Surfactant Protein B (SP-B) −/− Mice Are Rescued by Restoration of SP-B Expression in Alveolar Type II Cells but Not Clara Cells*

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Surfactant protein B (SP-B) mRNA and protein are restricted to alveolar Type II and Clara cells in the respiratory epithelium. In order to investigate the function of SP-B in these distinct cell types, transgenic mice were generated in which SP-B expression was selectively restored in Type II cells or Clara cells of SP-B −/− mice. The 4.8-kilobase murine SP-C promoter was used to generate transgenic lines which expressed human SP-B in Type II cells (mSP-C/hSP-B). Likewise, the 2.3-kilobase mouse CCSP promoter was used to generate transgenic mice which expressed human SP-B in Clara cells (mCCSP/hSP-B). mSP-C/hSP-B and mCCSP/hSP-B transgenic mice were subsequently bred to SP-B +/− mice in order to selectively express SP-B in Type II cells or Clara cells of SP-B −/− mice. Selective restoration of SP-B expression in Type II cells completely rescued the neonatal lethal phenotype in SP-B −/− mice. Expression of SP-B in some, but not all Type II cells of SP-B −/− mice, allowed postnatal survival, but resulted in significantly altered lung architecture and function. Selective restoration of SP-B expression in Clara cells of SP-B −/− mice resulted in respiratory dysfunction and invariable neonatal death, related to the complete absence of mature SP-B peptide in these mice. These results indicate that expression and processing of the SP-B proprotein to the mature peptide in Type II cells is absolutely required for lung function in vivo and that SP-B expression in Clara cells cannot substitute for this function.

Surfactant protein B (SP-B)1 is a critical component of pulmonary surfactant, a lipid-protein mixture which forms a film along the surface of the alveolar epithelium, and is absolutely required for maintenance of alveolar stability at low lung volumes. Surfactant phospholipid mixtures lacking the hydrophobic proteins SP-B and SP-C have poor surface film-forming properties, whereas surfactants containing SP-B as the sole protein component rapidly form a stable phospholipid film in vitro and restore lung function in surfactant-deficient preterm animals (1–3). Genetic ablation of the murine SP-B locus leads to acute respiratory distress syndrome at birth resulting in death within minutes (4). Likewise, mutations resulting in SP-B deficiency in human infants lead to severe respiratory distress and death in the neonatal period (5, 6). Intratracheal administration of exogenous SP-B to infants with hereditary SP-B deficiency failed to restore lung function, suggesting that SP-B may have functions in addition to promoting formation of a stable surface film in the alveolus (7). Consistent with this hypothesis, SP-B deficiency in mice and human infants was associated with failure to form lamellar bodies and altered pro-SP-C processing (4, 8).

Expression of SP-B is restricted to alveolar Type II cells and nonciliated bronchiolar epithelial (Clara) cells of the pulmonary epithelium (9, 10). In Type II cells, proteolytic processing of the SP-B proprotein is initiated in the multivesicular body with cleavage of an NH2-terminal propeptide to generate a processing intermediate of 25 kDa (11, 12). Subsequent cleavage of a COOH-terminal propeptide results in liberation of the hydrophobic mature peptide which forms homodimers of 18 kDa. Mature SP-B is stored with surfactant phospholipids in lamellar bodies, the contents of which are released into the alveolar airspace via basal and stimulus-induced secretion (reviewed in (13)).

In contrast to the well characterized SP-B biosynthetic pathway in Type II cells, little is known about synthesis and processing of SP-B in Clara cells. Technical problems in the isolation of pure populations of these cells has made study of SP-B processing by Clara cells difficult. Clara cells are the most abundant cell type within the conducting airways of the murine lung, comprising more than 50% of the epithelial cells lining the terminal bronchioles (14, 15). Expression of SP-B in cells at the terminal airway/alveolar junction suggests that under certain circumstances, Clara cells may contribute SP-B to the alveolar surfactant pool. In addition to a putative alveolar surfactant function, Clara cell SP-B may also promote formation of a surfactant film in the terminal bronchioles which may be important for maintaining the patency of small conducting airways. Finally, given the widespread distribution of Clara cells in the murine airway, it is possible that SP-B has function(s) that are independent of surfactant activity. In order to better understand the role of SP-B in Type II cells and Clara cells, we have generated transgenic mouse lines in which SP-B expression was ablated and then restored in a cell-specific manner.

MATERIALS AND METHODS

Targeted Expression of Human SP-B in Clara Cells of Transgenic Mice—The murine CCSP/hSP-B transgene, comprised of a 2.4-kb HindIII/XhoI fragment of the murine CCSP promoter (16) (a kind gift from Dr. Barry Stripp, Departments of Environmental Medicine, Pediatrics and Oncology, University of Rochester, Rochester, NY) ligated to a 1.6-kb XhoI/EcoRI fragment of the full-length human SP-B cDNA (17) and a 400-bp EcoRI/NcoI fragment containing the SV40 small t intron and
polyadenylation signal, was cloned into the pET21a vector (Novagen, Madison, WI). Prior to injection, the 4.4-kb transgene was released from the vector by SalI/NotI digestion and gel-purified using QIAEX II Gel Extraction Kit (Qiagen, Chatsworth, CA). Purified DNA was extensively digested against 5 units Tris (pH 7.4) and 0.1 μl EDTA and subsequently dialyzed into fasting buffer (24). After washing, the human SP-B was incubated overnight at 4 °C and diluted in 1:1000, 1:2000, or 1:500, 1:1000, or 1:2000 in blocking solution (24). Immunohistochemistry—Immunostaining for SP-B proprotein was performed with antisera 96189, 55522, or 55019 (Promega, Madison, WI) as described previously (24). Serial 5-μm paraffin sections from lung tissues were loaded onto polylysine-coated slides (Fisher, Atlanta, GA). Slides were deparaffinized, rehydrated in a series of graded alcohol, and treated with 3% H2O2 in methanol for 15 min to quench endogenous peroxidase. Non-specific staining was blocked with 2% normal goat serum in phosphate-buffered saline with 0.2% Triton X-100 for 2 h at room temperature. Slides were then incubated overnight at 4 °C with antisera diluted 1:500, 1:1000, or 1:2000 in blocking solution (24). After washing, the sections were blocked for 30 min at room temperature with biotinylated goat anti-rabbit secondary antibody diluted 1:200 in the blocking solution, followed by incubation for 30 min with the avidin-biotin-peroxidase complex using Vectastain Elite ABC Kit (Vector Laboratories, Inc., Burlingame, CA). The slides were color developed using 3,3′-diaminobenzidine-HCl and further enhanced with nickel cobalt ferrophosphate counterstain. The section is represented by the ultrathin cryosections photographed on a Microphot-FXA Nikon fluorescence microscope.

Electron Microscopy—Fetuses were collected by cesarean section at 18 days of gestation and the torso fixed in ice-cold 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (SCB) at pH 7.3 for 2 h. The fixed lungs were removed, cut into 1–2-mm² blocks, post-fixed in fresh fixed overnight at 4 °C, washed in 0.1 M SCB, and post-fixed for 1 h with 1% osmium tetroxide (Electron Microscopy Sciences, Ft. Washington, PA) in 0.1 M SCB for 2 h at room temperature. Tissues were dehydrated in a series of graded alcohol solutions and embedded in EMBed 812 (Electron Microscopy Sciences). Ultrathin sections were cut at 100 nm of thickness using a Reichert Ultracut E ultramicrotome (Reichert, Austria), and post-stained with 2% uranyl acetate and lead citrate. Alveolar Type II epithelial cells and Clara cells were photographed at ×9,000 using a JEOL 100-CXII electron microscope at 80 keV of accelerating voltage. Immunogold labeling of ultrathin cryosections was performed precisely as we have previously described (25).

Hypoxic Challenge—hSP-ChSP-B(mSP-B+/−) transgenic mice express a truncated human SP-B cDNA, encoding residues 1–279 of the protein (i.e. deletion of the 102 residue COOH-terminal propeptide), under control of the 3.7-kb human SP-C promoter (26). We have previously demonstrated that SP-B RNA is not detected in Clara cells of this transgenic line and alveolar surfactant pool size and phospholipid composition are not different from wild type littermates (26). Three 6-week-old hSP-ChSP-B(mSP-B+/−) mice and 3 wild type mice in the same genetic background (FVB/N) were exposed to 95% oxygen for 48 or 72 h as described previously (27, 28). Following exposure, mice were immediately sacrificed by a lethal injection of pentobarbital, rapidly exsanguinated, and the lungs perfused with 4% paraformaldehyde in phosphate-buffered saline. The lungs were excised, post-fixed in 4% paraformaldehyde overnight, and prepared for either hematoxylin and eosin staining or immunostaining with antibodies directed against the mature SP-B or proprotein. Control mice consisted of 3 room air-exposed littermates of the same genotype. For semiquantitative analysis, MetaMorph software (Universal Imaging Corporation, West Chester, PA) was used to acquire and store images of hematoxylin/eosin-stained sections from the right upper, right middle, right lower, left upper, and left lower lobes. Five sections from each lung were visualized with a ×20 objective lens and MetaMorph software used to assess total number of Type II cells, average Type II cell size, average septal wall thickness, total alveolar space, and average alveolar space. These measurements were scored by 3 masked observers and compared statistically by two-way ANOVA. Each observer also noted the degree of microhemorrhages and edudination/edematous (none, mild/minimal, severe).

RESULTS

Generation and Characterization of Transgenic Mice Expressing Human SP-B—SP-B is synthesized in both alveolar Type II cells and bronchial Clara cells. To define the functions of SP-B in each cell type, transgenic mice were generated in which the full-length human SP-B cDNA was targeted to the bronchial Clara cell (mCCSP/hSP-B) or the alveolar Type II cell (mSP-B) using either the murine C57 or the murine SP-C promoter. Of 49 offspring from mCCSP/hSP-B mice, 3 from a wild type littermate and 3 wild type mice in the same genetic background (FVB/N) were exposed to 95% oxygen for 48 or 72 h as described previously (27, 28). Following exposure, mice were immediately sacrificed by a lethal injection of pentobarbital, rapidly exsanguinated, and the lungs perfused with 4% paraformaldehyde in phosphate-buffered saline. The lungs were excised, post-fixed in 4% paraformaldehyde overnight, and prepared for either hematoxylin and eosin staining or immunostaining with antibodies directed against the mature SP-B or proprotein. Control mice consisted of 3 room air-exposed littermates of the same genotype. For semiquantitative analysis, MetaMorph software (Universal Imaging Corporation, West Chester, PA) was used to acquire and store images of hematoxylin/eosin-stained sections from the right upper, right middle, right lower, left upper, and left lower lobes. Five sections from each lung were visualized with a ×20 objective lens and MetaMorph software used to assess total number of Type II cells, average Type II cell size, average septal wall thickness, total alveolar space, and average alveolar space. These measurements were scored by 3 masked observers and compared statistically by two-way ANOVA. Each observer also noted the degree of microhemorrhages and edudination/edematous (none, mild/minimal, severe).

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SP-B gene were bred to achieve cell-specific hSP-B expression in the SP-B /− background.

Previous studies have suggested that the 2.3-kb mouse CCSP (16, 29) and the 4.8-kb mouse SP-C (30) promoters directs gene expression specific to Clara cells or alveolar Type II cells, respectively. To assess the specificity of hSP-B transgene expression from the murine CCSP and murine SP-C promoters, in situ hybridization was performed using human and murine SP-B probes (Fig. 1). In the adult wild type animal, murine SP-B mRNA was detected in alveolar Type II cells and bronchiolar Clara cells (Fig. 1A). In day 18 fetal mice expressing human SP-B under control of the murine CCSP promoter in the wild type background (mCCSP/hSP-B), human SP-B mRNA was detected in Clara cells but not in Type II cells (Fig. 1D), whereas murine SP-B mRNA was detected in both alveolar and bronchiolar cells (Fig. 1C). In the mCCSP/hSP-B mice, human SP-B mRNA was detected at higher levels in the proximal bronchiolar epithelium than in distal bronchioles on day 18 of gestation (not shown). When these mice were crossed into the SP-B /− background to generate mCCSP/hSP-B(mSP-B /−) mice, SP-B mRNA (not shown) and protein (Fig. 2) were detected in presumptive Clara cells but not in alveolar epithelial cells of the fetal lung, consistent with Clara cell restricted expression of SP-B protein.

In mSP-C/hSP-B(mSP-B /−) transgenic mice (mice expressing the human SP-B transgene under control of the mouse SP-C promoter in the SP-B /− background), human SP-B mRNA was detected only in Type II cells (Fig. 3B); murine SP-B mRNA was not detected (Fig. 3A). However, in contrast to the results for in situ hybridization analyses, SP-B protein was detected in some Clara cells. In mSP-C/hSP-B(mSP-B /−) transgenic line 1, staining for SP-B in Clara cells was extensive and only slightly less than that in Clara cells from wild type littermates (not shown); however, in a second mSP-C/hSP-B(mSP-B /−) transgenic line (line 2), SP-B staining was largely restricted to alveolar Type II epithelial cells and only a few Clara cells expressing SP-B protein were detected (Fig. 4). Immunohistochemical analysis of a third mSP-C/hSP-B(mSP-B /−) transgenic line (line 3) indicated that SP-B protein was detected in some, but not all, Type II cells (see below, “Type II Cell-restricted Expression” and Fig. 9). These results suggest that the site of transgene integration strongly influences expression from the 4.8-kb murine SP-C promoter.

Expression of Human SP-B in Clara Cells—Expression of both human and murine SP-B RNA was observed in Clara cells from mCCSP/hSP-B transgenic mice in the SP-B /+ background. mCCSP/hSP-B transgenic mice survived without overt evidence of respiratory pathophysiology and had normal body weight, lung weight, reproductive function, longevity, and normal gross lung structure, suggesting that expression of human SP-B in Clara cells did not significantly alter lung function (not shown). To determine the fate of SP-B synthesized by Clara cells, bronchoalveolar lavage from wild type and mCCSP/hSP-B transgenic mice was immunoblotted with antibody directed against the COOH-terminal propeptide of human SP-B (antibody 96189) (Fig. 5) or antibody (55522) directed against...
the full-length human SP-B (not shown). Both the 42-kDa SP-B proprotein and 25-kDa processing intermediate were detected in increased concentrations in lavage from mCCSP/hSP-B mice relative to wild type littermates, consistent with secretion and accumulation of human SP-B precursor in the airway of transgenic animals. These results also suggest that partial processing of the SP-B proprotein normally occurs in Clara cells.

Clara Cell-restricted Expression of Human SP-B—In order to restrict SP-B synthesis to Clara cells, both mCCSP/hSP-B transgenic lines were crossed with hemizygous SP-B +/- mice to produce mCCSP/hSP-B(mSP-B +/-) mice. Newborn mCCSP/hSP-B(mSP-B +/-) pups from both mCCSP/hSP-B parental transgenic lines died within minutes of birth, similar to findings in SP-B +/- mice (4). To further increase SP-B protein levels, the two mCCSP/hSP-B lines were interbred prior to crossing into the SP-B +/- background. mCCSP/SBP-B(mSP-B +/-) pups carrying multiple copies of the mCCSP/hSP-B transgene and expressing increased levels of SP-B protein (Fig. 6A) also died of acute respiratory dysfunction within minutes of birth. Western blots of lung homogenates from newborn mCCSP/hSP-B(mSP-B +/-) mice with antibody 96189 detected both the 42- and 25-kDa forms of SP-B (Fig. 6A); however, mature SP-B peptide (18 kDa) was not detected, even when 10-fold more protein was loaded on the gel (Fig. 6B), strongly suggesting that these pups died from the lack of mature SP-B peptide and that Clara cells did not completely process pro-SP-B to the active peptide. Mature SP-B peptide was readily detected in lung homogenates from wild type littermates (Fig. 6B). Consistent with the neonatal death of mCCSP/hSP-B(mSP-B +/-) mice, ultrastructural analyses of lungs from pups on day 18 of gestation indicated that Clara cell-restricted expression of hSP-B was associated with severe disruption of the surfactant biosynthetic pathway in Type II cells (Fig. 7). Lamellar bodies, the intracellular storage form of pulmonary surfactant, were never detected; instead, similar to SP-B +/- mice, numerous multivesicular bodies and composite bodies, containing disorganized membrane lamellae and vesicular elements, were observed in Type II cells. The ultrastructure of Clara cells was unremarkable (not shown). Overall, restricted expression of SP-B in Clara cells was not associated with postnatal survival, formation of mature SP-B peptide, or normal lamellar bodyogenesis.

Type II Cell-restricted Expression of Human SP-B—The mSP-C promoter was used to generate three mSP-C/hSP-B transgenic lines expressing both endogenous murine SP-B and human SP-B mRNA in Type II epithelial cells. Expression of human SP-B mRNA and protein in Type II cells did not alter lung structure or function in any of the three transgenic lines, consistent with a previous study in which a truncated human...
SP-B proprotein was expressed in Type II cells of transgenic mice (26). In the mSP-C/hSP-B transgenic mice, human SP-B proprotein was expressed to the mature peptide and formed heterodimers with endogenous mouse SP-B mature peptide (Fig. 8); lung morphology in these mice was normal (not shown). To generate mice in which SP-B was synthesized only in Type II cells, mSP-C/hSP-B transgenic mice were crossed into SP-B −/− background. Offspring from mSP-C/hSP-B(mSP-B −/−) transgenic line 2, in which SP-B expression was largely restricted to Type II cells, were normal in terms of body weight, lung weight, reproductive function, and longevity, indicating that decreased expression of SP-B in Clara cells was not associated with any overt pathophysiology (not shown); furthermore, lamellar body structure was indistinguishable from wild type littermates and there were no detectable ultrastructural abnormalities in Type II cells (not shown). The ultrastructure of Clara cells in mSP-C/hSP-B(mSP-B −/−) mice was also indistinguishable from that of SP-B −/+ littermates (not shown). Although Western blotting indicated equivalent levels of SP-B in all three transgenic lines (not shown), fewer alveolar cells stained for pro-SP-B than for pro-SP-C in mSP-C/hSP-B(mSP-B −/−) transgenic line 3, consistent with the lack of SP-B expression in some Type II cells of mice from this transgenic line (Fig. 9). Both enlarged and collapsed alveoli were observed, consistent with heterogeneity of alveolar inflammation (Fig. 9). While ultrastructural analyses revealed normal Type II cell morphology, some Type II cells had abnormal lamellar bodies with morphology similar to those from SP-B −/− mice, consistent with heterogeneity of alveolar inflammation. These results underscore the importance of SP-B in Type II cells and suggest that regional deficiency of SP-B may lead to heterogeneous inflation.

Role of Clara Cell SP-B during Hyperoxic Lung Injury—SP-B mRNA is markedly increased in Clara cells in response to hyperoxia (28) and SP-B −/+ mice are highly susceptible to oxygen induced injury in vivo (31). In order to test the hypothesis that SP-B production by Clara cells is critical for recovery from oxygen-induced lung injury, 6-week-old hSP-C/hSP-BΔ(mSP-B −/−) mice were exposed to 95% O2. By immunohistochemistry, SP-B protein was detected in Type II cells but not in Clara cells of hSP-C/hSP-BΔ(mSP-B −/−) mice (not shown) consistent with the results of previous in situ hybridization studies (26). There was no difference in gross lung structure between transgenic mice and wild type littermates with respect to septal thickening, microhemorrhages, alveolar inflammation, or cell infiltration, or Type II cell hyperplasia or hypertrophy after 48 or 72 h of O2 exposure. Mean survival times for wild type mice (7.5 days ± 0.5, n = 10) and transgenic mice (7.9 days ± 0.5, n = 10) were not different, suggesting that Clara cell SP-B may play a role in the protection against hyperoxic lung injury.

FIG. 7. Ultrastructure of type II cells in mCCSP/hSP-B(mSP-B −/−) and mSP-C/hSP-B(mSP-B −/−) transgenic mice. Electron microscopy was performed on ultrathin sections from lungs of mCCSP/hSP-B(mSP-B −/−) mice (A) and mSP-C/hSP-B(mSP-B −/−) mice (B) on day 18 of gestation. mSP-C/hSP-B(mSP-B −/−) transgenic line 2, in which expression of SP-B was largely restricted to Type II cells, was selected for these studies. Multivesicular bodies (arrow) and composite bodies (arrowhead) containing multivesicular bodies and membrane lamellae were detected in alveolar Type II epithelial cells of mCCSP/hSP-B(mSP-B −/−) fetuses. Mature lamellar bodies were never detected in mCCSP/hSP-B(mSP-B −/−) fetal lung, but were always detected in mSP-C/hSP-B(mSP-B −/−) mice (B, arrowhead). Bar, 1 μm.

FIG. 8. SP-B proprotein is processed to the mature peptide in mSP-ChSP-B(mSP-B −/−) mice. Five μg of lung homogenate from 6-week-old mice were subjected to SDS-PAGE under nonreducing electrophoretic conditions and analyzed by Western blotting with antibody 28031. Lane 1, wild type mice; lane 2, mSP-C/hSP-B transgenic mice; lane 3, mSP-ChSP-B(mSP-B −/−) mice. Mouse SP-B homodimer, Mr = 16,000, lane 1 migrates more quickly than human SP-B homodimer, Mr = 18,000 (lane 3). Mouse/human heterodimers were detected when the mSP-ChSP-B transgene was expressed in the wild type background (lane 2).
FIG. 9. Failure to restore SP-B proprotein expression in all Type II cells is associated with microatelectasis and alveolar dilatation. Immunohistochemistry was performed on serial lung sections from 6-week-old wild type mice (A and B) and mSP-C/hSP-B(mSP-B \( \sim \)) transgenic line 3 (C and D) using antibody 28061 to detect SP-B (A and C), or antibody 68514, to detect SP-C (B and D). Approximately equal numbers of SP-B and SP-C immunoreactive cells (arrowheads) were detected in alveoli of wild type mice (A and B), whereas the number of SP-B immunoreactive cells was notably decreased in mSP-C/hSP-B(mSP-B \( \sim \)) transgenic mice (C and D). In transgenic mice (D), clusters of SP-C immunoreactive Type II cells were detected, consistent with alveolar collapse (arrows). Enlarged alveolar space(S) were common in mSP-C/hSP-B(mSP-B \( \sim \)) mice (C and D). Magnification is \( \times 95 \) for all panels.

Discussion

SP-B is expressed in both Type II epithelial cells and nonciliated Clara cells of the pulmonary epithelium. Type II cells are restricted to the alveoli, whereas Clara cells are widely distributed in conducting airways of the mouse but are absent in alveoli. The increased abundance of Clara cells in terminal bronchioles suggests that Clara cells in this region may contribute SP-B to the alveolar surfactant pool. This hypothesis was tested by selectively replacing SP-B expression in either Clara cells or Type II cells of SP-B \( \sim \) mice. Restoration of SP-B in Clara cells was not sufficient to correct pulmonary dysfunction or neonatal lethality in SP-B \( \sim \) mice, whereas selective expression in Type II cells completely restored lung function in vivo. These results indicate that the neonatal lethality in SP-B \( \sim \) mice is due to the absence of SP-B expression in Type II cells and that expression of SP-B in Clara cells cannot replace this function.

The failure of selective expression of human SP-B in Clara cells to correct respiratory failure in SP-B \( \sim \) mice is likely caused by the lack of mature SP-B peptide in these animals. SP-B proprotein and processing intermediate (25 kDa) were detected by Western blotting and immunohistochemistry and were dramatically increased in mice carrying multiple copies of the transgene; however, complete processing to the mature SP-B peptide by Clara cells was not observed. These results are similar to those in the H441 pulmonary adenocarcinoma cell line, and provide further support for the Clara cell origin of this cell line (32). The most likely explanation for incomplete processing of SP-B in SP-B \( \sim \) mice and Clara cells of mCCSP/hSP-B(mSP-B \( \sim \)) mice is that these cells lack an endoprotease that cleaves the 25 kDa processing intermediate to generate the mature SP-B peptide. Given that lamellar bodies were never detected in mCCSP/hSP-BmSP-B \( \sim \) mice, it is unlikely that SP-B proprotein or the 25-kDa form secreted by Clara cells is internalized and processed by Type II cells. Incomplete processing of SP-B proprotein, complete absence of mature SP-B peptide, and consistent neonatal respiratory failure in mCCSP/hSP-B(mSP-B \( \sim \)) mice strongly suggest that, under resting conditions, Clara cells do not contribute SP-B to the alveolar surfactant pool.

In SP-B \( +/– \) mice exposed to 95% oxygen for 3 days lung compliance was markedly reduced relative to wild type littermates (31). Altered lung function was associated with increased severity of pulmonary edema, hemorrhage, and inflammation, lung permeability, and protein leakage into the alveolar space supporting the concept that SP-B deficiency exacerbates hyperoxic-induced lung injury. In SP-B \( +/– \) mice similarly exposed to hyperoxia, SP-B RNA was dramatically increased in bronchiolar cells and decreased in alveolar cells suggesting that production of SP-B in Clara cells may compensate for diminished expression in Type II cells during hyperoxic challenge (28). This hypothesis predicts that loss of SP-B expression in Clara cells would increase susceptibility to hyperoxic-induced lung injury; however, the complete loss of SP-B in Clara cells of hSP-C/hSP-B(mSP-B \( \sim \)) mice did not exacerbate hyperoxic-induced changes in mortality or lung structure relative to wild type littermates. These results suggest that expression of SP-B in Clara cells is not required for surfactant homeostasis in unchallenged mice or for recovery from or adaptation to hyperoxia.

It has been suggested that the patency of terminal bronchioles is maintained by the presence of a surfactant film similar to that in alveoli (33). Given the large numbers of Clara cells present in terminal bronchioles, it is possible that SP-B produced by these cells contributes to formation of a surface film. Consistent with this hypothesis, SP-B \( +/– \) mice, in which SP-B RNA and protein levels are decreased 50% compared with wild type littermates, exhibit air trapping related to collapse of small airways (34). However, the ability of SP-B to promote rapid formation of a stable phospholipid film is likely exclusive to the mature peptide which is not synthesized in Clara cells. Since Type II cells are the sole source of mature SP-B, any SP-B peptide in the terminal airways is likely derived from the alveolar surfactant pool; however, to date, there is no direct
evidence for movement of SP-B from alveoli to conducting airways.

Although the role of SP-B in Clara cells remains uncertain, it is clear that expression of SP-B in Type II cells is absolutely required for lung function in vivo. Expression of a full-length (this study) or truncated (26) human SP-B proprotein in Type II cells of SP-B−/− mice completely reversed neonatal respiratory dysfunction and lethality in these mice. The importance of SP-B expression in Type II cells is further supported by results in mSP-C/HSP-B/mSP-B−/− transgenic line 3 in which SP-B expression was restored in some, but not all, Type II cells. These mice had distended alveoli and septal thickening consistent with focal atelectasis. This outcome is similar to that in ventilated preterm lambs in which surfactant deficiency resulted in dilation of alveolar ducts with collapse of some adjacent alveoli (35). Taken together, these results suggest a model in which Type II cells lacking SP-B secrete surfactant with poor surface activity leading to collapse of the involved alveolus at the end expiration. The collapsed alveolus contributes to thickening of the alveolar wall, while the expelled air enters an adjacent alveolus resulting in distension (36). Although distended, this alveolus remains open at the end expiration because Type II cells in this unit express SP-B and, consequently, secrete a functional surfactant.

In summary, selective restoration of SP-B synthesis in Type II cells completely rescued the respiratory failure following birth of SP-B−/− mice. Expression of SP-B in some, but not all Type II cells of SP-B−/− mice, allowed postnatal survival, but resulted in significantly altered lung architecture and function. Synthesis of pro-SP-B in Clara cells of SP-B−/− mice resulted in respiratory dysfunction and invariable neonatal death, associated with the complete absence of mature SP-B peptide in the lungs of these mice. These results indicate that expression and processing of the SP-B proprotein to the mature peptide in Type II cells is absolutely required for lung function in vivo and that synthesis of pro-SP-B in Clara cells cannot substitute for this function.

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