Ethanol ingestion via glutathione depletion impairs alveolar epithelial barrier function in rats

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Guidot, David M., Katharina Modelska, Manuel Lois, Lucky Jain, I. Marc Moss, Jean-François Pittet, and Lou Ann S. Brown. Ethanol ingestion via glutathione depletion impairs alveolar epithelial barrier function in rats. Am J Physiol Lung Cell Mol Physiol 279: L127–L135, 2000.—We determined that rats fed a liquid diet containing ethanol (36% of calories) for 6 wk had decreased (P < 0.05) net vectorial fluid transport and increased (P < 0.05) bidirectional protein permeability across the alveolar epithelium in vivo compared with rats fed a control diet. However, both groups increased (P < 0.05) fluid transport in response to epinephrine (10−5 M) stimulation, indicating that transcellular sodium transport was intact. In parallel, type II cells isolated from ethanol-fed rats and cultured for 8 days formed a more permeable monolayer as reflected by increased (P < 0.05) leak of [14C]inulin. However, type II cells from ethanol-fed rats had more sodium-permeant channels in their apical membranes than type II cells isolated from control-fed rats, consistent with the preserved response to epinephrine in vivo. Finally, the alveolar epithelium of ethanol-fed rats supplemented with L-2-oxothiaxolidine-4-carboxylate (Propcysteine), a glutathione precursor, had the same (P < 0.05) net vectorial fluid transport and bidirectional protein permeability in vivo and permeability to [14C]inulin in vitro as control-fed rats. We conclude that chronic ethanol ingestion via glutathione deficiency increases alveolar epithelial intercellular permeability and, despite preserved or even enhanced transcellular sodium transport, renders the alveolar epithelium susceptible to acute edematous injury.

alcoholism; acute respiratory distress syndrome; endotoxin; lung

The acute respiratory distress syndrome (ARDS) is a common and devastating form of acute lung injury in which the air spaces become flooded with inflammatory cells and debris, leading to respiratory failure and, in ~50% of the cases, death. Diverse biological insults, including sepsis, trauma, and aspiration, may result in acute lung injury even in previously healthy people without a history of pulmonary disease. When initially described, ARDS was defined as a discrete disorder involving only the lung parenchyma (3). Based on an improved understanding of its clinical course, ARDS is now recognized both as the severe end of a wide spectrum of acute lung injury and as the pulmonary manifestation of a systemic disorder called the multiple organ dysfunction syndrome (25). Although the pathophysiology of the syndrome is extremely complex, recent studies have begun to identify specific mechanisms by which diverse biological insults result in diffuse alveolar damage.

For example, we recently determined that alcohol abuse is a comorbid variable that independently increases the incidence and severity of ARDS in at-risk patients (16). To identify mechanisms by which alcohol renders the lung susceptible to injury, we developed a rat model of ethanol-dependent acute lung injury (10). We determined that ethanol ingestion dramatically decreased alveolar epithelial levels of glutathione, a critical antioxidant in this microenvironment, and increased endotoxin-dependent acute edematous injury in isolated lungs that were perfused ex vivo (10). Importantly, glutathione replacement decreased endotoxin-mediated lung edema in ethanol-fed rats. We also 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 (10). This study provided the first evidence that chronic ethanol ingestion is toxic to the alveolar epithelium. However, although ethanol ingestion perturbed key functions of type II cells that were isolated and examined under cultured conditions and predisposed to endotoxin-mediated lung edema in isolated lungs that were perfused ex vivo, there was still no direct evidence that ethanol ingestion affected alveolar epithelial barrier function in vivo.

The alveolar epithelial barrier is a dynamic one and depends on active transport of water and ions, particularly sodium, out of the alveolar space and into the interstitium to keep the alveoli dry. This active transport is further increased during acute stress by mechanisms that include catecholamine-mediated enhancement of alveolar liquid clearance (15). Histologically, ARDS is characterized by diffuse alveolar damage with...
extensive epithelial cell destruction (4, 9), and disruption of this normally tight barrier results in flooding of the alveolar air space with proteinaceous fluid (27). Approximately 95% of the alveolar surface is lined by flat type I epithelial cells through which oxygen and carbon dioxide diffuse between the alveolar and capillary compartments. Alveolar type II cells, which comprise the remaining 5% of the alveolar epithelial surface, are cuboidal cells that have diverse functions in maintaining the unique microenvironment of the alveoli, including glutathione homeostasis and surfactant secretion. They are also the progenitors of type I cells and therefore are responsible for maintaining and/or restoring the alveolar structure after injury (2). Type II cells are sensitive to oxidative stress (6), and ARDS patients have multiple abnormalities related to alveolar type II cell function, including decreased glutathione (22) and decreased surfactant (8, 24) in their lung lavage fluid.

Based on our previous study, we hypothesized that ethanol ingestion could impair the ability of the alveolar epithelial cells to form the tight barrier on which normal alveolar function depends. Furthermore, we speculated that glutathione depletion and the subsequent oxidative stress on these cells could be at least partially responsible for any abnormalities that we identified. Therefore, we examined the net vectorial fluid transport across the alveolar epithelium in ethanol-fed and control-fed rats in vivo. We then extended these studies to isolated alveolar epithelial cell formation of monolayers in culture. Finally, we determined the effects of glutathione replacement on ethanol-mediated alterations of alveolar epithelial barrier function.

MATERIALS AND METHODS

Ethanol Feeding of Rats

Young adult male Sprague-Dawley rats (150–200 g; Charles River Laboratory) were fed the Lieber-DeCarli liquid diet with either ethanol (36% total calories) or an isocaloric substitution with maltose-dextrin (control diet) added. In some experiments, ethanol-fed rats had their liquid diet supplemented with Procysteine (1-2-oxothialoidine-4-carboxylate; generously provided by Transcend Therapeutics) at a final concentration of 0.35%. Rats were fed the liquid diet for 6 wk (range 5–7 wk) before death. Dietary intake was comparable in all groups, with all rats demonstrating consistent weight gain throughout the 6-wk feeding period. Ethanol-fed rats had slightly decreased rates of weight gain (10–20% less weight gain compared with control-fed rats), consistent with the well-studied effects of isocaloric ethanol feeding on intermediate metabolism (13).

Alveolar Type II Cell Isolation

Type II cells were isolated using a standard protocol (7). 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 bacteriological plastic dishes that had been coated previously with rat IgG. After a 1-h 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 contain ~90% alveolar type II cells (7) that are >90% viable by trypan blue exclusion. Type II cells were suspended in Dulbecco’s modified Eagle’s medium-nutrient mixture F-12 (Sigma) containing 5% fetal calf serum and maintained in culture at 37°C in 90% air-10% CO₂. Patch-clamp studies were performed between 24 and 96 h after harvest. For studies of epithelial barrier function, cells were maintained in culture for 8 days to form a monolayer before determination of permeability to [¹⁴C]inulin. After 8 days in culture, ~95% of the cells had assumed a flat appearance consistent with a type I cell morphology and no longer stained positively for surfactant protein B by immunostaining.

Determination of Glutathione Levels

To determine the levels of reduced glutathione in lung lavage fluid of intact rats and in freshly isolated type II cells, we used a variation of the high-performance liquid chromatography method presented by Martin and White (14). Briefly, each sample was extracted in 5% perchloric acid with 0.2 M boric acid and 0.5 M acetic acid (10 μM) as an internal standard. Iodoacetic acid was added, and the pH was adjusted to 9.0 ± 0.2. After incubation for 20 min to obtain S-carboxymethyl derivatives of thiols, dansyl chloride was added and the samples were incubated for 24 h in the dark. Samples were then separated on an amine column with the solvents described by Reed et al. (19). Fluorescence detection was used for separation and quantification of the dansyl derivatives. The dilution of the lung epithelial lining fluid by the saline lavage was estimated by concomitant measurements of urea in the plasma and lavage fluid (20). In the lung lavage fluids, glutathione levels are expressed as a concentration (in μM). In the isolated type II cells, glutathione levels are expressed as nanomoles per milligram of protein.

Determination of Alveolar Liquid Clearance and Bidirectional Protein Permeability In Vivo

Surgical preparation and ventilation. Rats were anesthetized with pentobarbital sodium (60 mg/kg ip). An endotracheal tube (PE-220) was inserted through a tracheostomy. Pancuronium bromide (0.3 mg · kg⁻¹ · h⁻¹ iv) was given for neuromuscular blockade. Catheters (PE-50) were inserted into both carotid arteries to monitor systemic arterial pressure and obtain blood samples. The rats were maintained in the left lateral decubitus position during the experiments and were ventilated with room air at a tidal volume of 3 ml, a rate of 60 breaths/min, and positive end-expiratory pressure of 2.5 cm H₂O.

Preparation of alveolar instillate. A 5% bovine albumin solution was prepared with Ringer lactate and adjusted with NaCl to be isomolar with the rat’s circulating plasma, as previously published (15). Anhydrous Evans blue dye (0.5 mg) was added to the albumin solution to confirm the location of the instillate at the end of the study, and 1 μCi of ¹²⁵I-labeled human serum albumin (Frostat Laboratories, Quebec, Canada) was also added to the albumin solution. The ¹²⁵I-albumin served as the alveolar protein tracer in all experiments. A sample of the instilled solution was saved for total
protein measurement, radioactivity counts, and wet-to-dry weight ratio measurements so that the dry weight of the protein solution could be subtracted from the final lung water calculation. In addition, in some studies, epinephrine (10^{-7} M) was added to the instillate.

**General protocol.** In all experiments, heart rate and systemic blood pressure were allowed to stabilize for 60 min after the surgery. The rat was placed in the left lateral decubitus position to facilitate liquid deposition into the left lung. A vascular tracer, 1 μCi of {\(^{131}\)I}-labeled human albumin, was injected into the blood 30 min before the instillation of an albumin solution in the distal air spaces of the lung to calculate the flux of plasma protein into the lung interstitium, as previously published (15). An alveolar tracer, 1 μCi of {\(^{125}\)I}-albumin in 3 ml/kg of the 5% bovine albumin solution, was instilled into the left lower lobe 30 min later to calculate the flux of protein from the air spaces into the circulating plasma. This tracer was instilled by using a 1-ml syringe and a polypropylene tube (0.5 mm ID) over 20 min.

At the end of the experiment (1 h after the beginning of the alveolar instillation), the abdomen was opened and the rats were exsanguinated with transecting the abdominal aorta. Urine was obtained for radioactivity counts. The lungs were removed through a median sternotomy. An alveolar fluid sample from the distal air spaces (0.1–0.2 ml) was obtained by gently passing the sampling catheter (PE-50 catheter, 0.5 mm ID) into a wedged position in the instilled area of the left lower lobe. After centrifugation, the total protein concentration and the radioactivity of the liquid sampled were measured. The right and left lungs were homogenized separately for radioactivity counts.

**Determination of albumin flux across endothelial and epithelial barriers.** Two different methods were used to measure the flux of albumin across the lung endothelial and epithelial barriers, as done before (15, 18). The first method measures residual {\(^{125}\)I}-albumin (the air space protein tracer) in the lungs as well as accumulation of {\(^{125}\)I}-albumin in plasma. The second method measures {\(^{131}\)I}-albumin (the vascular protein tracer) in the extravascular space of the lungs.

The total quantity of {\(^{125}\)I}-albumin (the air space protein tracer) instilled into the lung was determined by measuring duplicate samples of the instilled solution for total radioactivity counts (counts per minute per gram) and multiplying this value by the total volume instilled into the lung. To calculate the residual {\(^{125}\)I}-albumin in the lungs at the end of the study, the average radioactivity counts of two 0.5-μl samples obtained from the lung homogenate were multiplied by the total weight of lung homogenate. The {\(^{125}\)I}-albumin in the lung homogenate data was added to the recovered counts in the final aspirated distal air space fluid to calculate the quantity of instilled {\(^{125}\)I}-albumin that remained in the lungs at the end of the study. The {\(^{125}\)I}-albumin in the circulating plasma was measured from a sample of plasma obtained at the end of the experiment. The plasma fraction was accounted for by multiplying the counts per gram times the plasma volume (body weight × 0.07/1 – hematocrit).

The second method requires measurement of the vascular protein tracer {\(^{131}\)I}-albumin in the extravascular space of the lungs. We estimated the quantity of plasma that entered the instilled lungs by measuring the accumulation of the vascular protein tracer {\(^{131}\)I}-albumin into the extravascular space of the instilled lung using the equation of plasma equivalents previously described (15, 18). The extravascular lung plasma equivalents were determined by counting the total lung {\(^{131}\)I}-albumin (subtracting the fraction of {\(^{131}\)I}-albumin present in the blood retained in the lungs) and then dividing by the mean counts in the circulating plasma over the duration of the experiment, as done previously (15, 18).

**Determination of alveolar liquid clearance.** Changes in the concentration of the instilled nonlabeled bovine albumin and the instilled {\(^{125}\)I}-albumin over the study period (1 h) were used to measure liquid clearance from the distal air spaces, as done before (15, 18). There is a good correlation between the changes in the concentration of instilled nonlabeled bovine albumin and {\(^{125}\)I}-albumin. Furthermore, when the alveolar liquid clearance is calculated, the amount of {\(^{125}\)I}-albumin recovered in the plasma was subtracted from the initial instilled {\(^{125}\)I}-albumin value so as to correct for loss of {\(^{125}\)I}-albumin from the airway fluid. Finally, because some reabsorption may have occurred across distal bronchial epithelium, the term “alveolar” does not imply that all fluid reabsorption occurred at the alveolar level.

**Tracer binding measurement.** To determine {\(^{125}\)I} binding to albumin, trichloroacetic acid (20%) was added to all tubes, which were then centrifuged to obtain the supernatant for measurement of free {\(^{125}\)I} radioactivity. The results are expressed as a percentage of the unbound {\(^{125}\)I} radioactivity to the total amount of {\(^{125}\)I}-albumin radioactivity instilled. These fluid samples always had <1% of unbound iodine present.

**Determination of Alveolar Epithelial Barrier Function In Vitro**

Freshly isolated type II cells were resuspended at a density of 1 × 10^6 cells/ml Dulbecco’s modified Eagle’s medium. The cells were then plated on a 35-mm-diameter permeable microporous membrane (Transwell, Corning; 1-μm pore) and cultured for 8 days. The medium was changed every 48 h. The barrier function of the cell monolayers after 8 days in culture was determined by adding [\(^{14}\)C]inulin (100,000 dpm) to the medium covering the basolateral surfaces of the cultured cells. At multiple time intervals (15, 30, 60, or 120 min), the medium 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 and is expressed as a percentage.

**Determination of Apical Cation Channel Activity in Isolated Type II Cells**

All solutions were made with deionized water and then passed through an 0.2-μm filter (Gelman Sciences, Bedford, MA) before use. The bath and pipette solutions used in the cell-attached mode contained (in mM) 140 NaCl, 1 MgCl₂, 1 CaCl₂, 5 KCl, and 10 HEPES, pH 7.4 with 2 N NaOH. In the inside-out recordings, the pipette solution was the same, but the bath solution was changed to (in mM) 5 NaCl, 140 KCl, 4 CaCl₂, 5 EGTA, 1 MgCl₂, and 10 HEPES, pH 7.4 with 2 N KOH. The contents of the bathing and pipette solutions were varied as appropriate for specific protocols. Patch-clamp experiments to obtain single-channel recordings were carried out at room temperature. The pipettes were pulled from filamented borosilicate glass capillaries (TW-150, World Precision) with a two-stage vertical puller (Narishige, Tokyo, Japan). The pipettes were coated with Sylgard (Dow Corning) and fire polished (Narishige). The resistance of these pipettes was 5–8 MΩ when filled with pipette solution. After formation of a high-resistance seal (>50 GΩ) between the pipette and the cell membrane, channel currents were sampled at 5 kHz with a patch-clamp amplifier (Axopatch 200A; Axon Instruments, Foster City, CA) and filtered at 1 kHz with an eight-pole, low-pass Bessel filter. Data were recorded...
by a computer with pCLAMP 6 software (Axon Instruments). Current-amplitude histograms were made from stable continuously recorded data, and the open and closed current levels were determined from least squares fitted Gaussian distributions.

The single channel characteristics of the ion channel under study have been reported by us previously (12). The predominant sodium-permeant channel seen in apical membranes patches is a high-conductance (20.6 ± 1.1 pS), nonselective (Na\(^+\) to K\(^+\) permeability equals 0.97 ± 0.07) cation channel that is amiloride sensitive.

**Statistical Analyses**

Values shown represent the means ± SE, except for the cation channel densities (see Table 2). Values were compared by analysis of variance and corrected by Student-Newman-Keuls test for differences between groups, except for the cation channel densities for which \(\chi^2\) analysis with Bonferroni correction was performed. In all cases, \(P < 0.05\) was considered significant.

**RESULTS**

**Effects of Ethanol Ingestion With and Without Procysteine on Alveolar Epithelial Glutathione Levels**

We first determined the effects of ethanol ingestion with and without Procysteine supplementation on glutathione homeostasis in the alveolar epithelium. Procysteine is a synthetic precursor of glutathione synthesis. Rats fed the liquid diet with ethanol had lower \((P < 0.05)\) levels of glutathione in the lung lavage fluid (Fig. 1A) and in isolated alveolar epithelial type II cells (Fig. 1B) compared with rats fed the liquid control diet. In contrast, rats fed the liquid diet with ethanol that was supplemented with Procysteine had higher \((P < 0.05)\) levels of glutathione in the lung lavage fluid (Fig. 1A) and in isolated type II cells (Fig. 1B) compared with rats fed the liquid control diet. In addition, rats fed the liquid diet with ethanol had more \((P < 0.05)\) of their lung lavage fluid glutathione in the oxidized form (glutathione disulfide) than rats fed either the liquid control diet or rats fed the liquid diet with ethanol that was supplemented with Procysteine (Fig. 1C). Type II cells from control-fed and ethanol-fed rats that were placed in culture for at least 24 h had the same levels of glutathione as freshly isolated cells (data not shown), which is consistent with our long-standing experience that type II cells do not change their glutathione levels under routine culture conditions.

**Effects of Ethanol Ingestion With and Without Procysteine on Alveolar Epithelial Barrier Function In Vivo**

Alveolar liquid clearance. Rats fed the liquid diet with ethanol had lower \((P < 0.05)\) rates of alveolar liquid clearance in vivo than rats fed the liquid control diet (Fig. 2). In contrast, rats fed the liquid diet with ethanol that was supplemented with Procysteine had the same \((P > 0.05)\) rates of alveolar liquid clearance as rats fed the liquid control diet (Fig. 2). To determine whether ethanol ingestion affected catecholamine-mediated upregulation of alveolar liquid clearance, experiments were repeated with exogenous epinephrine.
(10^{-5} \text{ M initial concentration in alveolar instillate})
stimulation. Rats fed either the liquid control diet or
the liquid diet with ethanol that was supplemented
with Procysteine had higher (P < 0.05) rates of alveo-
lar liquid clearance after epinephrine stimulation than
unstimulated, control-fed rats (Fig. 2). In contrast,
although rats fed the liquid diet with ethanol retained
the ability to respond to catecholamines as reflected by
increased (P < 0.05) rates of alveolar liquid clearance
after epinephrine stimulation compared with those in
unstimulated, ethanol-fed rats, these rates were merely
the same (P > 0.05) as those in unstimulated,
control-fed rats (Fig. 2).

**Bidirectional protein permeability.** Rats fed the li-
quid diet with ethanol had approximately fivefold higher
(P < 0.05) rates of bidirectional protein permeability in
vivo than rats fed the liquid control diet. In contrast,
rats fed the liquid diet with ethanol that was supple-
mented with Procysteine had the same (P > 0.05) rates
of bidirectional protein permeability as rats fed the
liquid control diet. Shown in Table 1 are the plasma
levels of 125I-albumin expressed as a percentage of the
liquid control diet. Shown in Table 1 are the plasma
levels of 125I-albumin expressed as a percentage of the
total amount of tracer placed into the alveolar space at
the start of the experiment and the alveolar-to-plasma
ratios of 131I-albumin. A relatively higher value for
either tracer reflects relatively greater leak of protein
from the alveolar space into the vascular compartment
(in the case of 125I-albumin) or relatively greater leak
of protein from the vascular compartment into the
alveolar space (in the case of 131I-albumin).

**Effects of Ethanol Ingestion With and Without
Procysteine on Alveolar Epithelial Barrier
Function In Vitro**

Type II cells isolated from rats fed the liquid diet
with ethanol that were maintained in culture for 8
days formed a more permeable monolayer as reflected
by higher (P < 0.05) rates of [14C]inulin leak at 60
and 120 min of incubation than type II cells isolated
from rats fed the control liquid diet (Fig. 3). In contrast,
type II cells isolated from rats fed the liquid diet
with ethanol that was supplemented with Procysteine
formed as tight a monolayer as reflected by the same
(P > 0.05) rates of [14C]inulin leak at 15, 30, 60, and
120 min of incubation as type II cells isolated from
control-fed rats (Fig. 3).

**Table 1. Effect of ethanol ingestion on alveolar
bidirectional protein permeability in vivo**

<table>
<thead>
<tr>
<th>Experimental Group (diet)</th>
<th>Plasma Levels of Alveolar Protein Tracer 125I-Albumin (% of instilled)</th>
<th>Lung Levels of Vascular Protein Tracer 131I-Albumin (alveolar-to-plasma ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.2 ± 0.1</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.1 ± 0.3*</td>
<td>0.33 ± 0.05*</td>
</tr>
<tr>
<td>Ethanol + Procysteine</td>
<td>0.2 ± 0.1</td>
<td>0.07 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE of 5 or more determinations. *P < 0.05 compared with control.

**Fig. 3. Rates of [14C]inulin leak across epithelial monolayers formed
after 8 days in culture by type II cells isolated from rats fed the
Lieber-DeCarli liquid diet with ethanol, rats fed the Lieber-DeCarli
control liquid diet without ethanol, and rats fed the Lieber-DeCarli
liquid diet with ethanol but supplemented with Procysteine. Values
represent the percentage of [14C]inulin that diffused from the baso-
lateral surface to the apical surface of the monolayers after 30, 60,
and 120 min. Each value is the mean ± SE of 6 or more determina-
tions. *P < 0.05 compared with monolayers from control-fed rats.

**Effects of Ethanol Ingestion on Single Channel
Characteristics of Nonselective Cation Channels
in Apical Membrane Patches of Isolated Type II
Cells In Vitro**

Type II cells isolated from rats fed the liquid diet
with ethanol for either 2 or 6 wk and studied after 24 h
in culture were more likely (P < 0.02) to have active
cation (sodium-permeant) channels in their apical
membrane patches than type II cells isolated from
control-fed rats (38.9% of patches in cells from control-
fed rat cells had channel activity, whereas 83% and
78.6% of patches cells from ethanol-fed rats had channel
activity; Table 2). In contrast, the open probabilities
for the cation channels were the same (P > 0.05) in
all groups (Table 2). Taken together, these findings
indicate a twofold increase in the density of sodium-
permeant channels in the apical membranes of cells
isolated from ethanol-fed rats, with the relative activi-
ties of the individual channels as reflected by the open
probabilities the same in cells from control-fed and
ethanol-fed rats.

**DISCUSSION**

This study reveals a potential mechanism by which
alcohol abuse renders patients susceptible to acute
lung injury. Specifically, we determined that chronic
ethanol ingestion in rats impaired alveolar liquid clear-
ance and increased alveolar epithelial permeability to
protein in vivo. In parallel, alveolar epithelial type II
cells that were isolated from ethanol-fed rats and
maintained in culture formed a more permeable mono-
layer in vitro, suggesting a primary defect in the ability
of these cells to form tight intercellular junctions. In
contrast to these significant effects on intercellular
barrier function, the alveolar epithelium in ethanol-fed
rats retained the ability to increase alveolar liquid
clearance in response to adrenergic stimulation in vivo
and type II cells isolated from ethanol-fed rats had a
twofold increase in sodium-permeant channels in their
mediated susceptibility to acute lung injury. The first model to examine potential mechanisms of ethanol on this clinical observation, we developed an animal ARDS and the effects of alcoholism on the lung. Based on this clinical observation, we developed an animal model to examine potential mechanisms of ethanol-mediated susceptibility to acute lung injury. The first effect of alcohol on the lung that we identified was glutathione depletion within the alveolar epithelial environment. We determined that ethanol ingestion in rats decreased lung lavage levels of glutathione by >70% and increased acute edematous lung injury mediated by endotoxin and neutrophil activation (10). In that study, we also determined that glutathione levels in isolated alveolar epithelial type II cells were markedly reduced by ethanol ingestion. Furthermore, despite the fact that type II cell recovery and viability in ethanol-fed rats were comparable to those in control-fed rats, surfactant production and resistance to oxidative stress were decreased in vitro (10). These results were dramatic in that there previously had been no evidence that chronic ethanol ingestion had any effect on the lung parenchyma and in particular on a specific target cell within the alveolar epithelium. Alveolar epithelial type II cells regulate glutathione homeostasis in the alveolar space in addition to synthesizing and secreting surfactant, the phospholipid-protein material that minimizes surface tension within the alveoli. These cells also serve as stem cells that proliferate and differentiate into the flattened type I cells that form the majority of the alveolar epithelium, a function that is particularly critical in the response to acute alveolar injury (2). Therefore, significant impairment of type II cell function would certainly be expected to render the lung vulnerable to injury. However, as noted earlier, type II cells form just 5% of the alveolar epithelial surface, with the majority of the epithelial barrier served by type I cells, the flatter and less metabolically active cells that are derived from type II cells. Therefore, although we postulated that ethanol-mediated glutathione depletion in the type II cells ultimately led to abnormal alveolar epithelial barrier formation, we had no direct evidence that type I cell function was consequently impaired by ethanol-mediated glutathione depletion. Our current study extends those original findings and provides new insights into how chronic ethanol ingestion renders the entire alveolar epithelium (i.e., both the type II cells and the type I cells) susceptible to acute edematous injury.

This study also provides further evidence that glutathione depletion is mechanistically involved in ethanol-mediated susceptibility to acute lung injury. In our previous study, we determined not only that glutathione levels were low in the alveolar epithelium of ethanol-fed rats but also that glutathione replacement therapy that was given during the last week of ethanol ingestion significantly decreased endotoxin-mediated
edematous lung injury (10). In this study, treatment with Procysteine, a synthetic precursor that increases glutathione levels (11), restored normal alveolar epithelial barrier function in ethanol-fed rats. In contrast to our previous study (10), we administered the glutathione replacement throughout the course of ethanol ingestion, thereby preventing glutathione depletion. Procysteine treatment actually supranormalized glutathione levels in the alveolar epithelial cells and lining fluid and preserved epithelial barrier function even in the setting of continuous ethanol ingestion. Therefore, although we cannot exclude other unidentified effects of Procysteine, these data provide strong evidence that glutathione depletion, and not ethanol ingestion per se, is the principal cause of ethanol-mediated alveolar epithelial dysfunction. However, we do not know at present how glutathione depletion causes these permeability changes. Possibilities include oxidative damage to discrete targets within the intercellular junctions of the epithelial cells as well as more complex mechanisms such as alterations within the epithelial matrix that interfere with the attachment of epithelial cells to the basement membrane. Further studies are clearly needed to delineate how glutathione deficiency ultimately leads to alveolar epithelial dysfunction.

This current study also offers an explanation as to why alcohol abuse alone does not cause lung edema in the absence of an acute stress such as sepsis. Specifically, although ethanol ingestion leads to alveolar epithelial cell glutathione depletion and cellular dysfunction, it did not cause lung edema formation in the absence of endotoxemia in our rat model (10), and there is no clinical evidence that alcohol abuse alone causes lung edema in humans. Our new findings indicate that although chronic ethanol ingestion increases net intercellular transit of protein and ions (and thereby water), it also increases the density of cation (sodium-potassium-ATPase) channels in the apical membranes of alveolar type II cells and, in parallel, does not impair the ability to increase liquid clearance in response to catecholamines. Transcellular transport of sodium across the alveolar epithelium is driven actively by a Na\(^+\)-K\(^+\)-ATPase complex on the basolateral surface of the cell that works in concert with the apical cation channels (5, 23). Although we did not measure the activity of this complex, the fact that alveolar liquid clearance increased twofold in ethanol-fed rats indicates that the Na\(^+\)-K\(^+\)-ATPase function was preserved, if not upregulated, in parallel with apical cation channel density. Therefore, despite the significant effects of ethanol ingestion on intercellular permeability, the alveolar epithelium appears able to maintain relatively normal alveolar water content by increasing transcellular ion and water transport. However, the epithelial barrier function is significantly stressed by chronic ethanol ingestion as indicated by epinephrine-stimulated rates of alveolar liquid clearance that merely match unstimulated rates in control-fed rats such that it cannot respond adequately to an acute challenge such as sepsis. Figure 4 shows a proposed scheme for how chronic ethanol ingestion affects alveolar epithelial barrier function. Clearly, much work remains to be done to delineate the precise mechanisms by which ethanol mediates these effects as well as to fill in some obvious gaps in this scheme, including potential concomitant abnormalities in the adjacent alveolar endothelium.

Remarkably, until recently, there have been no previous reports on the effects of alcohol abuse on lung glutathione levels in humans. However, in addition to our findings in an animal model, we now have evidence that chronic alcohol abuse is associated with alveolar epithelial glutathione depletion in humans. Specifically, we performed bronchoalveolar lavage on 13 otherwise healthy alcoholics and determined that the glutathione levels in their alveolar epithelial lining fluid were decreased by more than 80% compared with those in nonalcoholic control subjects (17). Therefore, patients with long-standing alcohol abuse who present to the hospital with any acute illness (1, 13, 21) likely...
have chronic alveolar epithelial glutathione deficiency. Furthermore, if we extrapolate our findings in the animal model, these otherwise healthy-appearing patients will have significant impairment in alveolar epithelial barrier function and surfactant secretion, factors that would be predicted to increase their susceptibility to acute lung injury. At present, we do not know how rapidly the alveolar epithelial glutathione levels could be replaced in that setting and, even more importantly, how quickly the alveolar epithelium can restore its normal function once the glutathione levels are replaced. Clearly, further preclinical studies in animal models and in otherwise healthy alcoholic subjects will be necessary to design and initiate appropriate clinical trials in critically ill patients.

In summary, this study shows that chronic ethanol ingestion via glutathione depletion significantly impairs alveolar epithelial barrier function. Although further work is necessary to define the precise molecular structures that are damaged by glutathione depletion, we have identified that the normally tight intercellular junctions between the alveolar epithelial cells are impaired. Furthermore, despite a compensatory increase in active transcellular sodium and water transport that serves to counteract this defect and maintain relatively normal alveolar water content in the un-stressed state, the normal dynamics within the alveolar epithelium are clearly changed. Based on these observations, we speculate that during acute stresses such as sepsis or trauma, the chronic effects of alcohol abuse predispose the lung to greater degrees of alveolar flooding with protein and water, thereby exacerbating acute lung injury. This speculation is consistent with the epidemiological evidence that chronic alcohol abuse predispose the lung to greater degrees of alveolar epithelial barrier function. Although further studies will identify the precise molecular targets within the alveolar epithelium that are exposed to oxidative stress by ethanol-mediated glutathione depletion and will no doubt uncover additional critical consequences of alcohol abuse in other cell types within the lung as well as guide future clinical trials targeted to this vulnerable patient population.

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