Glutathione availability modulates alveolar macrophage function in the chronic ethanol-fed rat

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Brown LAS, Ping X-D, Harris FL, Gauthier TW. Glutathione availability modulates alveolar macrophage function in the chronic ethanol-fed rat. Am J Physiol Lung Cell Mol Physiol 292: L824–L832, 2007. First published November 22, 2006; doi:10.1152/ajplung.00346.2006.—We have previously demonstrated that chronic alcohol exposure decreases glutathione in the alveolar space. Although alcohol use is associated with decreased alveolar macrophage function, the mechanism by which alcohol impairs macrophage phagocytosis is unknown. In the current study, we examined the possibility that ethanol-induced alveolar macrophage dysfunction was secondarily decreased glutathione and subsequent chronic oxidative stress in the alveolar space. After 6 wk of ethanol ingestion, oxidant stress in the alveolar macrophages was evidenced by a 30-mV oxidation of the GSH/GSSG redox potential (P ≤ 0.05). For control macrophages, ~80% internalized fluorescent Staphylococcus aureus were added in vitro. In contrast, only 20% of the macrophages from the ethanol-fed rats were able to bind and internalize fluorescent S. aureus. This ethanol-induced decreased capacity for phagocytosis was paralleled by increased apoptosis. When added to the ethanol diet, the glutathione precursors pro cysteine or N-acetyl cysteine normalized glutathione and oxidant stress in the epithelial lining fluid as well as the alveolar macrophages to control values. This attenuation of oxidant stress was associated with normalization of macrophage phagocytosis and viability. These results suggested that decreased glutathione availability in the alcoholic lung contribute to alveolar macrophage dysfunction via oxidative stress, resulting in not only decreased function but decreased viability.

A HISTORY OF CHRONIC ALCOHOL abuse increases the risk for infection, particularly in the lung (2). Although many mechanisms are undoubtedly involved, the increased risk of respiratory infections by alcoholics is partially due to an impaired immune response of the resident alveolar inflammatory cell, the alveolar macrophage. This impaired response is due in part to decreased ability of alveolar macrophages to phagocytose and clear infectious particles from the airways (3, 11). Equally important is impaired release of proinflammatory cytokines, chemokines, and oxidant radicals required for microbial killing (28).

In the epithelial lining fluid (ELF) of the alveoli, the antioxidant glutathione (GSH) is essential for the detoxification of endogenous and exogenous oxidant radicals and protection of cells residing in the airway and alveolus. Under stressed conditions such as hypoxia, the alveolar macrophage rely on the ELF pool of GSH to provide amino acids for de novo GSH synthesis (9), to protect themselves from oxidant injury (20) and maintain membrane integrity during their respiratory burst (30). Thus availability of extracellular GSH or its precursor amino acids is essential to maintain intracellular macrophage GSH homeostasis necessary for optimal cell functioning. When macrophage GSH availability is limited, generation of high-energy nucleotides is impaired (30) and cellular functions such as phagocytosis and microbial clearance become compromised. When GSH availability in the ELF is limited, phagocytosis and the respiratory burst are compromised (32, 33).

A growing body of clinical and experimental evidence from our laboratory has demonstrated that alcohol abuse independently increased the risk and severity of acute respiratory distress syndrome (ARDS) (24, 27) and was associated with decreased GSH availability and subsequent oxidant stress in the ELF (26). In a rat model of chronic ethanol ingestion, this altered GSH homeostasis in the ELF (14) resulted in increased susceptibility to sepsis-induced acute lung injury as well as impaired alveolar type II cell function and viability (1, 5, 12). An important role for GSH availability as a predisposing factor to the development of acute lung injury was demonstrated by the ability of GSH precursors to attenuate injury in the rat model (4, 5, 14).

Since alveolar macrophage are dependent on GSH in the ELF, and chronic alcohol decreases this GSH pool, we hypothesized that alcohol-induced macrophage dysfunction was secondary to alcohol-induced decreased GSH availability in this extracellular pool. Thus maintenance of GSH in the ELF during chronic ethanol ingestion should protect against the ethanol-induced decreases in macrophage phagocytosis.

METHODS

Rat model of chronic ethanol ingestion. Weanling male Sprague-Dawley rats (175–250 g; Harlan, St. Louis, MO) were fed the Lieber-DeCarli liquid diet (Research Diets, New Brunswick, NJ) containing ethanol ad libitum for 6 wk (5). The ethanol content of the Lieber-DeCarli diet was 36% of the calories, comparative to the calories consumed by otherwise healthy alcoholics (19), and resulted in a blood alcohol of 0.08 ± 0.2%, a level considered legally intoxicated in the state of Georgia. Pair-fed controls were fed an isocaloric mixture of liquid diet without ethanol. Where appropriate, GSH precursors N-acetylcysteine (NAC; 0.163 mg/ml; Sigma, St. Louis, MO) or (−)-2-oxo-4-thiazolidinecarboxylic acid procysteine (PRO; 0.35%; Sigma, St. Louis, MO) or (−)-2-oxo-4-thiazolidinecarboxylic acid procysteine (PRO; 0.35%; Sigma, St. Louis, MO) or (−)-2-oxo-4-thiazolidinecarboxylic acid procysteine (PRO; 0.35%; Sigma, were added to the diet as previously described (5, 12). All animals were used in accordance with NIH guidelines (Guide for the Care and Use of Laboratory Animals) as described in protocols reviewed and approved by the Emory University Institutional Animal Care Committee.

Alveolar macrophage isolation and culture. After pentobarbital anesthesia, the trachea was cannulated and the rat lung lavaged with 1.5 ml of sterile phosphate-buffered saline (37°C, pH 7.4). The lavage fluid was centrifuged at 500 g for 8 min, and the cell pellet was...
resuspended in DMEM/F-12 medium with 2% fetal bovine serum plus penicillin and streptomycin (100 U/I each) and cultured (4 h, 37°C, 5% CO₂). The cell-free lavage fluid was saved for further analysis (see below). The cell population was 95% macrophage as determined by MAC1 staining (31) and Diff Quik analysis with cell viability >95% as determined by calcine-ethidium iodide staining. This method routinely yielded ~3.2 × 10⁸ alveolar macrophages per rat and did not vary between the experimental groups.

Glutathione and oxidant stress analysis. GSH and its oxidized moiety, GSSG, were determined in the lavage fluid and isolated alveolar macrophages by HPLC analysis as previously described by this laboratory (4). Briefly, the samples were immediately acidified with perchloric acid (5% total) containing the internal standard γ-glutamyl-glutamate (5 μM; final concentration). After derivatization with iodoacetic acid and dansyl chloride, the GSH and GSSG fractions were separated on an amino μBondaPak column by HPLC with fluorescence detectors (Waters). GSH and GSSG concentrations were determined relative to γ-glutamyl-glutamate. To control for dilution by the lavage procedure, the GSH and GSSG concentrations in the ELF were normalized via the area method (14). For the alveolar macrophages, the cellular GSH and GSSG concentrations were calculated based on the cell volumes obtained from the three-dimensional reconstruction of the confocal microscopy images (see below).

Redox potential calculations. The redox potential (Eₒ) of the GSH/GSSG pools in the lavage fluid and the macrophages were calculated with the Nernst equation, Eₒ = Eₓ + RT/nF ln [disulfide]/[thiol] (23), where Eₓ is the standard potential for the redox couple, R is the gas constant, T is the absolute temperature, n is 2 for the number of electrons transferred, and F is Faraday’s constant. The standard potential Eₓ for the 2 GSH/GSSG couple was ~−264 mV.

Macrophage phagocytosis. After 2 h of culture, macrophages were washed and fluorescein-labeled inactivated Staphylococcus aureus (Molecular Probes, Eugene, OR) were added in a 1:100 ratio (macrophage to bacteria) (22). After a 2-h culture period, cells were washed and phagocytosis of S. aureus was determined via quantitative digital analysis of fluorescence (QImaging, Burnaby, BC, Canada) and data analysis using Image-Pro Plus for Windows (Jandel Scientific). Values are presented as the mean relative fluorescent units per cell (± SE) as tallied from 10 experimental fields per set. Any macrophage containing green fluorescence was considered a cell with active phagocytosis. Macrophage phagocytosis and apoptosis staining were evaluated using confocal microscopy to localize the fluorescent staining. After respective staining and analysis as described above, images were obtained by laser confocal microscopy with Fluoview analysis (Olympus, Melville, NY). Representative photomicrographs at ×60 magnification were obtained at a depth of 3–5 μm in the Z plane of the macrophage. The percentage of macrophages with active phagocytosis was also determined in each field. Fluorescence depicting the location of the fluorescein-labeled bacteria was confirmed with confocal microscopy by 50% of the cell depth and the plasma membrane stained with peanut agglutinin lectin (PNA; 5 μg/ml; Sigma) (21). Background fluorescence of unstained macrophages was used to correct for autofluorescence. Cell volume was estimated from the computer analysis of the dimensions obtained in the X, Y, and Z planes of the alveolar macrophages before treatment with S. aureus.

Cell viability. Alveolar macrophages were fixed with 3.7% paraformaldehyde, and nonspecific binding was blocked with bovine serum albumin. Apoptosis was determined by staining for the cleaved moiety of the DNA repair enzyme poly(ADP-ribose) polymerase (PARP), an indicator of the apoptosis pathway (10, 17). The primary antibody for cleaved PARP (Oncogene, Cambridge, MA) was added in a 1:100 dilution, and the sample was incubated for 2 h. Cells were serially rinsed with phosphate-buffered saline, and the secondary antibody (anti-rabbit IgG; AlexaFluor) was added in a 1:200 dilution for 1 h. Apoptosis was also evaluated after staining with the ApopTag Plus kit (Oncor, Gaithersburg, MD), which stains TdT-mediated dUTP nick end labeling (TUNEL)-positive cells. The percentage of cells positive for apoptosis was tallied from least 10 experimental fields per set.

Statistical analysis. SigmaStat for Windows was used for statistical calculations. ANOVA was used to detect overall treatment differences. Statistical differences were detected using post hoc analysis (Student-Newman-Keuls), and P ≤ 0.05 was considered significant.

RESULTS

Chronic ethanol ingestion decreased GSH availability in the ELF. After 6 wk of ingestion of a liquid diet containing ethanol, GSH was decreased by 70% in the lavage fluid (P < 0.05; Fig. 1A), whereas the percentage of the oxidized moiety GSSG was increased 95% compared with controls (P < 0.05; Fig. 1B). When the GSH precursors NAC or PRO were added to the ethanol diet, GSH homeostasis was maintained, resulting in normalization of GSH (P ≤ 0.05; Fig. 1A). When added to the control diet, neither NAC nor PRO significantly altered the GSH pools (513 ± 102 and 526 ± 111 μM for control + NAC and control + PRO, respectively). In addition to normalization of the GSH availability in the ELF pool, NAC and PRO also prevented the ethanol-induced increases in GSSG (P ≤ 0.05; Fig. 1B). When the Nernst equation was used to calculate the redox state of the 2 GSH/GSSG pair in the ELF, chronic ethanol ingestion shifted the redox potential Eₒ from −205 ± 2 to 172 ± 3 mV (Fig. 1C). Addition of the glutathione precursors NAC or PRO to the ethanol diet prevented this shift in the redox potential of the ELF. Therefore, addition of these GSH precursors to the diet prevented the chronic oxidant stress in thelavage expected for the ethanol group.

GSH homeostasis in alveolar macrophages. Given the dependence of the alveolar macrophage on the alveolar extracellular GSH pool, we hypothesized that ethanol-induced chronic oxidative stress in the ELF would result in decreased GSH in the macrophage. Indeed, chronic ethanol ingestion decreased GSH by 66% when the data were expressed as nanomoles per 10⁶ cells (11.5 ± 0.6 vs. 4.2 ± 0.6 nmol/10⁶ cells; P ≤ 0.05). However, the cellular volume of macrophages from the ethanol-fed rats was statistically smaller than that of macrophages from control cells (661 ± 103 vs. 281 ± 89 μm³; P ≤ 0.05). When these cell volumes were used to calculate the cellular concentrations, ethanol decreased the cellular GSH concentration by 25% (P < 0.05 vs. control; Fig. 2A). In contrast, the concentration of the oxidized moiety GSSG was elevated fivefold when alveolar macrophages from ethanol-fed rats were compared with those from the pair-fed control group (P < 0.05 vs. control; Fig. 2B). When the Nernst equation was used to calculate the redox state of the GSH/GSSG pair in the ELF, chronic ethanol ingestion shifted the redox potential Eₒ by 30 mV toward a more oxidized state (P ≤ 0.05; Fig. 2C). First, addition of the glutathione precursors NAC or PRO maintained the cellular volumes (416 ± 111 and 509 ± 82 μm³) for ethanol + NAC and ethanol + PRO, respectively; P ≤ 0.05 compared with ethanol alone). Second, both NAC and PRO increased the cellular GSH concentration by 65% compared with the control group. In contrast, addition of NAC or PRO to the control diet did not significantly alter the cell volume or the GSH concentration (data not shown). Although addition of NAC or PRO decreased the GSSG concentration compared with the ethanol group, cellular GSSG was still increased twofold compared with the control group. Despite this increased GSSG, the increased GSH concentration resulted in a more reduced redox
potential in the ethanol + NAC or ethanol + PRO groups compared with the ethanol alone or control groups.

**Chronic ethanol ingestion impaired phagocytosis.** To examine the impact of chronic ethanol ingestion on phagocytosis, we incubated the isolated alveolar macrophages with FITC-labeled *S. aureus*. In the visualization of the Z stacks of a representative alveolar macrophage, there was greater fluorescence in the control cell compared with the alveolar macrophage derived from ethanol-fed rats (Fig. 3, A and B, respectively). To determine whether the FITC-labeled *S. aureus* was located within the cell, we labeled the plasma membrane with rhodamine-labeled PNA. When analyzed at 50% of the cell depth (Z plane) for the control cells, the FITC label was localized within the rhodamine-labeled plasma membrane (Fig. 4A). In contrast, the FITC label and the rhodamine label colocalized for the macrophages from the ethanol group, suggesting that the *S. aureus* was not internalized but bound to the macrophage periphery (Fig. 4B). When the software was used to scan at 50% of cell depth and 50% of cell diameter, most of the fluorescence in the control cell was located within the center of the macrophage and none was located at the outer 10 μm of the cell periphery (Fig. 5A). In contrast, most of the fluorescence was located at the plasma membrane for the macrophages derived from ethanol-fed rats (Fig. 5B). Confocal microscopy at 50% of the cell depth was then used to categorize the macrophages as 1) cells with no binding or internalization of...
S. aureus), 2) cells with S. aureus only bound to the cell periphery, and 3) cells with evidence of any S. aureus internalization. For the control cells, the percentage of macrophages positive for internalization of the S. aureus was ~90% (Fig. 6). In contrast, chronic ethanol ingestion induced a dramatic paralysis of S. aureus phagocytosis. Approximately 80% of the macrophage population from ethanol-fed rats had no externally bound or internalized S. aureus. Of the ethanol macrophages positive for S. aureus internalization, the relative fluorescent units located within the cell membrane were ~30% of those measured for the control cells (Fig. 7).

In vivo GSH precursors preserved macrophage phagocytosis during chronic ethanol ingestion. Given that the GSH precursors maintained GSH homeostasis in the ELF and the alveolar macrophages during chronic ethanol ingestion, the potential of these GSH supplements to protect cellular functions such as phagocytosis was then examined. In the visualization of the Z stacks of a representative alveolar macrophage, there was greater fluorescence in the control, ethanol + NAC, and ethanol + PRO groups (Fig. 3, A, C, and D, respectively) compared with the macrophages from the ethanol-fed rats. (Fig. 3B). To verify that the FITC-labeled S. aureus was located within the cell, we labeled the plasma membrane with rhodamine-labeled PNA. When analyzed at 50% of the cell depth, the FITC label was localized within the rhodamine-labeled plasma membrane for the control, ethanol + NAC, and ethanol + PRO groups (Fig. 4, A, C, and D, respectively) compared with minimal internalization by the macrophages from the ethanol group (Fig. 4B). When confocal microscopy at 50% of the cell depth was used to evaluate internalization of

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**Fig. 2.** Macrophage GSH (A), GSSG (B), and GSH/GSSG redox potential (C). Freshly isolated macrophages were obtained from rats after 6 wk on the experimental diet by bronchoalveolar lavage. GSH and GSSG were determined via HPLC. In A and B, the bars represent means ± SE for 7 or more rats. In C, the cell volumes were determined by confocal microscopy and used to calculate the cellular GSH and GSSG concentrations. The redox state values were calculated from the GSH and GSSG concentrations using the Nernst equation (see text). The data are expressed in a box plot with median line and quartiles; the standard errors are contained within the box. *P < 0.05 compared with control. **P < 0.05 compared with ETOH.
FITC-labeled *S. aureus*, the relative fluorescence units per cell were not statistically different among the control, ethanol + NAC, and ethanol + PRO groups (Fig. 7), suggesting that these GSH precursors preserved phagocytosis during chronic ethanol ingestion.

**Chronic ethanol ingestion decreased macrophage cell viability.** Given the alteration in extracellular and intracellular GSH homeostasis and attenuation of phagocytosis, we postulated that cell viability was also compromised. Compared with macrophages from pair-fed controls, apoptosis as assessed by PARP cleavage was increased threefold when the macrophages were derived from ethanol-fed rats (Fig. 8A). Similarly, a 2.7-fold increase in apoptosis was obtained in ethanol-exposed alveolar macrophages when the TUNEL assay was used as the marker of apoptosis (Fig. 8B). Addition of NAC or PRO to the diet protected the macrophages from ethanol-induced apoptosis.

**DISCUSSION**

Although inhibition of alveolar macrophage function by chronic alcohol abuse has been well described (8, 11, 28, 34–36), the mechanisms by which alcohol inhibits these functions are poorly understood. Previous studies by this laboratory have demonstrated that GSH was decreased by 80% in the ELF of otherwise healthy alcoholics (24). This decrease in GSH and accumulation of GSSG suggested that chronic alcohol abuse resulted in chronic oxidative stress in the alveolar space. In a rat model, previous studies in this laboratory showed that...
chronic ethanol ingestion decreased GSH in the lung tissue, the ELF, and alveolar type II cells (13). As observed previously, the current study demonstrated that chronic ethanol ingestion decreased GSH in the ELF by 70%. This decrease in GSH was accompanied by a twofold increase in the percentage of the glutathione pool present as the oxidized moiety GSSG. Potential mechanisms that could diminish GSH within the ELF after chronic alcohol exposure include decreased GSH synthesis and diminished transport and/or uptake of GSH by pulmonary cells to the alveolar space, as well as increased utilization for detoxification of free radicals.

Despite the oxidative stress generated during ethanol metabolism (18), addition of the GSH precursors NAC or PRO to the ethanol diet maintained GSH homeostasis in the ELF. NAC and PRO also prevented the increased GSSG. The capacity of NAC or PRO to prevent ethanol-induced oxidant stress in the ELF was further supported by the normalization of the redox potential for the GSH/GSSG thiol pair.

Regardless of the mechanisms by which chronic ethanol ingestion decreased GSH and increased GSSG in the ELF, the GSH/GSSG redox potential was 30 mV more oxidized than the control group. This inability of the reducing equivalents in the ELF to maintain the redox potential in the more reduced state suggested that the ELF was under chronic oxidant stress. A 30-mV change in the redox potential would be sufficient to cause an eightfold change in the ratio of reduced to oxidized forms of proteins with vicinal dithiols (15) such as protein disulfide isomerase (7), thioredoxin (29), or the Na^+/H^+ exchanger (16). Priming of these different parameters by alcohol-induced chronic oxidant stress superimposed on the reactive
oxygen species generated during the influx of neutrophils, eosinophils, and leukocytes into the airspace may contribute to the increased incidence and severity of ARDS associated with alcohol abuse (25).

In the macrophages, chronic ethanol ingestion decreased cellular GSH by 25% and increased the oxidized moiety GSSG fivefold. Although the ethanol-induced decreases in the macrophage GSH concentration were relatively modest, the increases in GSSG resulted in a redox potential that was 30 mV more oxidized than control macrophages. Therefore, cellular GSH homeostasis was significantly shifted to an oxidized state during chronic ethanol ingestion, even when optimal nutrition was maintained with the Lieber-DeCarli diet. Thus ethanol-induced oxidative stress was not restricted to the alveolar type

Fig. 5. Relative fluorescence units (RFU) of a cross section of alveolar macrophages incubated with FITC-labeled *S. aureus*. Freshly isolated macrophages were incubated with the fluorescent *S. aureus* and then fixed. The cell was then scanned at 50% cell depth and width, and the RFU were extracted from the data generated as the confocal microscope scanned across the X-axis (n = 5). A: representative image of a control macrophage. B: representative alveolar macrophage derived from an ethanol-fed rat.

Fig. 6. Chronic ethanol ingestion impaired the binding and internalization of *S. aureus*. Freshly isolated alveolar macrophages were incubated with FITC-labeled inactivated *S. aureus* (1:100). Confocal images were obtained at 50% of the Z plane, and the percentages of total macrophages with no binding, binding only (external), and internalization of *S. aureus* (internal) were determined. Bars represent mean percentages (± SE) of 6 rats as tallied from 100 cells/group. aP ≤ 0.05 compared with control.

Fig. 7. Alveolar macrophage internalization of FITC-labeled *S. aureus*. Freshly isolated alveolar macrophages were incubated with FITC-labeled inactivated *S. aureus* (1:100). Confocal images were obtained, and the fluorescence intensity was determined by quantitative digital analysis. Bars represent mean RFU/cell (± SE) of 6 rats as tallied from 10 experimental fields per set. aP ≤ 0.05 compared with control. bP ≤ 0.05 compared with ETOH.
II cell and the ELF (4) but also was extended to alveolar macrophages.

In the alveolar macrophages, NAC and PRO significantly increased the cellular GSH pool despite chronic ethanol ingestion. Despite this increase in cellular GSH, the concentration of GSSG was still increased. However, the increase in GSSG was small relative to the increase in GSH and resulted in a redox potential that was more reduced than in the ethanol-derived or control cells. Given that cells in the alveolar space are dependent on GSH in the ELF, availability of GSH in this pool is an important modulator of GSH homeostasis and oxidant stress in the macrophages. However, one cannot rule out the possibility that ethanol-induced oxidant stress in the macrophages also contributed to the oxidant stress and GSH consumption in the ELF.

As observed by others (8, 11, 28, 34–36), the current study demonstrated that chronic ethanol ingestion impaired alveolar macrophage phagocytosis. Compared with control macrophages, the number of alveolar macrophages positive for any phagocytosis of S. aureus was decreased by 75% in the macrophages derived from ethanol-fed rats. In addition, the capacity for internalization per cell also was decreased by 75% in the ethanol group. Thus chronic ethanol decreased the capacity of alveolar macrophages to clear pathogenic particles by decreasing the rate of phagocytosis by individual macrophages but also by decreasing the percentage of macrophages positive for phagocytosis. Addition of the GSH precursors NAC or PRO to the ethanol diet not only prevented oxidant stress but also prevented the ethanol-induced paralysis of alveolar macrophage phagocytosis. In other words, NAC and PRO maintained both the rate of internalization and the number of macrophages positive for internalization. This suggested that GSH availability and subsequent chronic oxidant stress in alveolar space was an important modulator of alveolar macrophage cellular functions such as phagocytosis. This maintenance of phagocytosis occurred even though ethanol was present throughout dietary period.

Since reactive oxygen species are of fundamental importance in both activation and execution of apoptosis, we proposed that the paralysis of macrophage phagocytosis also was accompanied by decreased cell viability. Our previously published data demonstrated that chronic ethanol ingestion increased apoptosis of alveolar epithelial type II cells (6). We hypothesized that ethanol-induced cellular dysfunctions also would include compromised cell viability, regardless of the cell type. Thus the observation that the percentage of cells unable to phagocytose was greater than the percentage of apoptotic cells suggested that the macrophages from ethanol-fed rats did not phagocytose because they were apoptotic per se. Rather, these studies suggested that chronic ethanol ingestion compromised general cell health and resulted in macrophage dysfunction including phagocytosis and cell viability, two indicators of cell function. As assessed by positive staining for PARP cleavage and TUNEL, chronic ethanol ingestion increased alveolar macrophage apoptosis approximately threefold. As suggested by the normalization of macrophage redox potential as well as phagocytosis, the addition of NAC and PRO to the ethanol diet also prevented ethanol-induced apoptosis.

In summary, results from this study add an important new dimension to the well-described dysfunction of alveolar macrophage that is characteristic of chronic ethanol exposure. Chronic ethanol ingestion decreased GSH availability in both the ELF and the alveolar macrophages and resulted in oxidant stress as evidenced by a 30-mV oxidation in both pools. Decreased GSH availability in the alcoholic lung contributed to alveolar macrophage dysfunction by impairing phagocytosis and increasing apoptosis. A reduction in both the number of alveolar macrophages with active phagocytosis as well as the rate of phagocytosis could play a role in the increased susceptibility to respiratory infections in subjects with a history of alcohol abuse. The ability of GSH precursors to block the toxic effects of ethanol on the alveolar macrophages suggests that chronic oxidative stress is an important modulator of macrophage function and availability. Additional studies are needed to determine whether GSH precursors will rescue the extracellular GSH pool or rescue both macrophage GSH pool and cell functions, as well as improve resistance to respiratory infections in this selected at-risk patient population.
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REFERENCES