Surfactant Protein A Is a Principal and Oxidation-sensitive Microbial Permeabilizing Factor in the Alveolar Lining Fluid*

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We have reported that surfactant protein A kills some Gram-negative organisms by increasing membrane permeability. In this study, we investigated the physiologic importance of this activity and the effect of oxidative stress on the antimicrobial functions of SP-A in vitro and in vivo. Concentrated bronchoalveolar lavage fluids from SP-A+/+ mice increased the permeability of the Escherichia coli K12 cell membrane to a greater extent than lavage from SP-A−/− animals. Similarly, calcium-dependent surfactant-binding proteins of SP-A+/+ mice increased membrane permeability more than those from SP-A−/− mice and produced greater zonal killing of agar-embedded bacteria in a radial diffusion assay. Exposure of human SP-A to copper-initiated surfactant phospholipid peroxidation or to free radicals generated by human neutrophils in vitro increased the level of SP-A-associated carbonyl moieties and blocked the permeabilizing function of the protein. We also found that exposure of mice to 90% O2 for 4 days, sufficient to lead to consumption of glutathione, oxidation of protein thiols, and accumulation of airspace protein-associated carbonyl moieties, blocked the permeabilizing activity of lavage fluid from SP-A+/+ mice. We conclude that SP-A is a major microbial permeabilizing factor in lavage fluid and that oxidative stress inhibits the antibacterial activity of SP-A by a mechanism that includes oxidative modification and functional inactivation of the protein.

Surfactant proteins A and D, also known as the pulmonary collectins, play important roles in the innate immune defense of the lung, including the agglutination, opsonization, and augmentation of intraphagocytic killing of a variety of inhaled pathogens (1). We have recently reported that the pulmonary collectins also directly inhibit the growth of microorganisms by collectin-mediated binding of model surfactant lipids to the microorganism surface (2). Nosocomial pneumonias are a common complication of prolonged intensive care unit stays. Supplemental oxygen therapy is extensively used in this setting, resulting in conditions that favor formation of reactive oxygen species (ROS)† and damage to airway cells and extracellular molecules in the alveolar lining fluid. We recently reported that SP-A is oxidatively modified and functionally inactivated, with respect to interaction with phospholipids, upon exposure of SP-A to lipid peroxidation, in vitro (4). We postulate that oxidative damage of SP-A may also affect the host defense activities of the protein. In this study, we examined the relative physiologic importance of the permeabilizing activity of SP-A in the lung, and the effects of oxidizing stimuli on the antimicrobial activity of SP-A and the alveolar lining fluid.

MATERIALS AND METHODS

Reagents—Bovine serum albumin, Chelex 100, cholesterol, cupric sulfate, disodium EDTA, and β-phenanthroline-sulfonic acid were from Sigma. The OxyBlot™ Protein Oxidation Detection Kit was from In-tergen (Purchase, NY). The BCA Protein Assay Reagent Kit was from Pierce (Rockford, IL). 1,2-Dipalmitoyl-sn-glycerol-3-phosphocholine, L-phosphatidylcholine from egg yolk, and 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine (18:0–18:2 PC) in chloroform were from Avanti Polar Lipids, Inc. (Alabaster, AL). The alkaline phosphatase substrate, EL979, was from Molecular Probes, Inc. (Eugene, OR). The thiourea-iodoacetamide probe, Thiouki, was from Calbiochem. All other chemicals were of analytical grade. Microcon YM-3 MWCO 3,000 Millipore (Bedford, MA) centrifugal filter devices were used for concentration of protein. Spectra/Por cellulose membranes MWCO 3,500 (Spectrum Laboratories, Inc., Rancho Dominguez, CA) were used for dialysis. SeaKem LEagarose was from FMC Bioproducts (Rockland, ME).

Mice—Swiss Black SP-A−/− mice (a gift of J. Whitsett and T. Korfhagen) were developed from embryonic stem cells after disruption of the mouse SP-A gene by homologous recombination and maintained by breeding with Swiss Black mice, as previously reported (5). The SP-A null allele was bred into the C3H/HeN background through nine generations, as described (2, 3). All comparisons made with the SP-A−/− mice were with age and strain-matched C3H/HeN controls. All animals were housed in positively ventilated microisolator cages with automatic recirculating water located in a room with laminar, high efficiency particulate-filtered air. The animals received autoclaved food, water, and bedding. Mice were handled in accordance with approved protocols through the Institutional Animal Care and Use Committee at the University of Cincinnati School of Medicine.

SP-A Purification—Human SP-A was isolated from patients with pulmonary alveolar proteinosis, a lung disease associated with the accumulation of surfactant lipids and proteins. Briefly, SP-A was purified by the method of Suwabe (6) from the cell-free surfactant pellet of bronchoalveolar lavage by serial sedimentation and resuspension in buffer containing 5 mM Tris, 150 mM NaCl, and 1 mM Ca2+, release by incubation with 2 mM EDTA, and adsorption of the recalified supernatant to mannose-Sepharose affinity columns. SP-A was eluted from the carbohydrate affinity column using 2 mM EDTA. The purified proteins were dialyzed for 2 days against daily changes of 2,000 volumes of 5 mM Tris (pH 7.4), 150 mM NaCl and for 1 day against 2,000 volumes of 5 mM Tris (pH 7.4), and stored at −20 °C. The EDTA content of all protein samples used was measured by the method of Kratohvil, with modifications as previously reported (4). The average final EDTA concentration was 5 μM, and was less than 25 μM in all SP-A reagents used.

Membrane Vesicle Preparation—Model surfactant lipids were used as substrates for lipid oxidation. Unsaturated liposomes (UL) were prepared by the method of Gregoriadis (7). In brief, an unsaturated lipid mixture (1,2-dipalmitoyl-sn-glycerol-3-phosphocholine, cholesterol, egg L-phosphatidylcholine, 18:0–18:2 PC (1:1:0.15:0.15, w/w/w/w)) was dis-

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‡ The abbreviations used are: ROS, reactive oxygen species; SP-A, surfactant protein A; UL, unsaturated liposomes; DNP, dinitrophenylhydrazine.
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Was representative of the peak obtained at the thiobarbituric acid adducts indicated that the absorbance at 540 nm was determined from the cell-free supernatant by centrifugation at 45,000 rpm using a 540-nm filter. An absorption scan (500–570 nm) of both sample/developer. Following incubation at 95 °C for 30 min and centrifugation at 16,000 × g for 15 min, an aliquot was read in a spectrophotometer using a 540-nm filter. An absorption scan (500–570 nm) of both sample/developer. Following incubation at 95 °C for 30 min and centrifugation at 16,000 × g for 15 min, an aliquot was read in a spectrophotometer using a 540-nm filter.

Analysis of Escherichia coli Cell Wall Permeability—The effect of the SP-A on E. coli cell wall integrity was assessed by determining permeability to cleavage activated, fluorescent alkaline phosphatase substrate, ELF97. E. coli (A600 nm = ~0.5 absorbance units) in 100 μl of 5 mM Tris and 150 mM NaCl was incubated with 100 μl ELF97 phosphatase substrate at 37 °C with 50 μg/ml SP-A from 20 to 90 min. The changes in fluorescence intensity were measured at excitation and emission wavelengths of 405 and 535 nm, respectively. Thiol-specific fluorophores were detected using a fluorescent plate reader with excitation and emission wavelengths of 405 and 535 nm, respectively. Thiol-specific fluorophore, ThioGlo1 (3). Low molecular weight substrates were used to determine total protein content.

Assays of Protein-associated Carboxyls—Oxidative modification of SP-A was determined by Western blot analysis (Oxyblot) using an antibody to 2,4-dinitrophenylhydrazine (DNPH)-derivatized carboxyl groups. SP-A was incubated with DNPH to modify protein-associated carboxyls. After size fractionation by 8–16% SDS-polyacrylamide gel electrophoresis, reducing conditions were electrophoretically transferred to nitrocellulose membranes. The membranes were sequentially incubated with a rabbit anti-DNP IgG and a horse-radish peroxidase-conjugated goat anti-rabbit IgG. Blots were developed by horseradish peroxidase-dependent oxidation of a chemiluminescent substrate and visualized using autoradiography.

Hyperoxic Treatment—Adult male and female C3HHeN mice (n = 15 previously reported (9). The lower limit of sensitivity of the assay was 0.20 ng/ml, and the linear range extended from 0.16 to 10.0 ng/ml.

Reduced Glutathione and Protein Thiol Measurements—Contents of GSH and protein thiols in the lavage fluid of mice were measured using the thiol-specific fluorophore, ThioGlo1 (3). Low molecular weight standards were separated from lavage by filtering through Microcon YM-3 MWCO 3,000 Millipore centrifugal filter devices. A 10-μl aliquot of lavage filtrate or lavage in 100 μl of Hepes-buffered saline solution was incubated with 10 μM ThioGlo1 in the absence or presence of the protein denaturing agent SDS. The GSH and thiol-containing proteins were detected using a fluorescent plate reader with excitation and emission wavelengths of 405 and 535 nm, respectively. Thiol-containing proteins were quantified by subtracting fluorescence from the thiol-specific fluorophore (i.e., signal obtained from the fluorophore in the absence of SDS) from total fluorescence (i.e., signal obtained from lavage in the presence of SDS).

Assays of Surfactant Pellet Preparation—After tracheal cannulation, lungs were lavaged 3 times with 1 ml of sterile 5 mM Tris and 150 mM NaCl. Lavages from four to five mice were pooled and centrifuged at 400 × g for 5 min at 4 °C to sediment the cells. Surfactant was separated from the cell-free supernatant by centrifugation at 45,000 × g for 6 h at 4 °C in the presence of 2 mM CaCl2. Proteins bound to the surfactant pellet in a calcium-dependent manner were released by incubation with 2 mM EDTA.

Radial Diffusion Assay of E. coli Viability—Inhibition of bacterial growth was assessed using a radial diffusion method (10). Molten SeaKem LE-agarose (0.8–1.0%) (FMC Bioproducts) in 16 ml of buffer containing 10 mM sodium phosphate and 1.0% LB medium was mixed with 150 μl of E. coli (A600 0.3 absorbance units) at a temperature of 40 °C and allowed to harden by cooling. Agar composed of tryptone (40 g/liter), yeast extract (20 g/liter), and agar (15 g/liter) was layered on top. Albumin, lysozyme (0.1 and 1 mg/ml), or concentrated lavage (1 mg/ml) were added to 5–15 wells bored in the agar. After overnight incubation at 37 °C, the plates were visually inspected for clearing around the wells.

Preparation of Human Neutrophils—Heparinized blood from healthy volunteers was separated by discontinuous density-gradient centrifugation (11) to obtain neutrophils. The granulocyte-enriched material at the interface was collected, centrifuged, and washed in Hank's balanced salt solution (pH 7.4). Contaminating erythrocytes were removed by hypotonic lysis, and the washed neutrophils were resuspended in Dulbecco's modified Eagle's medium.

Exposure of SP-A to Neutrophil ROS—Neutrophils (2 × 10⁶ cells) in Dulbecco's modified Eagle's medium were prewarmed to 37 °C for 5 min and luminol (1 mM) was added to a total volume of 0.1 ml. The oxidative burst was assessed by measurement of chemiluminescence (12) using a Victor II plate reader chemiluminometer (Turku, Finland). SP-A (100 μg/ml) was incubated with 2 × 10⁶ neutrophils in 0.1 ml of Dulbecco's modified Eagle's medium for 2 h at 37 °C and 5% CO₂ and the samples were analyzed for oxidative damage by immunoblot-based assessment of protein-associated carbonyls, proteolytic degradation using an anti-human SP-A antibody (9), and nitration using an nitrotyrosine antibody (Cayman Chemical).

Statistical Analysis—The analysis of variance test was used for comparisons between experimental groups. All data are presented as mean ± S.E. unless otherwise noted.

RESULTS

Antimicrobial Activity of Calcium-dependent Binding Proteins from the Surfactant Pellet of SP-A+/+ and SP-A−/− Mice—Experiments were performed to determine the direct effect of alveolar lavage fluids isolated from SP-A+/+ and SP-A−/− mice on E. coli growth using a radial diffusion method and the results are shown in Fig. 1. SP-A is intimately associated with surfactant phospholipids in the airspace, such that less than 1% of protein is found in the unbound state in the aqueous phase. Surfactant pellets were prepared from mice in the presence of calcium, in a manner that preserves the association between SP-A and sedimented phospholipid. Calcium-dependent binding proteins released from the surfactant pellet of SP-A+/+ mice (total protein 6.3 μg/well) produced dose-dependent zonal clearing; considerably more than the same concentration of protein eluted from the pellet of SP-A−/− mice. The EDTA containing, protein-free filtrate (molecular weight cutoff of 10,000) from the surfactant pellet of SP-A+/+ mice had little effect on E. coli growth. The data indicate that...
from SP-A permeabilization by lavage proteins from the SP-A-sufficient also a dose-dependent increase in the initial rate of membrane
the E. coli protein, lavage from SP-A cell wall in a dose-dependent fashion (Fig. 2). At 1 mg/ml lavage from SP-A
lated from genetically engineered mice. Concentrated lavage
permeability using this technique and airspace proteins iso-
3.8-fold greater than untreated
mice (0.2 mg/ml protein from lavage of SP-A+/+ mice (Δ), 1 mg/ml protein from lavage of SP-A+/− mice (○), or no protein (○) are shown in
panel A). The initial rate of E. coli cell wall permeability is shown in panel B. Data are mean ± S.E., n = 3. * p < 0.05. a.u., absorbance units.

calcium-dependent surfactant-binding proteins in SP-A+/+ mice have greater antibacterial activity than those from SP-A−/− mice.

Permeabilizing Activity of Lavage and Calcium-dependent Surfactant-binding Proteins—We have previously reported that pulmonary collectins induce protein leak in E. coli (2). A technique was developed to more rapidly assess cell wall permeability, using an impenetrant, fluorescent substrate (ELP97) for the periplasmic enzyme, alkaline phosphatase. We tested the physiological role of SP-A in induction of E. coli membrane permeability using this technique and airspace proteins isolated from genetically engineered mice. Concentrated lavage from SP-A+/+ mice increased the permeability of the E. coli cell wall in a dose-dependent fashion (Fig. 2). At 1 mg/ml lavage protein, lavage from SP-A+/+ mice induced the permeability of the E. coli cell wall 14.8-fold (p < 0.001) compared with 3.6-fold (p > 0.05) for lavage from SP-A−/− mice (Fig. 3A). There was also a dose-dependent increase in the initial rate of membrane permeabilization by lavage proteins from the SP-A-sufficient mouse strains, which was greater than that produced by lavage from SP-A−/− mouse strains (Figs. 2B and 3B). To further define the role of SP-A in permeabilization, we tested the antimicrobial activity of calcium-dependent surfactant-binding proteins from SP-A+/+ and SP-A−/− mice. We found that the EDTA eluate from the surfactant pellet of SP-A+/+ mice (0.2 mg/ml total protein) permeabilized E. coli to an extent that was 3.8-fold greater than untreated E. coli (p < 0.05), compared with 1.3-fold (p > 0.05) for the proteins eluted from the surfac-
tant pellet from SP-A−/− mice (0.2 mg/ml total protein) (Fig. 4A). There was also a significant increase in the initial rate of membrane permeabilization by proteins from the surfactant pellet of SP-A+/+ versus SP-A−/− mice (Fig. 4B). Taken together, these data are consistent with a role for SP-A in the permeabilizing activity of alveolar lavage fluid.

Effect of Lipid Peroxidation on Collectin Antimicrobial Activity—At a concentration of 50 μg/ml, human SP-A markedly increased intracellular penetration of ELP97, resulting in a time-dependent increase in fluorescence that began at −20 min and increased in a linear fashion (Fig. 5, A and B). SP-A was then oxidized using both chemical and cellular free radical sources and tested for antimicrobial activity. Oxidative damage of human SP-A was first assessed by a Western blot analysis technique that detects carbonyl adducts (13). Human SP-A had a detectable level of carbonyls at baseline, as previously re-
ported (14). Exposure of human SP-A to oxidizing conditions further increased the content of protein-associated carbonyls (Fig. 5C), to a greater extent when the protein was exposed to lipid peroxidation from incubation with 2 μM CuSO4 plus UL than to CuSO4 alone. There was no effect of UL alone on the carbonyl content of SP-A.

The ability of human SP-A to increase the permeability of the E. coli cell membrane was blocked by pre-exposure of SP-A to lipid peroxidation (Fig. 5, A and B). There was no effect of pre-exposure of human SP-A to lipids alone, or CuSO4 alone (at 4 μM), on the kinetics (Fig. 5A) or end point (Fig. 5B) of SP-A-induced membrane permeability. The data shown in Fig. 5 also indicate that, in the absence of human SP-A, there was no effect of incubation of the bacteria with lipids alone, CuSO4 alone, or lipids plus CuSO4 on intracellular penetration of alkaline phosphatase substrate.

The effect of physiological levels of ROS produced by activated granulocytes on SP-A antimicrobial function was next examined. Neutrophil-generated ROS oxidize the luminol sub-
strue and cause emission of chemiluminescence in a time-de-
pendent manner (Fig. 6A). Exposure of SP-A to ROS produced by human neutrophils resulted in an increase in protein-assos-
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FIG. 4. Proteins eluted from the surfactant pellet of SP-A+/+ mice increases the permeability of the E. coli cell wall to a greater extent than the eluate of the surfactant pellet of SP-A−/− mice. The kinetics of E. coli cell wall permeability in the presence of 250 μg/ml protein from the surfactant pellet of SP-A+/+ mice (□), 250 μg/ml protein from surfactant pellet of SP-A−/− mice (△), or no protein (○) are shown in panel A. The initial rate of E. coli cell wall permeability is shown in panel B. Data are mean ± S.E., n = 3. * and #, p < 0.05. a.u., absorbance units.

FIG. 5. LPO mediated oxidative damage of human SP-A blocks the collectin mediated permeability of the E. coli cell wall. SP-A was preincubated alone, with multilamellar phospholipids, with copper, or with multilamellar phospholipids plus copper for 24 h at 37°C. The kinetics of E. coli cell wall permeability upon exposure to: 1) SP-A alone (●); 2) SP-A preincubated with 20 μg/ml multilamellar phospholipids (○); 3) 20 μg/ml multilamellar phospholipids alone (+); 4) 4 μM CuSO4 alone (−); 5) SP-A preincubated with 4 μM CuSO4 (□); 6) SP-A preincubated with 20 μg/ml multilamellar phospholipids and 4 μM CuSO4, alone (●); 7) 20 μg/ml multilamellar phospholipids and 4 μM CuSO4, alone (○) and in the absence of any addition (×) are shown in panel A. The initial rate of E. coli cell wall permeability is shown in panel B. In panel C, oxidative modification of SP-A that occurred during 24 h exposure of SP-A at 37 °C as in 1–7 above was determined by Western analysis using an antibody to DNP-derivatized carbonyl moieties and quantification by densitometry. Data are mean ± S.E., n = 3. * and #, p < 0.05. a.u., absorbance units.

Associated carbonyls, consistent with oxidative damage (Fig. 6B).

Previous studies have shown that exposure of SP-A to high concentrations of oxidizing agents results in SP-A fragmentation (15). We found minor amounts (~7% by densitometry) of proteolytic degradation (Fig. 7) in the presence of human neutrophils. Exposure of SP-A to activated macrophages has been reported to result in nitration (16). There was no evidence of nitrosotyrosine formation (data not shown) in our neutrophil exposure experiments, however. Pre-exposure of SP-A to neutrophils decreased the permeabilizing effects of the protein compared with sham exposed SP-A (Fig. 8A). The initial rate of E. coli membrane permeability induced by neutrophil-exposed SP-A was considerably less than sham exposed SP-A (Fig. 8B).

These results are consistent with a significant effect of oxidation of SP-A. We cannot exclude the possibility that the minor amounts of proteolysis seen contributed significantly to the loss of permeabilizing activity.

The Effect of Hyperoxia on the Antimicrobial Activity of Alveolar Lining Fluid from SP-A+/+ Mice—To determine whether clinically relevant oxidant exposures affect the antimicrobial functions of SP-A in vivo, we next assessed the effect of hyperoxic exposure on the permeabilizing activity of the alveolar lining fluid. SP-A+/+ mice were exposed to air or to 90% oxygen for 96 h. These hyperoxic conditions were sufficient to cause consumption of glutathione (Fig. 9A, p < 0.01), a 3.5-fold decrease in protein thiols (Fig. 9B, p < 0.02), a 7.5-fold increase in the accumulation of total protein (Fig. 9C, p < 0.01), and marked increase in airway protein-associated carbonyl moieties (Fig. 10). The enzyme-linked immunosorbent assay-quantified concentrations of SP-A in the lavage fluid pre- and post-oxygen exposure were unchanged (29.5 ± 5.2 and 27.6 ± 2.4 ng/ml, respectively). Exposure of mice to hyperoxia did not result in nitration of lavage proteins, as assessed by immunoprecipitation followed by Western blot analysis with nitrotyrosine antibody (data not shown). The permeabilizing activity of lavage fluid isolated from the oxygen-exposed animals was markedly decreased compared with the control (air exposed) mice as shown by kinetic profile (Fig. 11A) and initial rate of
E. coli cell wall permeabilization (Fig. 11B).

Exposure of mice to hyperoxia resulted in significant airspace accumulation of serum proteins such as albumin (17). Under these conditions, protein inhibitors might interfere with the activity of SP-A. We found that the loss of permeabilizing activity of lavage fluid was not because of serum inhibitors of permeabilization because a 1:1 mixture of lavage from oxygen-exposed and sham treated animals was as active as a 1:1 mixture of buffer and lavage from sham treated animals (Fig. 12).

**DISCUSSION**

In this study, we examined the relative physiologic importance of SP-A in the membrane permeabilizing activity of alveolar lavage fluid, and the effect of oxidation on SP-A antimicrobial function. We found that lavage from SP-A−/− mice was much less disruptive to E. coli membranes than lavage from...
E. coli cell wall permeability induced by a 1:1 mixture of concentrated (1 mg/ml) lavage proteins from SP-A+/+ mice exposed to hyperoxia with lavage of sham exposed SP-A+/+ mice was similar to that caused by a 1:1 mixture of lavage from SP-A+/+ mice with buffer (○) (panel A). A no addition protein control is also shown (□). The initial rate of E. coli cell wall permeability is shown in panel B. Data are mean ± S.E., n = 3.

* p < 0.05.

SP-A+/+ mice, suggesting that SP-A is an important microbial permeabilizing protein in lavage. The permeabilizing activity of SP-A was dose dependent, and was blocked by oxidative damage to SP-A, in vivo and in vitro. We conclude that oxidative stress inhibits the antibacterial activity of SP-A by a mechanism that includes oxidative modification and functional inactivation of the protein.

The innate immune defense system of the lung consists of a variety of antimicrobial molecules and phagocytic cells (1), including peptides and proteins that opsonize and permeabilize bacteria (2) and fungal microorganisms (3). Several lines of evidence suggest that oxidative damage to surfactant components have critical effects on surfactant biophysical function, but less is known about the consequences of oxidative stress for collectin-mediated host defense (4, 18).

We have previously demonstrated that the SP-A exerts potent, macrophage-independent antibacterial activity against rough E. coli strains at physiologically relevant collectin concentrations, in vitro (2, 3). In this study, the demonstration that lavage from SP-A+/+ mice has greater permeabilizing and antiproliferative effects than that from SP-A−/− mice suggests an antimicrobial role for the protein, in vivo. Furthermore, a significant fraction of the permeabilizing activity of lavage fluid segregated with the surfactant pellet (and SP-A) upon centrifugation, rather than in the aqueous phase, which contains the non-phospholipid interacting antimicrobial peptides like lysozyme and defensins. The relative physiologic importance of the direct antimicrobial activities of the collectins versus their opsonic and macrophage modifying functions are important questions that were not addressed in this study.

Our results indicate that oxidative damage of surfactant proteins by lipid peroxidation or ROS generated by inflammatory cells in vitro inhibits the permeabilizing activity of SP-A. The changes in protein function were associated with oxidative modification of SP-A as assessed by carbonyl adduct formation, but not with detectable nitration of the protein. A minor amount of proteolytic degradation occurred upon exposure of SP-A to human blood neutrophils. A small amount of proteolysis can be associated with loss of protein function, especially for oligomeric proteins, and the degree of proteolysis can be underestimated because small fragments may not interact with the antibody. Nonetheless, loss of permeabilizing activity upon oxidation exposure is generally consistent with our previous observations regarding the functional consequences of
SP-A oxidative modification on lipid protein interactions (4).

Next we studied the in vivo effect of hyperoxic exposure on antibacterial properties of lavage from SP-A+/+ mice. Exposure of SP-A+/+ mice to 90% oxygen for 4 days resulted in significant decreases in permeabilizing activity of lavage, despite a 7.5-fold increase in total protein content. This loss of antimicrobial activity was not attributed to inhibitors of permeabilization that may have leaked into the airspace from the serum compartment. We speculate that similar oxygen tensions used to treat respiratory failure in patients may inactivate antimicrobial proteins such as SP-A in the alveolar lining fluid and render subjects more susceptible to infection. In summary, the data in this study indicate that SP-A is a principal permeabilizing protein in lavage and that oxidative stress as in vitro and in vivo results in protein damage and functional inactivation of antibacterial properties of SP-A.

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