Granulocyte/macrophage colony-stimulating factor treatment improves alveolar epithelial barrier function in alcoholic rat lung

Andres Pelaez,1,2 Rabih I. Bechara,1,2 Pratibha C. Joshi,1,2 Lou Ann S. Brown,3 and David M. Guidot1,2

1Atlanta Veterans Affairs Medical Center Pulmonary Section, Decatur 30033; and 2Division of Pulmonary, Allergy, and Critical Care Medicine and 3Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30322

Submitted 14 May 2003; accepted in final form 15 September 2003

Chronic alcohol abuse, in addition to its well-known toxicities in the liver, brain, and other organs, is now recognized as a comorbid variable that independently increases the incidence and severity of acute lung injury in critically ill patients. Specifically, alcoholic patients have an approximately threefold increased risk of developing acute respiratory distress syndrome (ARDS) in response to sepsis, trauma, or other acute inflammatory insults (13, 15). ARDS is a common and devastating disease process estimated to affect $\sim 75,000$–$150,000$ individuals per year in the United States alone (19). Although incremental improvements in the supportive care of these patients have improved the survival from this syndrome in the past decade or so, the mortality from ARDS remains unacceptably high at 40–60%, and there are no effective pharmacological therapies despite extensive research since the syndrome was first identified in 1967 (17). Diverse biological insults including sepsis, trauma, and aspiration lead to acute lung injury characterized by diffuse alveolar damage manifest by epithelial barrier disruption and flooding of the airways with proteinaceous fluid and consequent impairment of gas exchange.

To study the fundamental mechanisms underlying the epidemiological association between alcohol abuse and ARDS, we have developed a rat model of ethanol-mediated susceptibility to acute lung injury and determined that chronic ethanol ingestion produces multiple defects in alveolar epithelial function. For example, alveolar epithelial type II cells isolated from ethanol-fed rats formed more permeable monolayers (6) and had abnormal surfactant synthesis and secretion (5, 8) in vitro. In parallel, chronic ethanol ingestion in rats impaired alveolar epithelial barrier function in vivo, as reflected by decreased alveolar liquid clearance and increased permeability to protein (18). Another remarkable and previously unrecognized finding is that chronic ethanol ingestion produces significant oxidative stress, as reflected by markedly decreased glutathione levels within the alveolar epithelial lining fluid and in alveolar epithelial type II cells (8, 12). Furthermore, chronic dietary supplementation with glutathione precursors maintains glutathione levels and prevents alveolar epithelial barrier dysfunction both in vitro and in vivo (6). The potential clinical relevance of these findings in our rat model is reflected by our observation that otherwise healthy human alcoholic subjects have comparably decreased levels of glutathione in their lung lavage fluid (14). However, the chronic oxidative stress and consequent alveolar dysfunction in patients with longstanding alcohol abuse are likely intractable; as in our animal model, 1 wk of abstinence from alcohol combined with glutathione treatment is required to restore alveolar epithelial glutathione levels and surfactant secretion (5). Unfortunately, in the clinical setting, patients with or without alcohol abuse present acutely with sepsis, trauma, and/or other insults and develop ARDS within 24–72 h. Therefore, chronic strategies, such as alcohol abstinence and glutathione replacement, which may be appropriate to prevent long-term complications of alcohol abuse such as cirrhosis or neuropathy, hold limited promise for the treatment of critically ill patients whose acute lung injury is already in evolution when they present for medical care. It is known that those patients with ARDS who have relatively

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
intact alveolar epithelial function, as reflected by the ability to clear pulmonary edema fluid, have a better outcome than patients who cannot clear edema fluid (20). However, due to the heterogeneity and complexity of the syndrome, to date there are no specific pharmacological therapies that are efficacious in promoting alveolar epithelial barrier function per se in patients with ARDS. Furthermore, there have been no clinical trials targeted toward patients with alcohol abuse who have preexistent alveolar epithelial dysfunction and, therefore, might benefit the most from a treatment strategy designed to promote alveolar epithelial barrier function.

With this background, we have focused our research efforts on identifying novel treatment strategies that could improve alveolar epithelial function in the alcoholic lung. Recently, a phase II trial in a small group of patients with sepsis showed that 96 h of intravenous treatment with granulocyte/macrophage colony-stimulating factor (GM-CSF) was associated with improved indices of lung function and concomitant evidence of improved macrophage function (16). Provocatively, ethanol-induced defects in alveolar epithelial and macrophage function suggest that priming of these cells by GM-CSF signaling in the alveolar space could be impaired. Within the alveoli, GM-CSF acts as a trophic factor for the epithelium but also primes circulating monocytes that enter the alveolar space to undergo terminal differentiation into functional alveolar macrophages (17). This led us to speculate that treatment with GM-CSF could be particularly efficacious in alcoholic patients who have ethanol-induced alveolar epithelial barrier dysfunction. Therefore, in this study we asked whether GM-CSF treatment improves ethanol-mediated alveolar epithelial barrier dysfunction. To address this question, we examined the effects of GM-CSF treatment on alveolar epithelial barrier function in ethanol-fed rats with and without acute inflammatory stress induced by endotoxemia in vivo. We then extended those studies to identify a potential mechanism by which GM-CSF could improve alveolar epithelial function in vivo by determining the effects of GM-CSF treatment on the integrity of the physical barrier of alveolar epithelial monolayers derived from ethanol-fed rats in vitro.

MATERIALS AND METHODS

Ethanol feeding. All work was reviewed and approved by the Institutional Animal Care and Use Committee at the Atlanta VA Medical Center. Young adult male Sprague-Dawley rats (initial weight ~200 g; Charles River Laboratory, Wilmington, MA) were fed the Lieber-DeCarli liquid diet (Research Diets, New Brunswick, NJ) containing either ethanol or an isocaloric substitute with maltodextrin (control diet) added for 6 wk as we have used and published previously (2, 3, 5, 6, 18). Ethanol was added gradually, starting with 20% less weight to the dry weight was calculated and expressed (wet:dry) for each experimental determination. Distilled water was infused intranasally for three consecutive days. Briefly, rats were anesthetized, and a tracheostomy cannula was placed. Recombinant rat GM-CSF (Sigma, St. Louis, MO) or saline vehicle alone via intranasal instillation for three consecutive days. Briefly, all rats were anesthetized with 2% isoflurane so that any subsequent differences in lung liquid clearance or protein leak could not be attributed to the effects of repetitive inhalational anesthesia. Under this brief anesthesia, control-fed and ethanol-fed rats were treated with either GM-CSF (500 or 250 ng in 100 μl of saline) or saline vehicle alone (100 μl) by gently instilling the volume into one nostril with a pipette; rats then sniffed repetitively by a characteristic reflex that delivers a significant volume of the instilled liquid into the lower airways. On day 3 rats were subjected to lung liquid clearance (see Lung liquid clearance in vivo) after the third dose of GM-CSF or vehicle was given. For rats that were made endotoxic for 6 h (see Endotoxin treatment in vivo), the third dose of GM-CSF or vehicle was given at the time that the first of two doses of endotoxin was given intraperitoneally. Therefore, the total treatment period was ~54–56 h before lung liquid challenge in all rats in this study.

Endotoxin treatment in vivo. In selected experiments, GM-CSF-treated or vehicle-treated rats were injected with endotoxin (Salmonella typhimurium lipopolysaccharide; 2 mg/kg ip) and then 3 h later were given a second injection (also 2 mg/kg). Three hours after the second injection (6 h after the initial injection), the rats were anesthetized, and lung liquid clearance was determined. We have used this same protocol to induce endotoxemia in this model previously (2).

Lung liquid clearance in vivo. The rats were deeply anesthetized with pentobarbital sodium and a tracheostomy cannula was placed. A saline challenge (2 cc) was given intratracheally, and rats were then mechanically ventilated with a Harvard rodent ventilator with a tidal volume of 2.5 ml (~8 ml/kg body wt) at 60 breaths/min for 30 min. The lungs were then removed en bloc, the right lung was isolated and its bronchus tied with a suture, the bronchus was cut distal to the suture, and the right lung was weighed (wet weight) and then weighed after desiccation overnight at 70°C (dry weight). The ratio of the wet weight to the dry weight was calculated and expressed (wet: dry) for each experimental determination.

Determination of alveolar epithelial lining fluid protein. At the conclusion of the ventilatory period, the lungs were removed en bloc, and a lung lavage was performed (5 ml of saline × 3 with recovery of 7–10 ml in each rat). The fluid was centrifuged at 1,500 g for 10 min, and the supernatant was stored at ~80°C for subsequent protein analysis. Protein levels in the lung lavage fluid were performed using a bicinchoninic acid assay. A standard curve was prepared from known amounts of albumin to calculate the values of protein recovered in the bronchoalveolar lavage fluid. Total protein was calculated as the product of the concentration of protein and the amount of fluid recovered.

Alveolar epithelial cell isolation and formation of monolayers in vitro. With the use of a technique that we have used previously (2, 3, 6, 8), alveolar epithelial type II cells were isolated from control-fed and ethanol-fed rats. Briefly, rats were anesthetized, and a tracheostomy was placed followed by en bloc lung isolation. After buffer perfusion to remove intravascular blood elements, the lung airways were filled via the tracheostomy cannula with a solution containing porcine pancreatic elastase. The lung parenchyma was then cut away from the large airways and minced in a solution containing DNase and fetal bovine serum (to terminate the elastase reaction). The minced lung was then successively filtered through 100- and 20-μM nylon mesh, and the recovered cells were plated onto 100-mm bacteriologic plastic dishes that had been coated previously with rat IgG. After 1 h of incubation at 37°C, the nonadherent type II cells were gently aspirated from the plates to which the alveolar macrophages and other immune cells had adhered. Cells obtained by this method contained ~90% alveolar type II cells that were >90% viable by trypan blue exclusion. Freshly isolated type II cells were then resuspended at a density of 1 × 106 cells/ml of DMEM containing 10% serum and 3 × 106 cells/ml were plated onto each of 10-mm-diameter permeable microporous membrane (1-μm pore; Transwell, Corning) and cultured for a total of 8 days at 37°C in 90% air-10% CO2. In selected monolayers, recombinant rat GM-CSF (Sigma) was added to the
culture medium (10 ng/ml). The medium was changed every 48 h, and fresh GM-CSF was added with each medium change in the treatment groups. In some experiments, the number of cells per well after 8 days in culture was determined by trypsinizing the cells and counting the number of cells per well using a hemocytometer. Comparisons were made among monolayers derived from control-fed rats and ethanol-fed rats ± GM-CSF treatment in vitro.

**Determination of alveolar epithelial barrier function in vitro.** The barrier function of the cell monolayers after 8 days in culture was determined by adding [14C]inulin (100,000 dpm) to the media covering the basolateral surfaces of the cultured cells. At multiple time intervals (15, 30, 60, or 120 min), the media covering the apical surfaces of the monolayers were removed, and the radioactivity was determined. Leak was defined as the fraction of the initial radioactivity placed on the basolateral surface that appeared on the apical surface of the monolayer after 2 h and was expressed as a percentage.

**Statistical analysis.** Values shown represent the means ± SE of the mean. Values were compared by analysis of variance and corrected by the Student-Newman-Keuls test for differences between groups. P < 0.05 was considered significant.

**RESULTS**

**Effects of GM-CSF treatment via the upper airway on lung liquid clearance in ethanol-fed rats in vivo.** We first determined that the baseline wet:dry ratios in control-fed and ethanol-fed rats that were not challenged with saline were identical (Fig. 1). However, in response to the experimental saline challenge, ethanol-fed rats had decreased (P < 0.05) lung liquid clearance in vivo compared with control-fed rats, as reflected by increased lung tissue wet:dry ratios (Fig. 1).

![Fig. 1. Lung liquid clearance, as reflected by lung tissue wet:dry ratios 30 min after intratracheal challenge with 2 ml of saline, in rats fed either a control liquid diet or an isocaloric liquid diet with ethanol (36% total calories) for 6 wk, with or without treatment with recombinant granulocyte/macrophage colony-stimulating factor (GM-CSF) via the upper airway (500 ng in 100 μl of saline intranasally daily × 3). Untreated rats had 100 μl of saline instilled intranasally daily × 3. Rats were anesthetized with pentobarbital sodium, a tracheostomy was placed, and 2 ml of saline were administered intratracheally. Rats were then ventilated mechanically with room air for 30 min (tidal volume 2.5 ml, which was ~8 ml/kg and 2.5 cm positive end-expiratory pressure). The lungs were removed en bloc, the right lung was isolated, and its wet and dry weights were determined (wet:dry ratio) at 6 h of endotoxemia (induced by injecting 2 mg/kg of Salmonella typhimurium lipopolysaccharide intraperitoneally × 2) before saline challenge. Rats were anesthetized with pentobarbital sodium, a tracheostomy was placed, and 2 ml of saline were administered intratracheally. Rats were then ventilated mechanically with room air for 30 min (tidal volume 2.5 ml, which was ~8 ml/kg and 2.5 cm positive end-expiratory pressure). The lungs were removed en bloc, the right lung was isolated, and its wet and dry weights were determined (wet:dry ratio). Each value represents the mean ± SE of 6 or more determinations. *Significantly increased (P < 0.05) compared with endotoxemic control-fed rats; **significantly decreased (P < 0.05) compared with endotoxemic control-fed rats.**

Treatment with 500 ng of GM-CSF via the upper airway for three consecutive days significantly (P < 0.05) improved lung liquid clearance in ethanol-fed rats (Fig. 1), whereas pretreatment with a lower dose (250 ng) of GM-CSF for three consecutive days did not improve (P > 0.05) lung liquid clearance in ethanol-fed rats (not shown). In contradistinction, treatment with 500 ng of GM-CSF via the upper airway for three consecutive days had no effect (P > 0.05) on lung liquid clearance in control-fed rats (Fig. 1).

**Effects of GM-CSF treatment via the upper airway on lung liquid clearance in ethanol-fed rats after endotoxemia in vivo.** We next determined whether treatment with GM-CSF could be efficacious in the alcoholic lung even after a significant inflammatory stress such as endotoxemia. We examined the effects of the higher and effective dose of GM-CSF (500 ng × 3 days) via the upper airway on lung liquid clearance in rats that were made endotoxemic for 6 h before saline challenge with repetitive administration of S. typhimurium lipopolysaccharide intraperitoneally. We first determined that endotoxemia significantly (P < 0.05) decreased lung liquid clearance in both control-fed and ethanol-fed rats compared with lung liquid clearance in the control-fed and ethanol-fed rats not treated with endotoxin (shown in Fig. 1). As shown in Fig. 2, the lung wet:dry ratios in the control-fed and ethanol-fed rats after endotoxemia were 8.8 ± 0.2 and 10.4 ± 0.4, respectively, and were 6.3 ± 0.4 and 8.0 ± 0.2 in the nonendotoxic rats (shown in Fig. 1). However, lung liquid clearance was even more impaired (P < 0.05) in endotoxic, ethanol-fed rats.
compared with endotoxemic, control-fed rats (Fig. 2). Importantly, treatment with GM-CSF via the airway significantly ($P < 0.05$) increased lung liquid clearance in both control-fed and ethanol-fed rats after endotoxemia (Fig. 2). In fact, ethanol-fed rats treated with GM-CSF had the same ($P > 0.05$) lung liquid clearance after endotoxemia as comparably treated control-fed rats, and the lung liquid clearance in these rats was even greater ($P < 0.05$) than lung liquid clearance in endotoxemic, control-fed rats not treated with GM-CSF (Fig. 2).

**Effects of GM-CSF treatment via the upper airway on alveolar epithelial lining fluid protein levels in ethanol-fed rats after endotoxemia in vivo.** To further assess the effects of GM-CSF treatment on alveolar epithelial permeability in response to endotoxemia in vivo, we next measured total protein levels in the lung lavage fluid. Lung lavage fluid recovery was not significantly different among the treatment groups, so protein concentrations were expressed per volume of lavage fluid. As shown in Fig. 3, ethanol-fed rats had more ($P < 0.05$) protein leak into the alveolar space after endotoxemia and lung liquid challenge than comparably treated control-fed rats. In contrast, treatment with GM-CSF decreased ($P < 0.05$) protein leak into the alveolar space in both control-fed and ethanol-fed rats after endotoxemia and lung liquid challenge (Fig. 3), an effect that parallels the increased alveolar liquid clearance (shown in Fig. 2).

**Effects of GM-CSF treatment on alveolar epithelial barrier formation in vitro.** Alveolar liquid clearance in vivo is an integrated function that depends on active sodium transport as well as a relatively impermeable epithelium that prevents back leak of the edema fluid. Our previous work suggested that the primary defect in the alcoholic lung is increased permeability of the epithelium, whereas active sodium transport may actually be increased as a compensatory response (6). Consistent with those previous studies, GM-CSF treatment decreased alveolar epithelial protein leak after endotoxemia in vivo in this current study (as shown in Fig. 3). Therefore, we hypothesized that GM-CSF treatment improved alveolar liquid clearance in vivo by promoting a tighter alveolar epithelial barrier. To test this hypothesis, we extended these studies to an alveolar epithelial monolayer model in vitro so that we could assess the effects of GM-CSF on epithelial barrier integrity independently of sodium transport. We have employed this model previously to examine the effects of chronic ethanol ingestion and the associated glutathione depletion on alveolar epithelial permeability (6). These monolayers are derived from freshly isolated type II cells (see MATERIALS AND METHODS). Initially, cells that are plated in culture are cuboidal type II cells. After 6 days in culture, these monolayers form a mixture of cuboidal epithelial cells that express surfactant proteins (i.e., type II cells) and flattened epithelial cells that express aquaporins (i.e., they are phenotypically consistent with type I cells). In these experiments, all cells had the typical appearance of either alveolar epithelial type II cells or type I cells, and there were no cells in the monolayers that resembled fibroblasts. Furthermore, none of the cells stained positively with vimentin (not shown). As we have shown previously (6), alveolar epithelial monolayers derived from ethanol-fed rats were significantly ($P < 0.05$) more permeable, as reflected by $[14C]$inulin clearance in 2 h, than alveolar epithelial monolayers derived from control-fed rats (Fig. 4). In contrast, alveolar epithelial monolayers derived from ethanol-fed rats that were treated with GM-CSF in vitro were significantly ($P < 0.05$) less permeable than untreated monolayers derived from ethanol-fed rats (Fig. 4). In fact, epithelial monolayers derived from either control-fed or ethanol-fed rats treated with GM-CSF in vitro were significantly ($P < 0.05$) less permeable than untreated epithelial monolayers derived from control-fed rats, and both GM-CSF-treated groups actually had the same ($P > 0.05$) low permeability (Fig. 4). The effects of GM-CSF on alveolar epithelial monolayer integrity did not appear to be secondary to an increase in cell numbers. Specifically, there were no differences in the number of cells per well after 8 days in culture in monolayers derived from control-fed and ethanol-fed rats.
The incidence and/or severity of ARDS in septic patients, particularly in alcoholic patients who are at three times the risk for developing the syndrome as nonalcoholic patients (13, 15).

These findings complement and extend our previous studies on the effects of ethanol ingestion on alveolar epithelial barrier function and provide potential clues as to how chronic alcohol abuse increases the risk of developing acute lung injury in patients with sepsis or other critical illnesses. Previously, we determined that alveolar epithelial type II cells that were isolated from ethanol-fed rats had decreased surfactant production and were more susceptible to oxidant-mediated injury in vitro (8). We also determined that ethanol ingestion alters alveolar epithelial barrier function as reflected by increased protein leak across the alveolar barrier and decreased alveolar liquid clearance in vivo (6). Furthermore, an important finding was that ethanol ingestion dramatically decreased alveolar epithelial levels of glutathione, a critical antioxidant within the alveolar epithelium, and increased endotoxin-mediated acute edematous injury in isolated lungs that were perfused ex vivo (8, 12). Importantly, although chronic glutathione replacement in the ethanol diet prevents glutathione depletion and thereby maintains alveolar epithelial function in these experimental models (6, 8, 12, 18), it does not appear that acute glutathione replacement could rescue the alcoholic lung in the clinical setting. We have determined that N-acetylcysteine, the only approved glutathione precursor available for human use, does not maintain the critical mitochondrial glutathione pool during ethanol feeding and does not preserve surfactant production (5). This is consistent with previous clinical trials in which N-acetylcysteine therapy was minimally efficacious in patients with established ARDS (1, 10), although those trials were not directed toward patients with a history of alcohol abuse. Therefore, we need to identify therapies that can rapidly improve alveolar epithelial function despite chronic alcohol abuse that could then be tested in these high-risk patients. Such treatments could feasibly be given before the development of overt ARDS, since at-risk patients who present to the hospital after trauma or with signs of sepsis could be identified and treated before they exhibit respiratory failure. Furthermore, even if alcoholic patients develop ARDS, therapies that improve their alveolar epithelial function could potentially decrease the morbidity and mortality in these more vulnerable subjects (13).

This study suggests that one novel candidate for treating alcoholic patients at risk for developing ARDS is GM-CSF. GM-CSF is a 23-kDa, glycosylated, monomeric secreted polypeptide encoded by a 2.5-kb gene composed of four exons located near genes for other hematological growth family members both in humans (5q22-31) and mice (11q30-31) (17). Although GM-CSF was initially identified as an active factor present in lung cell-conditioned medium, it is also known that it is produced by multiple cell types, is present in serum and most tissues, albeit at low concentrations, and is also found in association with the extracellular matrix and as an integral membrane protein capable of stimulating growth of granulocytes and macrophages from cultured hematopoietic progenitors (17). GM-CSF functions as a growth factor predominantly for cells of the phagocytic lineage but also stimulates production of eosinophils, erythrocytes, megakaryocytes, and dendritic cells (17). Because of its stimulating effect on bone marrow, GM-CSF is now widely used therapeutically to ame-

**Table 1. Effects of GM-CSF treatment in vitro on cell number in alveolar epithelial monolayers after 8 days in culture**

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>Number of Cells Per Well, ×10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>Control + GM-CSF</td>
<td>1.6±0.3</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.6±0.1</td>
</tr>
<tr>
<td>Ethanol + GM-CSF</td>
<td>1.7±0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE of 4 determinations per experimental condition. GM-CSF, granulocyte/macrophage colony-stimulating factor.

DISCUSSION

We determined that treatment with GM-CSF via the upper airway for 3 days improved alveolar epithelial barrier function in ethanol-fed rats in vivo, as reflected by increased lung liquid clearance and decreased alveolar epithelial protein levels, even during acute endotoxemia. Lung liquid clearance in vivo is an integrated function that depends on active sodium transport as well as an intact epithelial physical barrier that prevents back leak of transported fluid. Our previous work suggested that the primary defect in the alcoholic lung is increased permeability of the epithelium, whereas active sodium transport may actually be increased as a compensatory response (6). Therefore, we hypothesized that GM-CSF treatment improved lung liquid clearance in vivo by promoting a tighter alveolar epithelial barrier. Consistent with that hypothesis, GM-CSF treatment decreased alveolar epithelial protein leak in parallel to improving net fluid clearance after endotoxemia in ethanol-fed rats. To further test this hypothesis, we extended these studies to an alveolar epithelial monolayer model in vitro so that we could assess the effects of GM-CSF on epithelial barrier integrity independently of sodium transport. GM-CSF treatment improved alveolar epithelial barrier function in vitro, as reflected by decreased permeability in monolayers derived from alveolar epithelial cells isolated from ethanol-fed rats. Although the salutary effects of GM-CSF treatment were most dramatic in the lungs and lung epithelial cells of ethanol-fed rats, GM-CSF treatment also improved liquid clearance and decreased protein leak in the lungs of control-fed rats after endotoxemia and improved alveolar epithelial barrier formation in vitro by cells isolated from even control-fed rats. Overall, this study provides the first evidence that GM-CSF treatment can affect alveolar epithelial function in vivo. Furthermore, these findings are also important because they provide the first evidence that the alveolar epithelial dysfunction caused by chronic ethanol ingestion can be reversed relatively acutely. A cardinal pathological finding in acute lung injury is diffuse alveolar epithelial damage and flooding of the airways with fluid. Furthermore, lung fluid clearance is impaired in the majority of patients with acute lung injury, and the ability to clear alveolar edema in patients with ARDS is associated with improved survival (20).

Because GM-CSF therapy is already used clinically to treat patients with bone marrow suppression and has shown promise in reducing acute lung injury in a recent phase II clinical trial of patients with sepsis (16), this study raises the exciting possibility that GM-CSF therapy could significantly decrease the incidence and/or severity of ARDS in septic patients, particularly in alcoholic patients who are at three times the risk for developing the syndrome as nonalcoholic patients (13, 15).
lorate chemotherapy-induced neutropenia and to stimulate hematopoietic recovery after bone marrow transplantation. Separate from its effects on progenitor cell proliferation, GM-CSF also influences the growth of nonhematopoietic cells such as the alveolar epithelium of the lung. Transgenic mice that overexpress GM-CSF have alveolar epithelial type II cell hyperplasia and increased lung size (9). This is consistent with previous studies showing that the trophic effect of GM-CSF is not only restricted to lung epithelium but also increases glutathione levels in injured skin epithelium (11) and promotes skin wound epithelialization (4, 7). Despite these previously identified effects of GM-CSF, it was surprising that in this study GM-CSF treatment increased alveolar epithelial barrier function in vitro without any apparent effect on cell number. GM-CSF may increase intercellular tight junctions or otherwise promote epithelial integrity without increasing cell number per se. Alveolar epithelial cells are essentially terminally differentiated cells, and it may well be that GM-CSF is not a growth factor for these cells but instead stimulates other functions that mediate barrier formation. Further investigations will be necessary to define such mechanisms, particularly since to date most investigations have focused on GM-CSF priming of alveolar macrophages rather than the alveolar epithelium. Regardless of the specific mechanism(s), this study raises the provocative possibility that GM-CSF could overcome many of such mechanisms, particularly since most investigations have focused on GM-CSF priming of alveolar macrophages rather than the alveolar epithelium.

In summary, this study shows that relatively acute treatment with GM-CSF significantly reverses chronic alveolar epithelial barrier dysfunction in ethanol-fed rats, even in the setting of a severe inflammatory stress such as endotoxemia. Although further work is necessary to define the precise mechanisms by which ethanol disrupts alveolar epithelial function, these novel findings suggest that pharmacological GM-CSF treatment could be an effective therapy for critically ill patients, and, in particular, critically ill alcoholic patients at high risk for developing acute lung injury. Many of these patients can be identified within hours of admission to the hospital and before developing overt ARDS (15). Our current study suggests that even 54 h of treatment with GM-CSF can profoundly improve alveolar epithelial function in a well-characterized animal model of ethanol-mediatated susceptibility to sepsis-induced acute lung injury. As GM-CSF therapy has already been tested in a clinical trial of patients with sepsis and showed promise in decreasing acute lung injury (16), these new findings reveal that GM-CSF has previously unidentified effects on alveolar epithelial integrity and could ultimately prove to be of greatest value in alcoholic patients who have preexisting alveolar epithelial dysfunction and are markedly susceptible to acute lung injury.

ACKNOWLEDGMENTS

The authors thank Raena Garcia, Robert Raynor, and Frank Harris for technical assistance.

GRANTS

This work was supported in part by National Institute on Alcohol Abuse and Alcoholism Grants R01-AA-11660 and AA-12197 (to D. M. Guidot and L. A. S. Brown), a Veterans Affairs (VA) Merit Review Award (to D. M. Guidot), and a national VA Career Development Award cofunded by the American Thoracic Society (to R. L. Bechara).

REFERENCES