The Role of Homodimers in Surfactant Protein B Function in Vivo*

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Surfactant protein B (SP-B) is detected in the airways as a sulfhydryl-dependent dimer (M₂ ~ 16,000). To test the hypothesis that formation of homodimers is critical for SP-B function, the cysteine residue reported to be involved in SP-B dimerization was mutated to serine (Cys²⁴⁸ → Ser) and the mutated protein was targeted to the distal respiratory epithelium of transgenic mice. Transgenic lines which demonstrated appropriate processing, sorting, and secretion of human SP-B monomer were crossed with SP-B +/− mice to achieve expression of human monomer in the absence of endogenous SP-B dimer (hSP-Bmon, mSP-B+/−). In two of three transgenic lines, hSP-Bmon, mSP-B+/− mice had normal lung structure, complete processing of SP-C proprotein, well formed lamellar bodies, and normal longevity. Pulmonary function studies revealed an altered hysteresis curve for hSP-Bmon, mSP-B+/− mice relative to wild type mice. Large aggregate surfactant fractions from hSP-Bmon, mSP-B+/− mice resulted in higher surface tension than surfactant from wild type mice. Surfactant lipids supplemented with 2% hSP-B monomer resulted in slower adsorption and higher surface tension than surfactant with 2% hSP-B dimer. Taken together, these data indicate a role for SP-B dimer in surface tension reduction in the alveolus.

Pulmonary surfactant is a complex mixture of phospholipids and proteins synthesized and secreted by the alveolar Type II epithelial cell. Pulmonary surfactant insufficiency leads to respiratory distress syndrome in newborn infants, a leading cause of morbidity and mortality among neonates worldwide. Substantial benefit is derived from treating affected infants with surfactant replacement preparations, particularly those containing the hydrophobic surfactant protein (SP)-1 B or SP-C (1). Hereditary SP-B deficiency in human infants results in intracellular packaging of surfactant phospholipids was disrupted, resulting in a virtual absence of lamellar bodies, the intracellular storage granule for surfactant. Intracellular processing of the surfactant protein C proprotein was disrupted, leading to accumulation of an SP-C processing intermediate (M₂ ~ 8,000) and reduced levels of SP-C mature peptide (M₂ ~ 3,500) in the alveolar surfactant pool. Collectively, these observations indicate that SP-B plays a critical role in the maintenance of pulmonary surfactant homeostasis.

SP-B is synthesized as a 381-amino-acid propropein in Type II cells and Clara cells of the distal respiratory epithelium. Ablation of SP-B expression in Clara cells does not affect lung function whereas ablation of SP-B expression in Type II cells results in lethal, neonatal respiratory dysfunction (4). In Type II cells, SP-B proprotein is routed to the multivesicular body where amino- and carboxyl-terminal peptides are sequentially cleaved to produce the 79-amino-acid mature SP-B peptide (5). The mature peptide shares sequence homology with saposin proteins A-D, placing it in the saposin-like protein (SAPLIP) family. Members of the SAPLIP family share a common secondary structure characterized by three conserved intramolecular cysteine bridges (6). SP-B differs from other members of this family in that it is more hydrophobic and forms sulfhydryl-dependent homodimers (7). The SP-B propein, M₂ ~ 42,000, and its major processing intermediate, M₂ ~ 25,000, are never detected in oligemic form, consistent with dimerization of the SP-B mature peptide following proprotein processing. In humans and mice, the mature peptide is detected exclusively as a homodimer (M₂ ~ 16,000), suggesting that dimerization may be important for pulmonary surfactant structure and/or function. The current study was undertaken to test the hypothesis that formation of SP-B homodimers is critical for SP-B function in transgenic mice.

MATERIALS AND METHODS

DNA Constructs—To generate a dimerization-deficient human SP-B construct (hSP-Bmon), site-directed mutagenesis was employed using a PCR-ligation-PCR protocol to substitute serine for the cysteine residue involved in dimerization of the mature peptide (Cys²⁴⁸ → Ser) (8). Primers were chosen which would amplify a 1.6-kb fragment of human SP-B cDNA that included the entire 381-amino-acid propropein sequence (5′-ATTACCCGTCGACCTCACTGAGGCTGAGGCGAT-3′ and 5′-CAGAGATTCGATATCAATCTTGGATGCGATGCATGGGGGTGGG-3′). Internal primers used in the early round of PCR resulted in the substitution of serine (TCA) for cysteine (TGC; upstream primer 5′-CTTCGTCGATGCTACCGTCTCGG-3′ and downstream primer 5′-TGTCGATGGCGCCCTCGTACCTCGACGTC-3′). This construct was cloned into the mammalian expression vector PCI-neo (Promega, Madison, WI) and subjected to bidirectional sequencing to verify the fidelity of the PCR product throughout the SP-B coding region and to confirm the mutation.
**Targeted Expression of Monomeric SP-B to the Distal Respiratory Epithelium of Transgenic Mice**—To generate mice that expressed the mutant peptide in a spatial and temporal manner similar to endogenous SP-B, hSP-B<sub>mon</sub> was cloned into the pUC-hSP-C expression vector (9). This vector contained the 3.7-kb human SP-C promoter fragment, which drove the SV40 small t intron (10). Distal transgenic expression was achieved by inserting the neomycin resistance gene (upstream primer 5'-CACAACAGACAATCG-3') into the vector, as described (9) and confirmed by Southern analysis using a <sup>32</sup>P-radiolabeled probe that recognized the small t intron and polyadenylation signals. The transgene was microinjected into fertilized FVB/N oocytes by the Children's Hospital Transgenic Core facility and founders were identified by transgene-specific PCR and confirmed by Southern analysis using a <sup>3</sup>2P-radiolabeled probe that recognized the neomycin resistance gene and confirmed by transgene-specific PCR and confirmed by Southern analysis using a <sup>32</sup>P-radiolabeled probe that recognized the neomycin resistance gene (upstream primer 5'-CACAACAGACAATCG-3') and the absence of the PCR product for murine SP-B (10). Six to eight hSP-B<sub>mon</sub> mice from three independent transgenic lines, indicating that the transgene was present in the distal respiratory epithelium of these transgenic mice. Expression of Monomeric SP-B in the SP-B Null Background—To generate animals that expressed monomeric human SP-B in the SP-B<sup>-/-</sup> background, transgenic mice (hSP-B<sub>mon</sub>, mSP-B<sup>-/-</sup>) were first crossed with SP-B<sup>-/-</sup> mice. Transgenic offspring with a single copy of the endogenous SP-B allele (hSP-B<sub>mon</sub>, mSP-B<sup>+</sup>/<sup>-</sup>) were subsequently crossed with SP-B hemizygous animals to generate offspring that contained the monomer transgene in the absence of endogenous SP-B (hSP-B<sub>mon</sub>, mSP-B<sup>-/-</sup>). The SP-B<sup>-/-</sup> genotype was confirmed by amplification of a 438-base pair PCR product corresponding to the neomycin resistance gene (upstream primer 5'-CACAACAGACAATCG-GCT-3' and downstream primer 5'-CAGTTCGCTGCGCCGAG-3') and the absence of PCR product for murine SP-B (10). Six to eight hSP-B<sub>mon</sub> mice from three independent lines (A, D, and E) were killed and surfactant protein expression assessed by Western analysis. To determine SP-B concentration in the air spaces (i.e. secreted SP-B), surfactant was isolated by bronchoalveolar lavage from two groups of five wild type and five hSP-B<sub>mon</sub>, mSP-B<sup>-/-</sup> mice (14). The surfactant pellets were extracted with chloroform/methanol/water (3:2:0.75) and the organic phase was recovered for isolation of SP-B by gel filtration over Sephadex LH60 in chloroform, methanol, 0.01 M HCl, 19:19:2 (v/v/v) (15).

**Lung Morphology in Transgenic Animals**—To assess lung morphology, lungs from four to six adult hSP-B<sub>mon</sub>, mSP-B<sup>-/-</sup> mice from lines A, D, and E, were inflation fixed for immunostaining and light microscopy. Immunostaining for surfactant proteins was performed with antisera directed against the carboxyl terminus of pro-SP-B, mature SP-B, and the amino terminus of pro-SP-C. To confirm the expression of the transgene, murine SP-B, and GM-CSF (hSP-B<sub>mon</sub>, mSP-B<sup>-/-</sup>, GM-<sup>-/-</sup>) were bred with GM-CSF null mice (GM<sup>-/-</sup>) to generate animals with single copies of the transgene, murine SP-B, and GM-CSF (hSP-B<sub>mon</sub>, mSP-B<sup>-/-</sup>, GM<sup>-/-</sup>). These animals were subsequently crossed to generate mice that produced only SP-B monomer in the GM-CSF null background (hSP-B<sub>mon</sub>, mSP-B<sup>-/-</sup>, GM<sup>-/-</sup>). Similar crosses were performed with a transgenic line that expressed human SP-B dimer in the absence of murine SP-B to generate mice that produced increased quantities of the human peptide (hSP-B<sub>mon</sub>, mSP-B<sup>-/-</sup>, GM<sup>-/-</sup>, GM<sup>-/-</sup>) (10). A PCR specific for the endogenous GM-CSF allele (5'-TGCCCCAACCCAGGAGGACG-3' and 5'-CCACCGAGAGCAAGCACA-3') was used to identify GM-CSF<sup>-/-</sup> animals. SP-B protein was isolated as described above.

**In Vitro Surface Tension Measurements**—To assess the surface tension reducing properties of surfactant, large aggregate surfactant was isolated from one group of wild type mice and one group of hSP-B<sub>mon</sub> mice. Surfactant was isolated by bronchoalveolar lavage from two groups of five wild type and five hSP-B<sub>mon</sub>, mSP-B<sup>-/-</sup> mice (14). A Wilhelmy balance with a platinum dipping stick was used to estimate minimum surface tensions achieved during five consecutive cycles of compression and expansion. The surface properties of SP-B monomer and dimer isolated from GM-CSF<sup>-/-</sup> mice were assessed by captive bubble surfactometry as described (19).

**Characterization of Human SP-B Monomer Transgenic Mice**—SP-B is synthesized as a preproprotein which is proteolytically cleaved to generate the 79-amino acid mature peptide. The mature peptide is detected exclusively as a homodimer in mouse bronchoalveolar lavage fluid. In order to identify the function of SP-B dimerization, transgenic mouse lines were generated in which the cysteine residue proposed to be responsible for mediating dimerization (7) was mutated to serine. Expression of the SP-B monomer was targeted to the distal respiratory epithelium using the 3.7-kb human SP-C promoter (Fig. 1A). Seven of 13 (54%) offspring from fertilized oocyte injections were transgene positive, as identified by both PCR and Southern blot analysis of tail DNA (not shown). Under nonreducing electrophoretic conditions, the SP-B homodimer (M<sub>r</sub> = 16,000) was detected in all animals whereas the SP-B monomer (M<sub>r</sub> = 8,000) was detected only in transgenic animals (Fig. 1B). This result confirmed that cysteine 248 was essential for SP-B homodimer formation. Monomeric SP-B protein was detected in offspring from three of the seven transgenic lines (A, D, and E). These mice (hSP-B<sub>mon</sub>, mSP-B<sup>-/-</sup>, GM<sup>-/-</sup>) survived without any overt evidence of respiratory pathophysiology and had normal body weight, lung weight, reproductive function, longevity, and lung structure (not shown), indicating that expression of SP-B monomer in the wild type SP-B background did not significantly alter lung function.

**RESULTS**

**Analysis of Lung Function**—To test lung function in vivo, pressure-volume curves were obtained for six wild type, seven hSP-B<sub>mon</sub>, mSP-B<sup>-/-</sup>, and five hSP-B<sub>mon</sub>, mSP-B<sup>-/-</sup>–5-week-old mice from the D transgenic line. Mice were killed with a lethal intraperitoneal injection of sodium pentobarbital and allowed to expire in a 100% oxygen chamber. The chest wall was completely opened, the trachea was cannulated, and connected to a syringe and pressure sensor (X-ducer; Motorola, Phoenix, AZ). The lungs were inflated in 75- or 100-μl increments to a maximum pressure of 30 cm H<sub>2</sub>O and deflated to negative pressure. The hysteresis ratio was calculated as the area bound by the inflation and deflation curves divided by the total area bound by the maximum and minimum values for pressure and volume area (software provided by Harvard Apparatus). Lung surfactant protein content and distribution were determined by gas chromatography and Western blotting with SP-B–specific antiserum confirmed the complete absence of SP-B dimer in hSP-B<sub>mon</sub>, mSP-B<sup>-/-</sup>.
mSP-B−/− mice (Fig. 2). To determine if there was increased mortality in animals expressing only SP-B monomer, a series of 10 crosses was performed in which a rescued male from transgenic line D (hSP-Bmon, mSP-B−/−) was crossed with hemizygous females (mSP-B+/−). The expected Mendelian inheritance pattern for mice alive at 6 weeks of age was 1:2:0 for rescued (hSP-Bmon, mSP-B−/−): hemizygous (mSP-B+/−): null (mSP-B−/−) animals. The observed frequencies were not significantly different from the expected frequencies (Table I). Overall, replacement of endogenous SP-B with SP-B monomer resulted in complete reversal of the neonatal lethal SP-B−/− phenotype.

**Lung Structure and Surfactant Protein Expression in hSP-Bmon, mSP-B−/− Mice—SP-B null mice (mSP-B−/−) have poor lung compliance, resulting in collapsed airways and alveoli (20). To test the ability of SP-B monomer to restore normal lung structure in mSP-B−/− mice, histological sections from hSP-Bmon, mSP-B−/− lungs were examined. Lung architecture in mice from transgenic lines D and E1 was histologically indistinguishable from wild type littermates (not shown). Immunostaining for SP-B in these animals revealed that the human SP-B monomer was appropriately expressed in Type II cells and non-ciliated bronchiolar epithelial cells, recapitulating the endogenous expression pattern for SP-B (not shown). Ultrastructure analyses revealed that animals which expressed only SP-B monomer (hSP-Bmon, mSP-B−/−) formed lamellar bodies, in contrast to SP-B−/− mice in which mature lamellar bodies were never detected (not shown) (3). In addition, levels of mature SP-C peptide, which were dramatically reduced in SP-B−/− mice, were normal in hSP-Bmon, mSP-B−/− mice (Fig. 3). Furthermore, the SP-C processing intermediate which accumulated in SP-B−/− mice was not detected in hSP-Bmon, mSP-B−/− mice, consistent with restoration of SP-C proprotein processing (Fig. 3).

The level of SP-B peptide in surfactant has been shown to be important in lung function (21, 22). The amount of monomeric SP-B protein in bronchoalveolar lavage fluid from transgenic line D hSP-Bmon, mSP-B−/− mice relative to wild type mice was estimated from amino acid composition analyses following organic extraction (23). In two separate experiments, bronchoalveolar lavage from wild type mice contained 0.36 and 0.25 μg of SP-B per mouse, whereas hSP-Bmon, mSP-B−/− mice contained 0.42 and 0.25 μg of SP-B per mouse. This result suggested that monomeric SP-B was able to function effectively when present at levels comparable to endogenous SP-B levels in wild type mice.

Consistent with the hypothesis that SP-B protein levels are critical for lung function, transgenic line E, which displayed variable hSP-B expression levels, had decreased survival. Offspring resulting from crosses with a transgenic male (E1) expressed high levels of SP-B monomer protein and survived in the mSP-B−/− background, whereas offspring from a transgenic female (E2) had low levels of monomeric protein in the SP-B null background and never survived the neonatal period (Fig. 4). This was demonstrated for five consecutive generations of animals in the SP-B−/− background, and was therefore not likely the result of independent integration sites. The most likely explanation for this finding is that the transgene had inserted into a site subject to genomic imprinting (24, 25). S1 nuclease analyses confirmed that levels of hSP-B mRNA in transgenic line E2 (hSP-B mRNA/β-actin mRNA = 0.5) were

**FIG. 2.** SP-B monomer reverses the neonatal lethal SP-B null phenotype. Four micrograms of total lung homogenates from adult mice were subjected to SDS-PAGE and Western blotting with mature SP-B antiserum under conditions that maintained cysteine bond integrity. The complete absence of SP-B expression (mSP-B−/−, lane 1) resulted in neonatal respiratory distress and death. hSP-Bmon, mSP-B−/− mice (lanes 4–6 and 9–11) that expressed SP-B monomer (arrowhead) but not endogenous SP-B (arrow) appeared healthy at 6 weeks, indicating reversal of the neonatal lethal SP-B−/− phenotype (lanes 4–6 were from transgenic line A; lanes 9–11 were from transgenic line D). SP-B dimer was detected only in animals that contained an intact endogenous SP-B allele in addition to the transgene (lanes 2, 3, 7, and 8).

**TABLE I**

|          | hSP-Bmon | mSP-B−/− | mSP-B+/− |
|----------|----------|-----------|-----------|
| Expected | 37       | 74        | 0         |
| Observed | 40       | 71        | 0         |

**FIG. 3.** Processing of SP-C proprotein is restored in hSP-Bmon, mSP-B−/− mice. Ten micrograms of protein from fetal day 18.5 total lung homogenates were subjected to SDS-PAGE under nonreducing electrophoretic conditions and analyzed by Western blotting with anti-mature SP-C antibody. SP-C mature peptide (arrowhead) was markedly reduced in fetal day 18.5 SP-B null mice (lanes 3 and 4) compared with wild type littermates (lanes 1 and 2). In mice that expressed SP-B monomer in the SP-B−/− background (lanes 5 and 6), mature SP-C levels were restored to normal levels. An SP-C processing intermediate (arrow) that accumulates in SP-B−/− mice was not detected in animals expressing transgenic SP-B monomer or endogenous SP-B dimer.
Overall, the results of a monomeric quaternary structure in this concentration range. Migrated with an apparent molecular mass of 9 kDa, indicating a lack of function. However, function in mice was restored when transgenic line E 2 were insufficient to restore normal lung function in mSP-B−/− mice. It is therefore likely that expression levels of SP-B monomer in transgenic line E 2 were insufficient to restore normal lung function in mSP-B−/− mice.

**Surfactant Function in hSP-Bmon, mSP-B−/− Mice**—To determine if the SP-B monomer was as effective as its dimer in reducing surface tension in the lung, pressure-volume curves were generated for wild type, hSP-Bmon, mSP-B+/−, and hSP-Bmon, mSP-B−/− mice (Fig. 5A). The hysteresis area was significantly decreased in hSP-Bmon, mSP-B−/− mice when compared with wild type and hSP-Bmon, mSP-B+/− mice (Fig. 5B). To test the hypothesis that surfactant from hSP-Bmon, mSP-B−/− mice had altered surface properties, the ability to reduce surface tension in vitro was tested using a Wilhelmy balance. Minimum surface tensions for five consecutive cycles were obtained for large aggregate surfactant fractions isolated from wild type and hSP-Bmon, mSP-B−/− mice (Fig. 6). The minimum surface tension achieved with wild type surfactant was less than 10 milliNewtons/m, consistent with previous studies (26). Surfactant from hSP-Bmon, mSP-B−/− mice generated higher surface tension upon compression. The effect of sulfhydryl-dependent dimerization of SP-B on the ability of surfactant phospholipids to adsorb to an air-liquid interface and reduce surface tension was directly tested (Fig. 7) by recombining purified SP-B monomer or dimer with a defined, synthetic phospholipid mixture. Data in Fig. 7A demonstrate that minimum surface tension was attained rapidly for all surfactant mixtures in the absence of cycling. However, surfactant lipids with 2% SP-B monomer (w/w) had a significantly slower rate of adsorption than did surfactant with 2% SP-B dimer (Fig. 7A). After multiple cycles of compression and expansion, minimum surface tension achieved with monomer surfactant was significantly higher than surfactant with SP-B dimer (Fig. 7B). These results indicate that, on a mass basis, SP-B monomer is not as effective as wild-type SP-B at reducing surface tension at an air-liquid interface. However, when the concentration of SP-B monomer was doubled relative to the concentration of SP-B dimer, a similar reduction in surface tension was achieved. Gel filtration of monomeric SP-B (approximately 10 μM) over Sephadex LH-60 in chloroform, methanol, 0.1 M HCl (19:19:2, by volume) showed that the molecule migrated with an apparent molecular mass of 9 kDa, indicating a monomeric quaternary structure in this concentration range. Overall, the results of in vivo and in vitro studies indicate that dimerization of SP-B is not absolutely required for lung function, but that SP-B dimerization contributes to optimal surfactant function.

![Image](http://www.jbc.org/)

**DISCUSSION**

The SP-B mature peptide is detected exclusively as a homodimer in humans and mice. It is the only member of the SAPLIP family that forms disulfide-dependent dimers, suggesting that dimerization of SP-B may be critical for its role in surface tension reduction at the alveolar air-liquid interface. To test this hypothesis, transgenic mouse lines were generated that expressed SP-B monomer in the distal respiratory epithelium. Under oxidizing conditions that maintained cysteine bridge integrity, transgenic protein was detected as a monomer, M, 8,000. This result confirmed the prediction from in vitro analyses that SP-B cysteine residue 248 participates in an
Expression of SP-B monomer protein in SP-B-deficient mice completely reversed the neonatal lethality associated with SP-B deficiency. Normal longevity was restored in these mice, indicating that the formation of SP-B dimer was not essential for survival in unchallenged animals. The role of SP-B dimerization in lung function was assessed by generation of pressure-volume curves. The hysteresis area of lungs containing only SP-B monomer was significantly decreased when compared with lungs containing equivalent amounts of SP-B dimer on a weight basis. This indicates that there was a loss of function in the absence of SP-B dimer that could not be compensated for by the presence of SP-B monomer. These data indicate that SP-B dimer is important in establishing normal lung hysteresis, likely due to an important role in surfactant function.

To further investigate the role of SP-B dimerization in surfactant film formation and surface tension reduction, the surface properties of large aggregate surfactant fractions isolated from hSP-Bmon, mSP-B+/−, and wild type mice were tested in vitro. As predicted from pressure-volume studies, surfactant from hSP-Bmon, mSP-B+/− mice was less able to reduce surface tension forces compared with wild type surfactant. Two explanations are possible: SP-B dimers contribute directly to the surface tension reducing properties of pulmonary surfactant, or SP-B dimerization is critical in the Type II cell for formation of an optimal surfactant protein-lipid complex. To distinguish between these possibilities, SP-B monomer and dimer were isolated from mice that produced large quantities of human SP-B dimer (hSP-Bmon, mSP-B+/−, GM-CSF+/−) or human SP-B monomer (hSP-Bmon, mSP-B+/−, GM-CSF−/−) and used to reconstitute surfactant preparations for in vitro analyses. Surfactant from GM-CSF+/− mice has been shown to have surface properties similar to equivalently high levels of wild type surfactant, providing a useful model for generating large quantities of functional surfactant proteins and lipids (14). SP-B levels for both transgenic lines in the GM-CSF+/− background were increased more than 100-fold when compared with wild type mice (data not shown). When equal masses (2% w/w) of monomer or dimer were combined with a DPPC/POPG mixture, the monomer preparation exhibited surface properties that were not significantly different from lipid alone. Since SP-B dimer consists of two covalently linked mature peptides, there were twice as many molecules of SP-B dimer than SP-B monomer. Increasing the concentration of SP-B monomer 2-fold (four times the molar quantity) resulted in normal surface activity. These data suggest that increased levels of SP-B monomer can compensate for diminished protein function.

The surface tension reducing properties of surfactant are very dependent upon concentration (26). The current study utilized low concentrations of surfactants (25 μg/μl) in order to better identify differences between the surface activities of SP-B dimer and SP-B monomer. At this dilution, the surfactant containing 2% SP-B monomer had relatively poor surface activity. However, it should be noted that at such low concentrations, even surfactant containing SP-B dimer did not result in surface tensions approximating zero, which are characteristic of higher surfactant concentrations.

Despite decreased surface properties in vitro, the SP-B monomer completely reversed the neonatal lethality in SP-B−/− mice. One explanation for this outcome is that SP-B is present in concentrations in excess of that required for optimal lung function. Consistent with this hypothesis, SP-B hemizygous mice survive and have only subtle changes in lung function; similarly, human carriers of a single null SP-B allele demonstrated no evidence of lung disease (27). In the hSP-Bmon, mSP-B−/− mice, SP-B monomer was present in similar or slightly higher levels compared with wild type mice. The results of in vitro analyses suggest that a decrease in monomer levels to

Figure 6. Surfactant from hSP-Bmon, mSP-B+/− mice results in increased surface tension. Large aggregate surfactant was isolated from wild type or hSP-Bmon, mSP-B+/− and added to saline for a final Sat PC concentration of 0.010 μmol/ml. A Wilhelmy balance was used to measure the minimal surface tension achieved by the large aggregate surfactant during five consecutive three-minute cycles. Results are shown for two separate experiments. Wild type surfactant reduced the surface tension to a greater extent than did surfactant from monomer rescued mice (closed circles, hSP-Bmon, mSP-B+/− surfactant; open circles, wild type surfactant).

Figure 7. Surface activity of purified SP-B monomer and SP-B dimer. Surface properties of the 2% SP-B (monomer or dimer)/DPPC/POPG mixture were assessed by captive bubble analysis. The mixture containing SP-B monomer had slower adsorption (A) and higher minimum surface tension (B) than the mixture containing SP-B dimer. When the concentration of SP-B monomer was doubled to 4%, the mixture had similar surface activity to 2% SP-B dimer (○, DPPC/PG; □, 2% dimer SP-B/DPPC/PG; Δ, 2% monomer SP-B/DPPC/PG; □, 4% monomer SP-B/DPPC/PG; *, p < 0.05 versus SP-B monomer; t, p < 0.0001 versus SP-B monomer by t test).

intermolecular disulfide bridge and mediates SP-B homodimerization (7). No overt phenotype was observed in hSP-Bmon, mSP-B+/− mice, consistent with previous studies demonstrating that expression of human SP-B did not alter lung structure or function in wild type mice (4, 10).

Expression of SP-B monomer protein in SP-B−/− mice completely reversed the neonatal lethality associated with SP-B monomer deficiency. The formation of SP-B dimer was not essential for survival in unchallenged animals. The role of SP-B dimerization in lung function was assessed by generation of pressure-volume curves. The hysteresis area of lungs containing only SP-B monomer was significantly decreased when compared with lungs containing equivalent amounts of SP-B dimer on a weight basis. This indicates that there was a loss of function in the absence of SP-B dimer that could not be compensated for by the presence of SP-B monomer. These data indicate that SP-B dimer is important in establishing normal lung hysteresis, likely due to an important role in surfactant function.

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50% of wild type levels may result in a significant decrease in lung function. An alternative explanation for normal survival of hSP-B
mon, mSP-B→/→ mice is that the role of SP-B in surface tension reduction is partially redundant with a similar role for SP-C (28). In SP-B null mice, SP-C mature peptide levels were greatly decreased; whereas in hSP-B
mon, mSP-B→/→ mice, SP-C mature peptide was restored to levels comparable to wild type mice. Therefore, mature SP-C protein may be critical for lung function in hSP-B
mon, mSP-B→/→ mice.

Incomplete SP-C processing in humans with hereditary SP-B deficiency and mSP-B→/→ mice resulted in a relative deficiency of mature SP-C peptide in the airways. Lamellar body formation in Type II cells was also disrupted, indicating that SP-B plays an important role in the intracellular processing and packaging of pulmonary surfactant. The mechanisms of lamellar body formation and packaging of surfactant proteins B and C in the multivesicular body are unknown, although it is likely that SP-B plays a pivotal role. In vitro studies demonstrated that the SP-B mature peptide is both fusogenic and lytic, and these properties may contribute to lamellar body formation (29). SP-B dimerization occurs concomitantly with protein processing in the multivesicular body, suggesting that SP-B dimer may be necessary for packaging of surfactant phospholipids during the transition from MVB to lamellar body. However, this study demonstrated that lamellar bodies formed in the absence of SP-B dimers and processing of SP-C to the mature peptide was complete in hSP-B
mon, mSP-B→/→ mice. Since the SP-B monomer was sufficient for correction of both lamellar body formation and SP-C proprotein processing, it is likely that the primary function of SP-B dimerization is related to surface tension reduction.

Previous work in this laboratory suggested that SP-B must be expressed in all Type II cells to restore normal lung structure and function (4). In the current study, one transgenic line was established that demonstrated expression of human SP-B monomer in a subset of Type II cells (A line, data not shown). In hSP-B
mon, mSP-B→/→ mice from this line, there were two populations of Type II cells, one with normal lamellar bodies and one with abnormal lipid inclusions that resembled those found in SP-B→/→ mice. The SP-C processing intermediate that accumulates in the absence of SP-B was detected in lungs from hSP-B
mon, mSP-B→/→ mice in this transgenic line. Lungs of adult hSP-B
mon, mSP-B→/→ offspring from this line had altered lung structure, with greatly widened air spaces. Taken together, these data support the hypothesis that SP-B production is required in all Type II cells, and that this requirement cannot be met through the presence of exogenous (i.e. airway) surfactant. A model has been proposed in which alveoli containing Type II cells deficient in SP-B have a tendency to collapse due to surfactant interactions with poor surface activity (4).

In summary, expression of SP-B monomer reversed the neonatal lethality and restored SP-C processing and lamellar body formation in SP-B→/→ mice. Monomer rescued mice revealed no overt phenotype, bred successfully, and experienced normal longevity. These results indicate that formation of SP-B dimers is not essential for surfactant function. However, hSP-B monomer was associated with altered lung hysteresis in vivo and altered surface properties in vitro. Taken together, these studies indicate a role for SP-B dimer in establishing normal lung hysteresis, and suggest that SP-B dimerization may be required for optimal lung function.

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The Role of Homodimers in Surfactant Protein B Function in Vivo
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