Superoxide dismutase moderates basal and induced bacterial adherence and interleukin-8 expression in airway epithelial cells

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Arita, Yuko, Ansamma Joseph, Hshi-Chi Koo, Yuchi Li, Thomas A. Palaia, Jonathan M. Davis, and Jeffrey A. Kazzaz. Superoxide dismutase moderates basal and induced bacterial adherence and interleukin-8 expression in airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 287: L1199–L1206, 2004.—Bacterial infection of the tracheobronchial tree is a frequent, serious complication in patients receiving treatment with oxygen and mechanical ventilation, resulting in increased morbidity and mortality. Using human airway epithelial cell culture models, we examined the effect of hyperoxia on bacterial adherence and the expression of interleukin-8 (IL-8), an important mediator involved in the inflammatory process. A 24-h exposure to 95% O2 increased Pseudomonas aeruginosa (PA) adherence 57% in A549 cells (P < 0.01) and 115% in 16HBE cells (P < 0.01) but had little effect on Staphylococcus aureus (SA) adherence. Exposure to hyperoxia, followed by a 1-h incubation with SA, further enhanced PA adherence (P < 0.01), suggesting that hyperoxia and SA colonization may enhance the susceptibility of lung epithelial cells to gram-negative infections. IL-8 expression was also increased in cells exposed to both hyperoxia and PA. Stable or transient overexpression of manganese superoxide dismutase reduced both basal and stimulated levels of PA adherence and IL-8 levels in response to exposure to either hyperoxia or PA. These data indicate that hyperoxia increases susceptibility to infection and that the pathways are mediated by reactive oxygen species. Therapeutic intervention strategies designed to prevent accumulation of intracellular reactive oxygen species may reduce opportunistic pulmonary infections.

LUNG INFECTION IN CRITICALLY ill patients continues to be a major clinical problem. The estimated prevalence of nosocomial pneumonia ranges from 10 to 65%, with fatality rates >25% in most studies (21). The incidence of ventilator-associated pneumonia (VAP) is 43–60% higher among patients with adult respiratory distress syndrome (2, 8, 29). Furthermore, pneumonia can be demonstrated in as many as 67% of lungs from mechanically ventilated patients histologically examined at autopsy (28).

Colonization of the upper airway by a variety of bacterial species, including gram-positive and enteric gram-negative bacteria, occurs before invasive pulmonary infection has developed. The most common pathogens in VAP are Pseudomonas aeruginosa (PA) and Staphylococcus aureus (SA), with some data suggesting a progression of infection with SA leading to subsequent PA infections (33). VAP usually develops within 10 days after the onset of adult respiratory distress syndrome, with colonization of the lower airway preceding VAP in two-thirds of cases (8). Adherence of bacteria to airway epithelial cells is a key element in the initial stages of infection, leading to colonization of the respiratory tract and to subsequent invasive pulmonary infections.

Severely ill hospitalized patients are often exposed to supraphysiological concentrations of oxygen. Reactive oxygen species (ROS) can injure the lung by overwhelming endogenous antioxidant defenses, causing the release of inflammatory mediators, damage to epithelial cell surfaces, and impairment of bacterial clearance (20, 27). Although numerous studies have demonstrated that damage to the epithelium exposes receptors on the basolateral surface, leading to increased bacterial adherence (7, 22, 32), no studies have examined the molecular pathways induced by hyperoxia on bacterial adherence.

Several studies have suggested that acute and chronic lung injury from hyperoxia and mechanical ventilation may be ameliorated by the administration of the antioxidant superoxide dismutase (SOD) (6, 17, 36). Three isoforms of SOD have been identified: cytosolic copper-zinc SOD (CuZnSOD), extracellular CuZnSOD, and mitochondrial manganese SOD (MnSOD). Studies in transgenic mice and cell culture models have demonstrated that targeted overexpression of MnSOD to airway epithelial cells confers protection from hyperoxia-induced lung injury (16, 38). Although these studies have shown benefits in the reduction of hyperoxic injury, there have been no studies addressing whether this strategy will help moderate or prevent hyperoxia-related infection.

Interleukin-8 (IL-8) is a potent neutrophil chemokine that has been associated with increased morbidity, mortality, and chronic lung inflammation. It is transcriptionally regulated and induced in response to a variety of stimuli, including infection and oxidant stress (see Ref. 31 for review). However, the potential interaction of hyperoxia and PA in the induction of IL-8 expression has not been examined. In this report, cell culture models of airway and alveolar epithelium were used to study the effects of hyperoxia on bacterial adherence, IL-8 expression, and the ability of MnSOD to modulate these processes.

MATERIALS AND METHODS

Generation of stable cell lines, cell culture, and cell viability assays. Human adenocarcinoma alveolar epithelial cells, A549 (ATCC, Manassas, VA), were maintained in F12-K medium supple-
mented with 10% fetal bovine serum, 1% glutamine, and 100 U/ml penicillin, and 100 U/ml streptomycin (GIBCO BRL, Rockville, MD) and maintained at 37°C in 5% CO2-95% room air (RA). Human bronchial epithelial cell line 16HBE (kindly provided by D. Gruenert, University of Vermont) were maintained in Dulbecco’s modified medium supplemented with 10% fetal bovine serum, 1% glutamine, 100 U/ml penicillin, and 100 U/ml streptomycin (GIBCO BRL) at 37°C in 5% CO2-95% RA (3, 20).

Stable cell lines were generated by transfection of A549 cells with the pWESOD2 construct, which contains the full-length human MnSOD cDNA driven by the cytomegalovirus (CMV) promoter (16). Cells with genomic incorporation were initially selected based on antibiotic resistance (G418-sulfate, GIBCO Biochemical) and screened as previously described (16). All isolates expressed comparable amounts of MnSOD, and two independent cell lines (SOD2#3 and SOD2#5) were randomly selected for use in these studies. MnSOD activity in SOD2#3 and SOD2#5 was increased 1.7- and 1.4-fold, respectively, compared with the vector or wild-type A549 cells. These cell lines were maintained in media described below, supplemented with G418-sulfate (800 μg/ml). All experiments were performed in the absence of G418.

Hyperoxic conditions were generated in sealed humidified chambers flushed with 5% CO2-95% O2 (20). Membrane permeability was determined by exclusion of trypan blue dye, and cells were counted with a hemacytometer. Mitochondrial activity was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma, St. Louis, MO) assay, which measures mitochondrial dehydrogenase activity (37) as recommended by the manufacturer. Recombinant adenovirus-mediated expression of MnSOD. Recombinant adenovirus (rAd) was used for the transient induction of MnSOD expression (transduction). Viral transduction was performed with a replication-deficient rAd, Ad.CMV MnSOD, constructed and grown at the University of Iowa as previously described (40). A rAd containing LacZ (Ad.CBlaC) was constructed and grown in the Vector Core at the Institute for Human Gene Therapy (Philadelphia, PA) and used as transduction controls (11). Expression was induced 1 day posttransduction and remained elevated for 4 days posttransduction in A549 cells (data not shown). For this reason, A549 cells were seeded at subconfluence and then transduced with Ad.CMV MnSOD or Ad.CBlaC viron particles in complete media. Cells were washed, and fresh media were added after 17 h. Cells were then harvested and seeded at 5.5 × 104/cm2 for adherence or IL-8 assays 4 days posttransduction (see below). MnSOD enzyme activity was assayed at the time of experiment in duplicate cultures.

Bacterial strains. Nonmucoid laboratory strain of PA (PAO-1) and SA (ATCC 25903) were grown in tryptic soy broth. Bacteria were metabolically labeled with [35S]methionine for 18 h and then washed extensively with PBS before use.

Adherence assay. Adherence to epithelial cells was assessed with a modified radiolabel assay as described previously (15). Briefly, A549 cells were seeded onto 12-well dishes at a density of 5.5 × 104/cm2 and allowed to adhere overnight. 16HBE cells were seeded onto 12-well dishes at a density of 8.8 × 104/cm2. Cells were then exposed to 95% O2 or RA at 37°C for 24 h. The radiolabeled bacteria (106 per well) were then added onto the cell layer and incubated for 1 h. Cells were washed with PBS to remove nonadherent bacteria and lysed with 1 N NaOH, and lysates were counted in a liquid scintillation counter. For pretreatment experiments, cells were exposed to either RA or hyperoxia for 24 h and then exposed to nonradiolabeled PA or SA (106 per well) for 1 h. The cells were extensively washed with PBS, and an adherence assay was performed.

IL-8 assay. The cell culture supernatant from the control and experimental groups were collected, and particulate was removed by centrifugation (350 g for 10 min). IL-8 expression was assayed colorimetrically with the IL-8 ELISA kit (Beckman Coulter, Brea, CA) at an optical density of 450 nm using 550 nm as a reference filter with a Beckman ELISA reader. Samples were diluted to ensure that the readings were within the linear range of the assay.

Electron microscopy. Cells were seeded onto 35-mm dishes, incubated in either RA or 95% O2, and fixed in 2.5% (vol/vol) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 1 h at 4°C. The cells were then postfixed in buffered 1% osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in LX112 (Ladd, Burlington, VT). Thin sections were stained with uranyl acetate and lead citrate and examined on a Zeiss EM 10 transmission electron microscope.

Statistical analysis. All data are reported as means ± SD. Statistical analysis was performed using Student’s t-test or ANOVA with Fisher’s paired least-significant difference post hoc analysis using StatView version 5.01 (SAS Institute, Cary, NC). P values were considered significant at <0.05.

RESULTS

Effects of hyperoxia on mitochondrial structure and bacterial adherence. To determine whether oxidant-induced stress pathways also affect bacterial adherence, we exposed A549 and 16HBE cells to 95% O2 for 24 h and assayed for the adherence of PA and SA. Previous studies have established that this is a nonlethal exposure (20). Exposure of A549 cells to hyperoxia resulted in a 57.1 ± 2.7% increase of PA adhesion (Table 1) (P < 0.01, ANOVA). This effect was even greater with exposure of 16HBE cells (115.4 ± 20.4%, P < 0.001, ANOVA). In contrast, hyperoxic exposure resulted in only a modest increase in SA adhesion to either A549 cells (16 ± 0.3%, P < 0.01) or 16HBE cells (6.5 ± 1.8%, P = not significant). These results demonstrate that hyperoxia increases the adherence of PA to lung epithelial cells and that the increase was significantly greater in airway compared with alveolar cells. Furthermore, hyperoxia induced more of an increase in PA adherence than in SA adherence in both epithelial cell types.

Several studies have addressed the effect of prolonged exposure to hyperoxia on epithelial cells (1, 14, 20, 24). These studies have demonstrated that hyperoxia inactivates mitochondrial enzymes (e.g., succinate dehydrogenase, aconitase, etc.), inducing growth arrest and cell death. The majority of these studies have focused on subconfluent cultures that allowed assessment of both growth arrest and cell death. However, the experiments described here were performed with confluent cultures, which precludes our ability to assess the effect on growth arrest; therefore, the effect of a short (24 h) exposure on mitochondrial enzymes and cell death was assessed. The MTT assay, which assesses mitochondrial dehydrogenase activity, was performed to determine whether oxidant-induced stress pathways also affect mitochondrial enzymes and cell death. The majority of these studies have established that this is a nonlethal exposure (20). Exposure of A549 cells to hyperoxia resulted in a 57.1 ± 2.7% increase of PA adhesion (Table 1) (P < 0.01, ANOVA). In contrast, hyperoxic exposure resulted in only a modest increase in SA adherence to either A549 cells (16 ± 0.3%, P < 0.01) or 16HBE cells (6.5 ± 1.8%, P = not significant). These results demonstrate that hyperoxia increases the adherence of PA to lung epithelial cells and that the increase was significantly greater in airway compared with alveolar cells. Furthermore, hyperoxia induced more of an increase in PA adherence than in SA adherence in both epithelial cell types.

Table 1. Effect of hyperoxia on bacterial adherence and mitochondrial dehydrogenase activity

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>PA Adherence</th>
<th>SA Adherence</th>
<th>Dehydrogenase Activity</th>
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<tr>
<td>A549</td>
<td>157.1±2.7*</td>
<td>116.0±0.3*</td>
<td>67.4±2.9*</td>
</tr>
<tr>
<td>16HBE</td>
<td>215.4±20.4†</td>
<td>106.4±1.8</td>
<td>56.2±5.0†</td>
</tr>
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Adherence and MTT activity are normalized to room air (RA) values and expressed as the percent increase ± SD (n = 6 per group, RA controls = 100%). Bacterial adherence was assayed in cells cultured in either RA or 95% O2 for 24 h using Pseudomonas aeruginosa (PA; PAO1) or Staphylococcus aureus (SA; ATCC 25903) reference strains and are normalized as described (see MATERIALS AND METHODS). The effect of 24-h exposure to 95% O2 on mitochondria was determined with the MTT assay. *P < 0.01 and †P < 0.001 relative to RA controls.
drogenase activity, was used as an early marker of oxidant stress in mitochondria. As shown in Table 1, MTT activity decreased to 67.4% (P < 0.01) of baseline in A549 and 56.2% (P < 0.001) in 16HBE cells compared with RA controls after 24 h of hyperoxia exposure. The effect on viability was examined next. A 24-h exposure to 95% O2 minimally reduced cell viability in both cell types (Table 2). These data indicate that hyperoxia induces oxidant stress but not widespread damage during a 24-h exposure. Several reports suggest that damage to the epithelial layer results in unmasking of receptors on the basolateral surface. To determine the effect of these exposures on the ultrastructure of the cell, hyperoxia- and RA-exposed A549 and 16HBE cells were examined by electron microscopy. In a previous study, we (20) had demonstrated that exposure of subconfluent A549 cells to 95% O2 for 6 days resulted in cellular and nuclear swelling. As shown in Fig. 1, a 24-h exposure induced some mitochondrial swelling (see arrows) relative to RA controls; however, there were no disruptions in the tight junctions noted (see Fig. 1, insets). Although not conclusive, these data suggest that the majority of the damage is limited to the mitochondria.

Next, we determined whether hyperoxia-induced pathways interact synergistically with bacterial colonization to further predispose epithelial cells to gram-negative infection. A549 and 16HBE cells were exposed to hyperoxia for 24 h and treated with either unlabeled PA or SA for 1 h, and then the adhesion assay was performed with labeled PA for 1 h as described above. Exposure of A549 cells to hyperoxia followed by preincubation with PA led to an additional 22.9% increase in PA adherence (P < 0.01) compared with cells exposed to hyperoxia alone (Fig. 2A). When A549 cells were preincubated with SA, PA adherence increased by 30.0% (P < 0.01) compared with cells exposed to hyperoxia alone (Fig. 2A). Similarly, PA adhesion increased by 13.7% in 16HBE cells (P < 0.001) with PA pretreatment, whereas preincubation with SA increased subsequent PA adherence by 30.6% (P < 0.0001) compared with cells treated with hyperoxia alone (Fig. 2B). In contrast, preincubation with PA did not enhance SA adherence in either cell line in RA or in hyperoxia (data not shown). In RA control cells, bacterial pretreatment did not increase subsequent PA adhesion (data not shown). Together, these results suggest that SA colonization promotes subsequent PA colonization only when epithelial cells are exposed to hyperoxia, presumably due to an upregulation of PA receptors by ROS.

Overexpression of MnSOD improves mitochondrial function. A549 cells with chromosomal integration of an exogenous copy of the MnSOD cDNA driven by the CMV promoter were generated. The resulting stable cell lines (SOD2#3 and SOD2#5) were then tested for their ability to protect cells exposed to 95% O2. Because hyperoxia inactivates mitochondrial enzymes and MnSOD is localized in the mitochondria, the effect of MnSOD overexpression on mitochondrial func-

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Viability, %</th>
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<tbody>
<tr>
<td>RA</td>
<td>98.1±1.2</td>
</tr>
<tr>
<td>95% O2</td>
<td>98.0±0.7</td>
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Values represent the percent excluding trypan blue ± SD (n = 9 per group). Confluent cultures were exposed to RA or 95% O2 for 24 h. Cells were harvested, and percent viable cells were determined by trypan blue exclusion.
Fig. 2. Hyperoxia induces bacterial adhesion in lung epithelial cells. A549 (A) and 16HBE (B) cells were exposed to 95% O2 for 24 h. A subset of cells were then exposed to either no bacteria, Pseudomonas aeruginosa (PA), or Staphylococcus aureus (SA) for 1 h in RA at 37°C. After these pretreatments, adherence of PA was measured by radiolabeled PA as described (39). Adherent PA (dpm) were normalized to the epithelial cell number, and numbers were plotted by average of 6 experiment points. *P < 0.01, **P < 0.001, and #P < 0.0001, relative to RA control.

Fig. 3. Manganese SOD (MnSOD) prevents hyperoxia-induced inhibition of mitochondrial activity in A549 cells. pWE4#5, SOD2#3, and SOD2#5 cell lines were exposed to either RA or 95% O2 for 24 h. Dehydrogenase activity was determined by the MTT assay per manufacturer’s instructions. Values represent percentage of dehydrogenase activity in hyperoxia-treated cells relative to the RA control. Values were plotted by average of 8 experiment points from 2 independent experiments. *P < 0.05 and #P < 0.001, relative to empty vector control (ANOVA).

Fig. 4. MnSOD reduces O2-induced increases in bacterial adhesion. As illustrated in Fig. 4A, stable cell lines overexpressing MnSOD had a significant reduction of bacterial adherence in RA (21–48%, P < 0.02) compared with vector controls. Although PA adherence did increase when the SOD cells were exposed to hyperoxia, it was reduced by an average of 55% (P < 0.0001) compared with vector controls exposed to hyperoxia. To confirm that these effects were not a result of clonal differences, A549 cells were transduced with Ad.CMV MnSOD to increase expression transiently. MnSOD activity at a multiplicity of infection of 75 viral particles per cell resulted in a twofold higher level of expression compared with nontransduced cells. In transduced cells, basal (RA) levels of bacterial adhesion were reduced by an average of 58% in SOD transduced cells compared with controls (P < 0.001, ANOVA) (Fig. 4B). When exposed to 95% O2, bacterial adhesion was reduced by an average of 50.8% in SOD-transduced cells relative to Ad.CBLacZ controls (P < 0.001, ANOVA). These results with two different methods demonstrate that a moderate increase of MnSOD activity can effectively reduce PA adherence in lung epithelial cells both in RA and after prolonged exposure to hyperoxia. The reduction of adherence detected in RA-exposed cells overexpressing MnSOD suggests that reducing the basal level of ROS production helps moderate the subsequent response in hyperoxia.

Effect of hyperoxia and MnSOD on IL-8 levels. Because IL-8 is an important proinflammatory chemokine associated with lung inflammation and infection, we hypothesized that hyperoxia would induce IL-8 expression. To assess IL-8 expression, 16HBE and A549 cells were exposed to hyperoxia and IL-8 levels were assayed by ELISA. In 16HBE cells, basal levels of IL-8 were 72.4 ± 0.1 pg/ml, with hyperoxia inducing expression by 127.4% (P < 0.0001, Fig. 5A). The basal level of expression in A549 cells was 22.1 ± 1.1 pg/ml, and levels increased by 61.3% after hyperoxic exposure (P < 0.001) (Fig. 5B). In 16HBE cells, both basal and induced levels of IL-8 per cell were significantly higher than in A549 cells (P < 0.0001).

Because PA adherence increases after exposure to hyperoxia and PA adherence induces an IL-8 response (9), we predicted that the combination of PA and hyperoxia would result in an additive response. To test this hypothesis, 16HBE and A549 cells were exposed to either RA or 95% O2 for 24 h followed by a 1-h incubation with PA. As shown in Fig. 5A, the addition of PA to 16HBE cells (RA) induced a 50% increase in IL-8 expression (from 72.4 to 108.8 pg/ml, P < 0.01). Exposure to hyperoxia followed by PA exposure increased IL-8 production by 180% (269.2 pg/ml) relative to RA controls and 124.5% relative to cells exposed to hyperoxia alone (216.0 pg/ml, P < 0.001). Similar results were obtained with A549 cells (Fig. 5B).

The addition of PA to A549 cells increased expression by 69% compared with RA control (from 22.1 to 37.5 pg/ml, P < 0.001). Exposure to hyperoxia before PA exposure resulted in
a 134% (51.9 pg/ml) increase in IL-8 production relative to RA controls \( (P < 0.0001) \) and corresponding to an additional 72.5% (16.1 pg/ml) relative to cells exposed to hyperoxia alone \( (P < 0.001) \). The induction of IL-8 by exposure to hyperoxia in 16HBE cells was more prominent than in A549 cells, supporting the observation that bronchial epithelial cells were more susceptible to hyperoxia and that IL-8 serves as a marker for cell stress.

The effects of MnSOD overexpression on IL-8 production were then examined. MnSOD-overexpressing cell lines demonstrated reductions in both basal and induced levels of expression \( (P < 0.0001 \text{ relative to } pWE4#5, \text{ Fig. 6A}) \). In MnSOD-overexpressing cells, the stimulated levels of IL-8 were reduced to 41.8% with PA treatment, to 19.6% with hyperoxia exposure, and to 58.7% with both hyperoxia and PA treatment. Although the reduction of IL-8 levels was evident, IL-8 expression was still induced in a similar manner by exposure to either PA or hyperoxia. The patterns were similar in both MnSOD cell lines. However, to ensure that this was not a cloning anomaly, we tested the effect of transiently expressing MnSOD. As illustrated in Fig. 6B, an identical pattern was seen with MnSOD reducing the basal and stimulated levels of IL-8 in transiently induced cells \( (P < 0.0001 \text{ relative to } \text{Ad.CBLaCZ, Fig. 6B}) \). A reduced basal level of expression indicates that ROS signaling is involved in regulation of IL-8 expression, a response that is moderated by MnSOD. The induction by all stimuli was observed in the MnSOD overexpressors, but levels of IL-8 were well below RA-exposed control cells \((pWE4#5 \text{ and Ad.CBLaCZ})\). These data indicate that both hyperoxia and bacterial infection increase inflammatory cytokine production in lung epithelial cells in an additive fashion, with increased MnSOD activity dampening the overall response.

**DISCUSSION**

Data indicate that hyperoxia increases PA adherence in lung epithelial cells, suggesting an upregulation of specific receptors responsible for the increase in PA adherence. Indeed, several adhesion-receptor interactions have been identified for PA \((12, 22, 25, 26, 30)\). The increases in SA adherence, although statistically significant in A549 cells, were moderate in comparison. Because both SA and PA strains can bind to asialoganglioside GM1 \((22)\) and their adherence levels differed in hyperoxia, it is unlikely that the increased adherence was due to an increase in this receptor, and further characterization is needed to identify the receptor(s) responsible. Cells exposed to hyperoxia followed by incubation with SA demonstrated a greater increase in PA adherence than those exposed to hyperoxia alone. This suggests that SA infection (or colonization) might further contribute to secondary PA infection in ventilated patients.

Hyperoxia generates ROS that cause oxidation of macromolecules, including proteins \((34)\), lipids \((35)\), and DNA \((14)\). The decrease in MTT activity found in cells exposed to hyperoxia is not surprising, considering that this assay measures mitochondrial dehydrogenase activity and hyperoxia is known to inhibit key metabolic enzymes \((13, 34)\). Although
this forces cells to utilize glycolysis to generate ATP (34), ATP is not depleted in these cells after 24 h of hyperoxia and is not a limiting factor (data not shown). As shown in electron micrographs, mitochondria were swollen under these exposure conditions in both airway and alveolar cells. It is unclear whether inhibiting these metabolic enzymes directly affects the ability of epithelial cells to clear bacteria.

Our results suggest that the increased susceptibility of the lower respiratory tract to gram-negative bacterial colonization is an early event (with airway cells significantly more susceptible to bacterial adherence and colonization than alveolar cells in both RA and in 95% O2). The deleterious effects of hyperoxia have been observed from various experimental models that demonstrate that hyperoxia can potentiate *Ureaplasma urealyticum* pneumonia in newborn mice and impair pulmonary clearance of PA in adult mice (4, 10). Johanson et al. (19) demonstrated an increased mortality from acute PA infection in hamsters, when infected hamsters were subsequently exposed to hyperoxia. If the increased mortality was due to damage, one would predict that preexposure would increase the mortality rate. However, a 4-day preexposure to hyperoxia did not result in a further increase of mortality compared with those exposed concurrently. Our data demonstrating that hyperoxia increases susceptibility to infection by increasing bacterial adherence without loss of tight junction integrity support the notion that the increase is pathway driven and not merely a function of oxidant damage to the epithelial layer. This initial trigger could lead to an inflammatory cascade, resulting in increased damage to the epithelium followed by colonization and subsequent infection.

In this report, an additive induction with the combination of hyperoxia and PA adherence was found. Exposure to hyperoxia induces signal transduction pathways, resulting in the rapid activation of the NF-κB (24) and a biphasic activation of AP-1 transcription factors (23). Given the role of these transcription factors in oxidative stress and the ability of MnSOD to reduce their activation (40), it is likely that these factors play a central role in the regulation of infection and inflammation in the lung. Studies to delineate the molecular pathways involved in their regulation are underway.

A moderate increase in MnSOD activity significantly reduced bacterial adherence and IL-8 expression in response to hyperoxia. The mechanism of this protection is unclear, but inactivation of mitochondrial enzymes was evident during O2 exposures and overexpression of MnSOD protects mitochondria, suggesting that protection of the mitochondria ameliorated cell stress and reduced subsequent bacterial adherence. We tested this notion by studying cells with increased expression of the CuZnSOD, which also demonstrated a similar but slightly less significant reduction of bacterial adherence than that of MnSOD (data not shown). It should be noted that transduction with Ad.CMV MnSOD increases both mitochondrial and cytosolic SOD levels; therefore, it is difficult to address the specific contributions of mitochondrial protection in these experiments. These results suggest that the reduction of bacterial adherence could be due to mitochondrial protection from oxidative damage or through moderation of the ROS-mediated signaling. H2O2 is a potent second messenger (18) that can affect transcriptional activation and cell cycle regulation. Because SOD catalyzes the conversion of superoxide to H2O2, scavenging H2O2 by the coexpression of other antioxidants (e.g., catalase or glutathione peroxidase) may further affect the response. Whether the source of the ROS is from normal respiration or from other ROS-generating enzyme systems (e.g., NADPH oxidase and xanthine oxidase), the scavenging of other ROS may also suppress the induced expression of PA receptors. These hypotheses will require further testing. Taken together, our data suggest a potential role of antioxidant enzymes in prevention of not only hyperoxic lung injury but also subsequent bacterial infection. Animal models of hyperoxic injury and human trials in premature infants treated with hyperoxia and mechanical ventilation have consistently demonstrated less lung injury and improved outcome with the prophylactic use of recombinant human CuZnSOD (5, 6). These studies, together with our results, demonstrate that reducing oxidant stress will reduce adherence and therefore
may prevent colonization of the airways and subsequent bacterial infection.

In summary, cell culture models of airway epithelium were used to examine the role of hyperoxia on bacterial adhesion, IL-8 expression, and the response of these events to antioxidants. Hyperoxia increased the susceptibility of epithelial cells to bacterial adhesion and resulted in an additive induction of IL-8 when bacteria (PA) were present. MnSOD was able to significantly reduce hyperoxia-induced increases in bacterial adhesion and IL-8 expression and minimize mitochondrial damage. This has important implications in the development of therapeutic interventions using antioxidants to prevent bacterial lung infection in patients receiving supplemental oxygen.

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