Depletion of pulmonary EC-SOD after exposure to hyperoxia

TIM D. OURY,1 LISA M. SCHAEFER,1 CHERYL L. FATTMAN,1 AUGUSTINE CHOI,2 KAREN E. WECK,3 AND SIMON C. WATKINS3
Departments of 1Pathology, 2Medicine, and 3Cell Biology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania 15261

Received 10 January 2002; accepted in final form 3 May 2002


NUMEROUS STUDIES HAVE SHOWN that reactive oxygen species are important mediators in the pathogenesis of acute lung injury. Many investigators (9, 36, 40–43) have found that augmentation of antioxidant enzymes is protective in animal models of acute lung injury. In addition, reports (13, 16, 21, 44) show decreased amounts of antioxidants and elevated levels of prooxidants in both patients with and animal models of adult respiratory distress syndrome (ARDS), which suggests that an oxidant-antioxidant imbalance contributes to the pathogenesis of acute lung injury.

In addition to reactive oxygen species, acute inflammation also contributes to acute lung injury. The acute inflammatory response results in parenchymal, epithelial, and endothelial injury that leads to an influx of protein-rich fluid to the airspaces. Notably, the most proximal signals leading to uncontrolled activation of this acute inflammatory response are unknown (8).

The antioxidant enzyme extracellular superoxide dismutase (EC-SOD) is highly expressed in the lungs compared with other tissues (18, 30, 31). This enzyme has been shown to significantly protect against hyperoxic lung injury (3, 12), which suggests that extracellular superoxide contributes to the pathogenesis of this disease. Notably, hyperoxia-induced neutrophil recruitment was found to be significantly inhibited in transgenic animals with elevated levels of EC-SOD in the lungs (12). These results suggest that extracellular superoxide contributes to acute lung injury perhaps via the recruitment of inflammatory cells.

EC-SOD is a 135-kDa heparin-binding protein that exists primarily as a tetramer of four identical subunits (17, 29). This tetramer consists of two dimers, each of which contains a disulfide bond that links the heparin-binding domains in the COOH terminus of two subunits together (11, 29, 30). EC-SOD is found predominantly in the extracellular matrix of tissues and to a lesser extent in extracellular fluids (18, 19, 28, 35). The heparin-binding domain of EC-SOD interacts with heparin sulfate in the extracellular matrix of tissues and cell surfaces. This affinity to heparin sulfate is believed to be important in determining the specific location of EC-SOD in the extracellular matrix and allowing it to act as an efficient antioxidant (30). The heparin-binding affinity of EC-SOD can be modulated through proteolytic removal of the heparin-binding domain. This proteolytic regulation of heparin affinity is believed to be important in regulating the distribution of EC-SOD in various tissues (10, 30, 34).

Hyperoxic lung injury is known to lead to elevated levels of activated proteinases (6, 32, 39). This study tests the hypothesis that increased proteolytic activity during hyperoxia exposure will result in a depletion of EC-SOD from alveolar interstitial spaces due to proteolytic removal of the heparin-binding domain of EC-SOD. Notably, hyperoxia-induced proteolysis of EC-SOD may enhance hyperoxic pulmonary injury by altering the oxidant-antioxidant balance in alveolar spaces. This proteolytic activity may contribute to the oxidant-antioxidant imbalance that is associated with this injury. Exposure to 100% oxygen for 72 h resulted in a significant decrease in EC-SOD levels in the lungs and bronchoalveolar lavage fluid of mice. This correlated with a significant depletion of EC-SOD from the alveolar parenchyma as determined by immunofluorescence and immunohistochemistry. EC-SOD mRNA was unaffected by hyperoxia; however, there was an increase in the ratio of proteolyzed to uncut EC-SOD after hyperoxia, which suggests that hyperoxia depletes EC-SOD from the alveolar parenchyma by cutting the heparin-binding domain. This may enhance hyperoxic pulmonary injury by altering the oxidant-antioxidant balance in alveolar spaces.

The heparin-binding domain of EC-SOD interacts with heparin sulfate in the extracellular matrix of tissues and cell surfaces. This affinity to heparin sulfate is believed to be important in determining the specific location of EC-SOD in the extracellular matrix and allowing it to act as an efficient antioxidant (30). The heparin-binding affinity of EC-SOD can be modulated through proteolytic removal of the heparin-binding domain. This proteolytic regulation of heparin affinity is believed to be important in regulating the distribution of EC-SOD in various tissues (10, 30, 34).

Hyperoxic lung injury is known to lead to elevated levels of activated proteinases (6, 32, 39). This study tests the hypothesis that increased proteolytic activity during hyperoxia exposure will result in a depletion of EC-SOD from alveolar interstitial spaces due to proteolytic removal of the heparin-binding domain of EC-SOD. Notably, hyperoxia-induced proteolysis of EC-SOD may enhance hyperoxic pulmonary injury by altering the oxidant-antioxidant balance in alveolar spaces. This proteolytic activity may contribute to the oxidant-antioxidant imbalance that is associated with this injury. Exposure to 100% oxygen for 72 h resulted in a significant decrease in EC-SOD levels in the lungs and bronchoalveolar lavage fluid of mice. This correlated with a significant depletion of EC-SOD from the alveolar parenchyma as determined by immunofluorescence and immunohistochemistry. EC-SOD mRNA was unaffected by hyperoxia; however, there was an increase in the ratio of proteolyzed to uncut EC-SOD after hyperoxia, which suggests that hyperoxia depletes EC-SOD from the alveolar parenchyma by cutting the heparin-binding domain. This may enhance hyperoxic pulmonary injury by altering the oxidant-antioxidant balance in alveolar spaces.
SOD. This will enhance the oxidant-antioxidant imbalance in the lung and further contribute to hyperoxic lung injury.

**EXPERIMENTAL PROCEDURES**

**Materials.** Xanthine oxidase was purchased from Boehringer Mannheim (Indianapolis, IN). Eosin Y, phloxine B, concanavalin A Sepharose, xanthine, and equine partially acetylated cytochrome c were purchased from Sigma (St. Louis, MO). Mayer's hematoxylin and Clear Rite were purchased from Fisher Scientific (Pittsburgh, PA). The diaminobenzidine (DAB) staining kit was purchased from Vector Laboratories (Burlingame, CA). The Immunon immunohistochemistry kit was purchased from Shandon (Pittsburgh, PA). Rabbit anti-mouse albumin was purchased from ICN Biochemicals (Aurora, OH). Rabbit anti-actin antibody was purchased from Oncogene Research Products (Boston, MA).

**Oxygen exposures.** All animal experiments were reviewed and approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Eight-week-old C57BL/6 mice (body wt 20–25 g) were purchased from Taconic (Germantown, NY). On arrival, the animals were allowed to acclimate for 7 days before experimentation and were fed rodent chow and water ad libitum. Animals were exposed for 72 h to hyperoxia (>99% O2) at a flow rate of 12 l/min in a 3.7-ft² Plexiglas exposure chamber and were supplied with rodent chow and water ad libitum throughout the exposure. Control animals were placed in similar cages and maintained at normoxia for the entire exposure. At the end of the exposure, the mice were given a lethal dose of Nembutol (5 mg ip).

Where specified, mouse lungs were subjected to bronchoalveolar lavage by intratracheal instillation and then removal of 1 ml of 0.9% saline. Lungs were inflation-fixed with formalin and processed for either paraffin embedding (immunoperoxidase studies) or frozen sectioning (immunofluorescence studies). Alternatively, lungs were removed and stored at –80°C for enzyme-activity assays, Western blot analysis, or RNA analysis.

Two separate experiments were performed. In the first experiment, there were four mice in each group. Blood was sampled from all mice at the time of lethal injection. Bronchoalveolar lavage fluid (BALF) was obtained from three mice in each group, and the left lungs from all mice were inflation-fixed in formalin, paraffin embedded, and used for immunoperoxidase studies. The right lungs from all mice were used for enzymatic activity and Western blot analysis. In the second experiment, there were six mice in each group. Blood was sampled from all mice at the time of lethal injection. BALF was obtained from four mice in each group, and the left lungs from all mice were inflation-fixed with 2% paraformaldehyde in PBS for 2 h followed by overnight incubation in 30% sucrose in PBS. Frozen sections of these lungs were used for the immunofluorescence studies. The right lungs from four mice in each group were used for enzymatic activity and Western blot analysis. The right lungs from two mice in each group were used for DNA isolation and quantitative RT-PCR analysis.

**DNA quantification.** DNA was quantified in lung homogenates using bisbenzamide (Hoechst 33258; Molecular Probes, Eugene, OR) as previously described (15).

**Measurement of BALF protein.** Total protein in the BALF was measured using the Coomassie Plus protein assay (Pierce, Rockford, IL).

**Analysis of EC-SOD activity.** Before removal, lungs from treated and control mice were perfused with 5 ml of PBS through the right ventricle of the heart. The lungs were homogenized in 3 ml of 50 mM potassium phosphate with 0.3 M potassium bromide, pH 7.4. After separation from Cu,Zn-SOD and MnSOD via concanavalin A Sepharose chromatography (20), EC-SOD activity was measured by inhibition of partially acetylated cytochrome c reduction at pH 10.0 as previously described (7). The total protein concentration in the homogenates was determined by the Coomassie Plus protein assay.

**Western blot analysis.** Proteins from lung homogenates, BALF, or sera were subjected to SDS-PAGE on 10% polyacrylamide gels under reducing conditions and were electrophoretically transferred to an Immobilon-P membrane (Millipore, Bedford, MA). The membranes were blocked overnight at 4°C with 5% nonfat milk in PBS [20 mM potassium phosphate (pH 7.4) with 0.15 M potassium chloride]. Membranes were then incubated with rabbit anti-mouse EC-SOD antibody (11, 27, 38) or nonimmune IgG in PBS with 0.3% Tween 20 and subsequently incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG. Antibody detection was performed using an enhanced chemiluminescence detection system (Amersham). Densitometry was performed on the resulting autoradiograph using a Kodak DC120 zoom digital camera and Kodak Digital Science 1D analysis software (version 3.0).

**Immunohistochemistry.** Mouse lungs were inflation-fixed with 10% buffered neutral formalin (VWR, West Chester, PA) for 4 h followed by overnight fixation in 70% ethanol at room temperature. After fixation, the tissues were processed for paraffin embedding. Serial 4-μm-thick sections of the paraffin-embedded tissues were cut (2 sections per slide). Sections were then deparaffinized and labeled for EC-SOD using an indirect immunoperoxidase method as part of the Immunon immunohistochemistry kit. Briefly, sections were first incubated in 6% H2O2 in methanol to inactivate endogenous peroxidases, and antigen retrieval was then performed with 0.1% pepsin in 0.01 N HCl. To reduce background staining and nonspecific binding, sections were incubated for 1 h in Immunon MaxiTags protein-blocking solution before being incubated for 60 min with a polyclonal antibody to mouse EC-SOD (11). Sections were then incubated with a biotinylated rabbit anti-mouse IgG and streptavidin-HRP. Primary and secondary antibody dilutions were determined empirically and made in Immunon dilution buffer. As a control, one serial section on each slide was labeled with preimmune rabbit serum. To enhance the strength of the staining, sections were incubated in avidin-biotin complex reagent (Vector Laboratories). All slides were developed using DAB for 1 min at 25°C. The reaction was stopped with water, and the slides were counterstained with Mayer’s hematoxylin. All sections compared for labeling intensities were labeled simultaneously to avoid staining differences owing to day-to-day variability.

Five fields from each slide were photographed at ×40 magnification under oil immersion. Field selection was based on the following criteria: no major blood vessels, no airways containing cuboidal epithelium, and no alveolar collapse. MetaMorph software (Universal Imaging) was used to view and quantitate EC-SOD labeling intensity in the fields. The entire field from each image was selected for analysis, and thresholds were determined for dark areas of the field (i.e., positive labeling for EC-SOD). The pixel intensity of the dark threshold areas was determined as a percentage of the total threshold area for each image.

**Immunofluorescence studies.** Mouse lungs were perfused with PBS. The lungs were then inflation-fixed with 2% paraformaldehyde in PBS for 1 h, washed in PBS, infused with 30% sucrose in PBS overnight, and frozen in liquid nitrogen-
cold isopentane. Sections (5 μm) were cut on a Microm cryostat and mounted on polylysine-coated slides (Fischer, Pittsburgh, PA). To reduce nonspecific labeling, sections were washed three times in PBS that contained 0.5% BSA and 0.15% glycine, pH 7.4 (buffer A) followed by a 30-min incubation with purified goat IgG (50 μg/ml) at 25°C and three additional washes with buffer A. The sections were then incubated for 60 min with a primary antibody to mouse EC-SOD (1 μg/ml; Ref. 11) and washed three times in buffer A before being incubated for 60 min in the fluorescence-labeled secondary antibody Alexa 488 (1–2 μg/ml; Molecular Probes) mixed with rhodamine-phalloidin (Molecular Probes) to stain the actin cytoskeleton of all cells. The sections were then washed six times (5 min/wash) in buffer A, mounted in Gelvatol, and coverslipped.

Using a Leica TCSNT laser confocal microscope, 10 random fields were imaged for each condition using selection criteria as described (see Immunohistochemistry). The magnification, photomultiplier tube voltage, laser voltage, and pinhole were fixed for the entire experiment. Images were taken blindly by an experienced confocal microscopist at a 1,024 × 1,024-pixel resolution with a ×40 1.0-NA oil-immersion objective. Images were stored as 8-bit TIFF files for each illumination condition.

Using fixed imaging conditions in combination with confocal microscopy, the same optical volume was collected for all samples and thus direct comparisons could be made between samples. Because it is important to measure EC-SOD staining in relationship to the alveolar parenchyma within the sample, phallodin staining was used as a counterstain to delineate all of the cells in the field. Because the entire blood volume was removed by perfusion before fixation, the only cells that were actin positive in the sample (apart from occasional free macrophages) were alveolar epithelial and vascular endothelial cells. The actin-positive image was therefore used to generate a binary mask against which the EC-SOD staining was quantified. Isolated actin-positive cells such as the macrophages were eliminated from the mask using a size classifier that excluded small (<25 μm diameter) objects from the mask. The actin mask was compared with the EC-SOD image using a Boolean MINIMUM statement. The threshold was obtained for this image to show EC-SOD-positive structures (fixed for all images), and an area of positivity within the total actin-positive mask was generated. Comparing this area with the actin-positive area (100%), a relative abundance of EC-SOD staining within the entire cellular field was calculated. All measurements and processing steps were performed using MetaMorph software.

**EC-SOD mRNA quantification.** Lungs were removed from the mice, flash frozen in liquid nitrogen, and stored at −80°C. RNA was obtained by first homogenizing the tissue in a denaturing and solubilizing solution (that contained 4 M guanidine isothiocyanate, 25 mM sodium citrate, pH 7, 0.5% sarcosyl, and 0.1 M β-mercaptoethanol) and then isolating the RNA via several acid-phenol-chloroform extractions as previously described (5).

Total mouse-lung RNA was quantitatively assayed for EC-SOD mRNA using a LightCycler instrument (Roche Molecular Biochemicals, Indianapolis, IN). The LightCycler RNA amplification kit SYBR Green I (Roche Molecular Biochemicals) was used to measure RNA in a one-step RT-PCR reaction in real time. Primers to mouse EC-SOD [GenBank accession no. U38261; sense (bases 279–300), 5′-TCTTGTGT-TCTACGGCTTGCTAC-3′; antisense (bases 452–431), 5′-CTCCATCCAGATCTCCAGCCT-3′] at a final concentration of 0.5 μM were used in the reaction with 0.5 μg of total mouse lung RNA and 6 mM final concentration of MgCl₂.

The reactions were cycled on the LightCycler instrument with the following parameters: the reverse transcription reaction was carried out at 55°C for 20 min followed by denaturation at 95°C for 30 s. PCR amplification (50 cycles) of the resulting cDNA was then performed using a 65°C annealing temperature for 10 s and a 72°C extension temperature for 13 s followed by denaturation at 95°C. Amplification curves were obtained through use of the LightCycler quantification software program. Product specificity was assured by analyzing the products for the correct melting point as well as via electrophoresis of the products with and without digestion using the restriction enzyme HaeIII (which cuts at position 306) to ensure appropriate product size and restriction-enzyme pattern. As an additional control, samples were pretreated with RNase before RT-PCR to ensure that the product was specific to EC-SOD mRNA and not due to DNA contamination.

**RESULTS**

**Hyperoxia exposure results in depletion of pulmonary EC-SOD.** After exposure to 100% oxygen for 72 h, lungs were removed from the mice and processed for analysis of EC-SOD activity. Oxygen exposure resulted in significantly elevated levels of protein in BALF compared with room-air control animals (room air, 0.17 ± 0.3; oxygen, 2.95 ± 0.44 mg/ml), which is consistent with hyperoxia-induced injury. Oxygen-exposed mice were found to have significantly reduced EC-SOD activity compared with normoxic control mice (Fig. 1A) when the activity was expressed per unit of total protein. A similar depletion of EC-SOD activity was also evident when the values were expressed per whole lung (408 ± 24 and 338 ± 23 U/lung for room air- and oxygen-exposed animals, respectively) and per unit of DNA (67.4 ± 0.7 and 34.8 ± 2.5 U/mg of DNA for room air- and oxygen-exposed animals, respectively). There was also a 75% loss of EC-SOD from the BALF of oxygen-exposed mice compared with control mice (Fig. 1A) when the activity was expressed per unit of total protein. A similar depletion of EC-SOD activity was also evident when the values were expressed per whole lung (408 ± 24 and 338 ± 23 U/lung for room air- and oxygen-exposed animals, respectively) and per unit of DNA (67.4 ± 0.7 and 34.8 ± 2.5 U/mg of DNA for room air- and oxygen-exposed animals, respectively). There was also a 75% loss of EC-SOD from the BALF of oxygen-exposed mice compared with control mice (Fig. 1A). Western blot analysis showed a significant ~30% loss of EC-SOD protein from the lungs (Fig. 1B, left) of oxygen-exposed animals compared with control animals and a 73% loss of EC-SOD protein from the BALF (Fig. 1B, right) of oxygen-exposed animals. Densitometric analysis of the ratio of full-length (Fig. 1B, top band) to proteolized (lacking heparin-binding domain; Fig. 1B, bottom band) EC-SOD indicated that there was a 1.5-fold increase in the ratio of proteolyzed to full-length EC-SOD in the lungs of oxygen-exposed mice compared with normoxic controls and a threefold increase in this ratio in the BALF of oxygen-exposed animals compared with controls. This increase in proteolyzed-to-full-length EC-SOD suggests that there is increased proteolytic removal of the heparin-binding domain of EC-SOD in response to hyperoxia.

**Hyperoxia leads to depletion of serum EC-SOD.** Sera were collected from control and hyperoxia-exposed animals at the time of death, and EC-SOD levels were assessed by Western blot analysis. Densitometric analysis of EC-SOD revealed that hyperoxia exposure also significantly depleted EC-SOD from the sera of exposed animals compared with control animals (Fig. 2).
Hyperoxia does not alter EC-SOD mRNA expression. RNA was purified from the lungs of control and hyperoxia-exposed animals, and EC-SOD mRNA levels were measured using quantitative real-time RT-PCR. No differences in EC-SOD mRNA levels were observed between control and oxygen-exposed animals (Fig. 3).

Hyperoxia leads to depletion of EC-SOD from alveolar parenchyma. Using both peroxidase-based immunohistochemical localization methods and immunofluorescence methods, we quantitated the level of EC-SOD staining in the alveolar matrices in the lungs of control and oxygen-exposed mice. These studies revealed a significant loss of EC-SOD labeling in the alveolar parenchyma after oxygen exposure when both methods of analysis were used (Fig. 4). Notably, mean actin-staining intensity in our immunofluorescence studies...
crossing point depicts the cycle at which linear amplification of the product begins and is shown for each group. Bars represent high and low values for each group.

showed no significant difference in actin levels between control and oxygen-exposed animals (data not shown). In addition, quantification of nuclear labeling in the immunofluorescence studies also showed no difference in oxygen- vs. room air-treated animals (data not shown).

**DISCUSSION**

An imbalance in the ratio of oxidants to antioxidants in the lung is believed to contribute to the pathogenesis of acute lung injury. Reactive oxygen species such as superoxide and peroxynitrite are thought to contribute to lung injury as a result of exposure to high levels of oxygen. The antioxidant enzyme EC-SOD has previously been shown to significantly protect against hyperoxic lung injury by scavenging superoxide in both transgenic (12) and knockout (3) models, which suggests that this enzyme may play an important protective role against hyperoxia-induced acute lung injury. The study presented here demonstrates that hyperoxia leads to a significant decrease in pulmonary EC-SOD protein levels. These results support our hypothesis that alterations in EC-SOD activity during the early stages of injury contribute to the pathogenesis of hyperoxic lung injury.

Using a mouse model in which animals were exposed to >99% oxygen for 72 h, significant changes in EC-SOD activity and protein levels in the lungs were observed. Hyperoxic mice had significantly reduced EC-SOD activity in both the lung tissue and BALF compared with normoxic control mice (see Fig. 1A). This decrease in activity was accompanied by loss of EC-SOD protein from the lungs (Fig. 1B, left) of oxygen-exposed animals compared with control animals. Also, there was a significant loss of EC-SOD protein from the BALF (Fig. 1B, right) of the hyperoxic animals.

We have previously hypothesized that proteolysis of the heparin-binding domain of EC-SOD may contribute to the loss of the enzyme from the extracellular matrix (30). Densitometric analysis of the full-length and proteolyzed EC-SOD demonstrated an increase in the ratio of proteolyzed to full-length EC-SOD in both the lung and the BALF of oxygen-exposed mice compared with normoxic controls. We have previously shown in a model of bleomycin-induced pulmonary fibrosis that proteolysis of the heparin-binding domain of EC-SOD results in the loss of the protein from the extracellular matrix of the lung and accumulation in the BALF (10). In contrast, although hyperoxia exposure did lead to increased proteolysis and a loss of EC-SOD from the alveolar matrix (see Fig. 4), there was also a depletion of the enzyme from the BALF (see Fig. 1A). Although no histological evidence of injury was seen in the animals, we speculated that because of the “leakiness” of the vascular endothelium, which develops in response to hyperoxic injury, the EC-SOD may be accumulating in the blood. However, we did not see accumulation of the protein in the sera of hyperoxic mice (see Fig. 2). It is possible that EC-SOD entered the bloodstream after proteolysis; however, renal clearance of the enzyme prevented noticeable increases in the blood (1). It is also possible that there is just increased total degradation of the protein that accounts for its loss. Quantitative analysis of EC-SOD mRNA revealed no change in message expression for EC-SOD (see Fig. 3), which suggests that changes in gene expression did not contribute to the loss of enzyme activity in the hyperoxia-exposed animals. Although the final location of the enzyme is not known, our findings suggest that proteolytic removal of the heparin-binding domain in response to hyperoxia contributes to the loss of EC-SOD from the extracellular matrix and cell surfaces of the alveolar parenchyma.

In addition to protein and activity analyses of EC-SOD, the subcellular localization of EC-SOD protein under normoxic and hyperoxic conditions was determined. Figure 4 shows that EC-SOD in normal lung tissue is located primarily in the extracellular matrix with additional labeling on membrane surfaces of bronchial epithelial cells and alveoli. On exposure to high levels of oxygen, there is a significant loss of EC-SOD from alveolar parenchyma (see Fig. 4). Notably, the alveolar parenchyma is also the critical site where hyperoxic injury occurs. The depletion of EC-SOD from the alveolar parenchyma (through proteolytic cleavage of the heparin-binding domain) may be significant, because the association of EC-SOD with type I collagen in the alveoli has been speculated to be important in preventing superoxide-mediated destruction of collagen (10, 28). The loss of EC-SOD from the extracellular space may compromise the ability of the lung to provide adequate antioxidant defense and thus contribute to hyperoxic lung injury.

The protease(s) responsible for the removal of the heparin-binding domain of EC-SOD is currently unknown. It is known, however, that many different proteases are released into the extracellular matrix during the pulmonary inflammation and matrix remodeling that is associated with acute lung injury (6, 32, 39). It is possible that one or more of these pro-
teases targets specific residues in the heparin-binding domain of EC-SOD and thus releases EC-SOD from the extracellular matrix. Loss of EC-SOD from the matrix may in turn serve to promote collagen degradation and perhaps increase oxidative injury to alveoli, as the enzyme is no longer present in its proper protective location. Type I collagen is sensitive to degradation by the superoxide anion both directly (14, 24–26) and indirectly (2, 37) through the activation of latent collagenases in neutrophils. In addition, collagen fragments are known to be both chemoattractants and activators of neutrophils (4, 22, 23, 33). Therefore, increased production or decreased scavenging of superoxide, which results in collagen degradation, may accelerate inflammatory responses and tissue destruction through neutrophil recruitment and activation.

In conclusion, this study demonstrates that exposure to hyperoxic conditions alters the oxidant-antioxidant balance in the extracellular matrix of the lung via proteolysis of EC-SOD. Loss of EC-SOD from the alveolar parenchyma may contribute to superoxide-mediated damage in this area. In addition, lower EC-SOD levels may contribute to inflammatory reactions by allowing increased degradation of collagen by superox-
ide, which results in the recruitment of inflammatory cells into the alveolar spaces. This redistribution of EC-SOD may contribute to the pathogenesis of hyperoxic lung injury.

This work was supported by National Heart, Lung, and Blood Institute Grants RO1-HL-63700 (to T. D. Oury) and 1F32-HL-10439-02 (to C. L. Fattman).

REFERENCES


