2 (MIP-2), and tumor necrosis factor-α (TNF-α) in BALF were measured by using Quantikine (R&D Systems, Minneapolis, MN).

Lung mechanics. For lung mechanics, mice were anesthetized with 0.1 ml/10 g of a mixture containing xylazine (2.0 mg/ml) and ketamine (40 mg/ml). Two-thirds of the dose was given to induce anesthesia, with the remaining given when the animals were attached to the ventilator. A tracheotomy was performed, and a polyethylene cannula (1.0 cm, 0.023 cm ID) was inserted. Mice were ventilated with a tidal volume of 8 ml/kg at a rate of 450 breaths per minute, with an end-expiratory pressure of 2 hPa, by using a custom-designed ventilator (flexiVent, Scireq, Montreal, PQ, Canada). This ventilator allowed measurement of lung function using a modification of the low-frequency forced-oscillation technique (8, 9). Respiratory input impedance was measured between 0.25 and 20 Hz by applying a composite signal containing 19 mutually prime sinusoidal waves during pauses in regular ventilation. Measurements were made at mean pressure of 2 and 5 hPa, and small-amplitude oscillatory signal produced pressure oscillations around the chosen pressure. For each measurement, the ventilator was paused to the mean pressure for 1 s to ensure pressure equilibration. The expiratory valve was then closed, and the oscillatory forcing function was applied by the piston. Because the mouse was exposed to a closed system during the measurements, changes in lung volume were avoided. The constant-phase model described by Hantos et al. (9) was used to partition respiratory input impedance into components representing the mechanical properties of the airway resistance, tissue resistance, and tissue elastance. Hysteresivity describes the mechanical coupling between tissue resistance and elastance and is calculated as hysteresivity = tissue resistance/tissue elastance. The calibration procedure removes the impedance of the equipment and tracheal tube. The results reported represent the mechanical properties of the mouse lung alone.

Surface activity. Large-aggregate surfactant was isolated from BALF by centrifugation at 40,000 g for 15 min over a 0.8 M sucrose in 0.9% NaCl cushion. The large-aggregate surfactant was recovered at the sucrose interface. The surface activity of three pools (3 mice/pool) of large-aggregate surfactant was measured from mice of each genotype with a captive bubble surfactometer (27) at 37°C. The concentration of each sample was adjusted to 3 nmol Sat PC/μl, and 3 μl of the surfactant were applied to the air interface of 25.6 ± 0.5 μl bubble by microsyringe. Surface tension was measured every 10 s for 300 s, equilibrium surface tension was measured, and then bubble pulsation was started. The minimum surface
tension after 65% bubble volume reduction was measured at the fifth pulsation. To study the stability of smaller bubbles, 3 μl of 0.3 nmol Sat PC/μl isolated large-aggregate surfactant was applied to 1.3 ± 0.1 μl volume bubble, and surface tension were recorded for 12 min (8).

**Statistics.** Values are means ± SE. Differences between the two groups were determined by a two-tailed Student’s t-test. Between-group comparisons were made by ANOVA followed by the Student-Newman-Keuls multiple-comparison procedure.

**RESULTS**

**Alveolar SP-B and SP-C pool sizes.** The amount of the SP-B and SP-C in alveolar lavage samples relative to Sat PC was estimated by Western blot and normalized to the quantity of SP-B and SP-C in (SP-B+/−, SP-C+/−) mice, which was given the value of 1 (Fig. 1).

Alveolar SP-B content in (SP-B+/−, SP-C+/−), (SP-B+/−, SP-C+/−), and (SP-B+/−, SP-C−/−) mice was decreased to ~40% compared with wild-type mice (P < 0.01 vs. wild type). In heterozygous SP-C-deficient mice, (SP-B+/−, SP-C+/−), the SP-C pool size was ~60% of that for wild-type mice (P < 0.001).

**Hyperoxia.** There were no statistical differences in body weight among the different genotypes. The mean body weight for all mice was 25.5 ± 0.4 g. There were no deaths on day 3 of hyperoxia in any group. After 3 days of exposure to 95% O2, body weight was reduced by 20% in all groups (mean body weight = 20.6 ± 0.4 g).

**Pressure-volume curve.** The protein content of lung tissue after alveolar lavage was similar in all the genotype groups, both in air and after 95% O2, with mean value of 9.5 ± 0.4 mg/mouse. Therefore, lung volumes (ml) at each pressure were normalized to total lung tissue protein (Fig. 2). Lung volumes at maximum pressure (Vmax) for mice breathing air were similar in all five genotype groups when compared by ANOVA. The Vmax for three groups in air with heterozygous SP-B alleles [(SP-B+/−, SP-C+/−), (SP-B+/−, SP-C+/−), and (SP-B+/−, SP-C−/−)] was larger than that for wild-type mice when analyzed by t-test (P < 0.05). As previously demonstrated in heterozygous SP-B mice (5, 30), air volumes at 5 and 0 cmH2O on the deflation limb for (SP-B+/−, SP-C+/−) mice were higher than for the groups with wild-type SP-B alleles, (SP-B+/+, SP-C+/+), and (SP-B+/+, SP-C+/−) mice (P < 0.05 by ANOVA). Similarly, (SP-B+/−, SP-C+/−) and (SP-B+/−, SP-C−/−) mice showed higher air volume than wild-type mice at 5 cmH2O on the deflation limb (P < 0.05 by t-test). After exposure to 95% O2 for 3 days, the pressure-volume curves were not altered in (SP-B+/−, SP-C+/+) and (SP-B+/−, SP-C+/−) mice. Hyperoxia altered lung pressure-volume curves in all groups, with heterozygous SP-B+/− alleles resulting in lowered lung volumes and reduced hysteresis after 3 days in 95% O2. The percent reductions in lung volumes at Vmax were 40% in (SP-B+/−, SP-C−/−), 37% in (SP-B+/+, SP-C−/−), and 19% in (SP-B+/+, SP-C+/−) mice. In these three groups of mice in which SP-B reduced, the decrease in Vmax and hysteresis caused by hyperoxia tended to be more severe when SP-C content was decreased [P < 0.05 (SP-B+/−, SP-C−/−) vs. (SP-B+/+, SP-C+/+) mice].

**Protein in alveolar lavage.** Total protein in BALF was measured as an indicator of proteinaceous alveolar edema (Fig. 3) after hyperoxia. For 95% O2 groups, body weight before exposure to hyperoxia was used to normalize the total amount of protein in BALF. Total protein in alveolar lavage samples was low for all the groups in air and was increased almost twofold by hyperoxia (P < 0.01 vs. air). After 3 days in 95% O2, the highest alveolar protein levels (P < 0.05) were observed in (SP-B+/−, SP-C−/−) mice.

In all mice housed in room air, both (SP-B+/−, SP-C−/−) and (SP-B+/+, SP-C−/−) mice had subtle but significantly higher amounts of alveolar protein than the two groups with wild-type SP-B alleles, (SP-B+/+, SP-C+/−) and (SP-B+/+, SP-C−/−) mice (P < 0.05).

**Alveolar cytokines.** IL-6, IL-1β, MIP-2, and TNF-α were measured in BALF from O2-exposed mice (Fig. 4). TNF-α was not detectable in any sample (data not shown). IL-6 was significantly increased after hyperoxia in (SP-B+/−, SP-C+/−), (SP-B+/+, SP-C−/−), and (SP-B+/+, SP-C−/−) mice and was significantly higher.
in (SP-B<sup>+/−</sup>,SP-C<sup>−/−</sup>) mice. IL-1β was significantly increased in BALF from (SP-B<sup>+/−</sup>,SP-C<sup>−/−</sup>) mice only. Mean MIP-2 concentrations were highest in (SP-B<sup>+/−</sup>,SP-C<sup>−/−</sup>) mice, although the difference among the groups was not statistically significant. During O<sub>2</sub> exposure, BALF cytokines were increased most markedly in (SP-B<sup>+/−</sup>,SP-C<sup>−/−</sup>) mice, revealing a possible protective role for SP-C during hyperoxia.

Sat PC content. Sat PC in alveolar lavage and total lung (alveolar lavage plus lung tissue after lavage) are shown in Fig. 5. In air, alveolar and lung Sat PC pool sizes were similar in all the genotypes. Sat PC content was decreased in alveolar lavage samples after 3 days of exposure to 95% O<sub>2</sub> (P < 0.05) except in (SP-B<sup>+/−</sup>,SP-C<sup>−/−</sup>) mice. A modest decrease (P < 0.05) in total lung Sat PC was observed in (SP-B<sup>+/−</sup>,SP-C<sup>+/+</sup>) and (SP-B<sup>+/−</sup>,SP-C<sup>−/−</sup>) mice after hyperoxia.

Baseline lung mechanics. Mice were sent from Cincinnati to Perth, Western Australia for measurement of lung mechanics. Numbers of (SP-B<sup>+/−</sup>,SP-C<sup>−/−</sup>) mice were inadequate for study at the time of shipment; thus, it was not possible to measure lung mechanics in this group. However, lung mechanics in this genotype (SP-C-deficient mice) was studied previously (8), demonstrating lower hysteresivity at low mean pressure compared with wild-type mice. Measurements made at increasing mean pressures at 2 and 5 hPa showed no significant changes in airway resistance, tissue resistance, and tissue elastance between the groups studied. Airway resistance was 0.37 ± 0.04 for (SP-B<sup>+/−</sup>, SP-B<sup>+/−</sup>, SP-C<sup>−/−</sup>), SP-C<sup>−/−</sup>) mice. IL-1β was significantly increased in BALF from (SP-B<sup>+/−</sup>,SP-C<sup>−/−</sup>) mice only. Mean MIP-2 concentrations were highest in (SP-B<sup>+/−</sup>,SP-C<sup>−/−</sup>) mice, although the difference among the groups was not statistically significant. During O<sub>2</sub> exposure, BALF cytokines were increased most markedly in (SP-B<sup>+/−</sup>,SP-C<sup>−/−</sup>) mice, revealing a possible protective role for SP-C during hyperoxia.

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SP-C/H11001/H11001/H11001/H11001/0.32/H11006/0.03 for (SP-B/H11001/H11002/H11002, SP-C/H11002/H11002/H11002/H11002), and 0.34/H11006/0.03 for (SP-B/H11001/H11002/H11002, SP-C/H11002/H11002/H11002/H11002) mice. Baseline hysteresivity at 2 hPa was similar in all the groups studied (Fig. 6). As expected, hysteresivity was increased as pressure was increased from 2 to 5 hPa. Interestingly, hysteresivity was significantly decreased in (SP-B/H11001/H11002/H11002, SP-C/H11002/H11002/H11002/H11002) mice at mean pressure of 5 hPa compared with (SP-B/H11001/H11001/H11001, SP-C/H11001/H11001/H11001/H11001) mice (\(P < 0.05\)), indicating reduced viscoelasticity of the lung at lower lung volumes.

Surface activity. Using a captive bubble surfactometer with a bubble size of 26 \(\mu\)l, we produced higher equilibrium surface tension from surfactant from (SP-B/H11001/H11002/H11002, SP-C/H11002/H11002/H11002/H11002) mice compared with other groups (Table 1). There were no statistical differences in minimum surface tension among the groups, but marked variations were seen in three groups with reduced SP-B. The observed difference in altered hysteresivity of lung mechanics was considered to reflect altered surface activity in (SP-B/H11001/H11002/H11002, SP-C/H11002/H11002/H11002/H11002) mice at low mean pressure or small alveolar volume. Therefore, surfactant was applied to a bubble of minimum size (1.3 \(\mu\)l), and the stability of the microbubble was recorded over time. Surfactant from SP-C/H11001/H11001/H11001 and SP-C/H11001/H11002/H11002 mice stabilized the microbubble (Fig. 7). Microbubbles made...
with surfactant from SP-C-deficient mice, whether (SP-B<sup>+/+</sup>,SP-C<sup>−−</sup>) or (SP-B<sup>++</sup>,SP-C<sup>−−</sup>), were unstable, with rapid changes in bubble shape and surface tension occurring during the 14-min study period. More experiment-to-experiment variations were seen in the SP-C<sup>−−</sup> surfactant samples.

**DISCUSSION**

In SP-B<sup>++</sup> mice, pressure-volume curves and inflammatory cytokine, alveolar protein, and Sat PC concentrations responses to lung injury caused by hyperoxia were markedly increased in the absence of SP-C. Mechanical coupling between tissue resistance and elastance (hysteresivity) was decreased at low lung volumes, and surfactant function (the ability to stabilize micro bubbles) was altered in SP-C<sup>−−</sup> compared with SP-C-replete mice. Thus decreased levels of SP-B revealed a physiological role of SP-C in maintenance of lung function during hyperoxia and the role of SP-C in stabilizing the alveolus at low lung volume.

SP-C and SP-B are hydrophobic peptides that enhance the surface activity of surfactant phospholipids. SP-C and/or SP-B increased the surface activity of phospholipid mixtures in vitro (1, 24, 29, 34) and restored lung function in surfactant-deficient immature lungs (10, 25) and adult lungs with inactivated surfactant function (15). Commercially available surfactant used for surfactant treatment (Survanta), containing phospholipids and bovine SP-C with lesser amounts of SP-B, are highly useful as surfactant replacement for treatment of respiratory distress syndrome. Likewise, synthetic surfactants made with recombinant human SP-C and phospholipid mixtures (Venticute) are highly functional in surfactant-deficient lungs in vivo (6, 20, 21). These surfactants dramatically improve lung function of patients with respiratory distress syndrome or acute respiratory distress syndrome as well as animal models with surfactant deficiency or altered surfactant function. The improvement in lung function with SP-C-rich surfactant is similar to the activity of surfactants containing physiological levels of SP-C and SP-B (e.g., CLSE, Infasurf).

Although in vitro studies and the successful use of SP-C-containing surfactants for replacement therapy supported an important role for SP-C in surfactant function, the distinct contribution of each protein has not been understood. Initial analyses of lung function of SP-B (5) and SP-C (8) knockout mice did not provide clear insight into the role of SP-C for lung function in vivo. SP-C knockout mice grow normally without apparent pulmonary abnormalities. Concentrations of other surfactant proteins, including SP-B, were not altered in BALF. Because SP-C-deficient mice die of respiratory failure immediately after birth, it has not been possible to study SP-C in the absence of SP-B. Furthermore, the processing of SP-C precursor is altered in SP-B<sup>−−</sup> mice and infants. An aberrant form of pro-SP-C is produced, SP-B<sup>−−</sup> mice and individuals being deficient in both SP-B and SP-C in the alveolus. We hypothesized that SP-B alone was sufficient for surfactant function but that transgenic mice with

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Table 1. *Surface tension of isolated surfactant (bubble size 23 μl)*

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Values are means ± SE (in mN/m); n = 4 pools/group. SP-B and SP-C, surfactant proteins-B and -C; ST, surface tension. *P < 0.05 vs. other groups.

**Fig. 7. Stability of microbubble.** Three pools of large aggregate fractions (3 mice/pool) of surfactant from each genotype group (A–E) were used to assess stability of surface tension measurements at a low bubble volume (1.3 μl). Symbols represent 3 individual measurements. Stable surface tension generated with surfactant isolated from the mice with half or full amount of SP-C: (SP-B<sup>+/+</sup>,SP-C<sup>+/+</sup>) (A1), (SP-B<sup>++</sup>,SP-C<sup>+/+</sup>) (C), and (SP-B<sup>+/−</sup>,SP-C<sup>+/+</sup>) (D) mice. Surface film generated from SP-C<sup>−−</sup> mice (in both SP-B<sup>++</sup> and SP-B<sup>−−</sup>-alleles) was unstable.
~50% reduced amounts of SP-B in the alveolar lavage (SP-B^{+/−}) might reveal the contribution of SP-C in response to hyperoxic stress.

As previously reported for (SP-B^{+/−}, SP-C^{+/+}) mice (4), in air, lung volumes on the deflation limb of the pressure-volume curve were higher in the mice with reduced levels of SP-B [(SP-B^{+−}/−), (SP-B^{+/−}, SP-C^{+/+}), and (SP-B^{+−}/−}, SP-C^{−/−})] compared with the mice with normal amounts of SP-B [(SP-B^{+/+}, SP-C^{+/+}) and (SP-B^{+−}/−}, SP-C^{−/−}) mice]. After exposure to 95% O₂ for 3 days, lung volumes on the pressure-volume curve were decreased in all SP-B^{+/−} mice. In contrast, lung volumes were unchanged by O₂ exposure in the SP-B^{+/+} mice, with either SP-C^{+/−} or SP-C^{−/−}.

In the SP-B^{+/−} mice, decreased levels of SP-C (both SP-C^{+/−} and SP-C^{−/−}) further perturbed pressure-volume relationships, which were worsened after hyperoxia. During O₂ exposure, the increase in total protein and cytokines in BALF was most severe in SP-B^{+/−} mice in the absence of SP-C compared with all other genotypes studied. The current study provides important information regarding the role of SP-C during hyperoxic stress in mice with genetic deficiency of SP-B. These results suggest that other stresses known to reduce SP-B levels in BALF may lead to altered lung function and that the outcome may well be affected by SP-C levels.

To assess tolerance to hyperoxia, we exposed mice to 95% O₂ for 3 days. The 3-day exposure period was chosen because recent studies in mice indicated that SP-B^{+/−} mice (30), SP-A^{−/−} mice (13), and wild-type mice tolerated 3 days of hyperoxia with increased total protein in BALF and increased protein permeability. Pressure-volume curves were altered in SP-B^{+/−} mice, but no changes in lung function were seen in SP-A^{−/−} mice. Sat PC was reduced during exposure to O₂ in the present study, and reduced amounts of surfactant phospholipid after hyperoxia were shown in other species (2, 12, 19). Likewise, decreased surfactant secretion was noted after 48-h exposure to 95% O₂ in vitro (32) and in our present study. Reduction in Sat PC was more marked in BALF than in the lung. After hyperoxia, alveolar macrophages were activated and expressed cytokine mRNAs (3). Each cytokine has a different time course of response to inflammation. TNF-α is a fast responder and returns to baseline more rapidly than other cytokines (3). BALF was collected at 72 h of hyperoxia, in the present study, and TNF-α was not increased at that late time point. IL-6 has been reported to increase in BALF after hyperoxia (3), and IL-6 was significantly increased by hyperoxic exposure of (SP-B^{+/−}, SP-C^{−/−}) mice. We did not characterize inflammatory cells in BALF, but increased cytokines and total protein strongly suggest a larger inflammatory response in the (SP-B^{+/−}, SP-C^{−/−}) mice than in the other groups.

SP-B and SP-C play important roles in the formation, maintenance, and function of the surface film. The results of in vitro studies suggest that SP-B is more effective in lowering minimum surface tension whereas SP-C is more effective in stabilizing the surface film (24, 26). The mechanisms underlying these functions are not clear but may include SP-C-mediated recruitment of lipids into the expanding surface film from a surfactant reservoir in the subphase (7, 26, 28). Surfactant from mice with normal levels of SP-C and 50% of the normal amount of SP-B exhibited stable surface tensions consistent with rapid transport of Sat PC from the reservoir to the gap in the surface film resulting from lipid squeeze out; in contrast, surfactant lacking SP-C produced an unstable surface film in which surface tension fluctuated. Surface film instability was only detected when the bubble radius was very small and packing of phospholipids was consequently very high. This outcome, coupled with lowered hysteresis in lung of SP-C^{−/−} mice at low mean airway pressure, suggests that SP-C stabilizes surfactant activity at low lung volumes. Under normal conditions, SP-B plays a dominant role in surfactant function; however, under conditions of stress and decreased levels of SP-B, SP-C can help maintain lung volumes during lung injury when alveolar capillary leak inhibits surfactant activity and levels of surfactant lipids are decreased.

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REFERENCES


