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Fetal Alcohol Exposure Impairs Alveolar Macrophage Function *via* Decreased Glutathione Availability

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ABSTRACT

Immature function of the alveolar macrophage increases the risk of pulmonary infections in premature newborns. *In utero* alcohol increases fetal systemic oxidative stress. Because the premature lung is deficient in glutathione (GSH), we hypothesized that chronic *in utero* alcohol (ethanol) exposure exacerbates the oxidative stress within the developing lung, thereby impairing alveolar macrophage function. Additionally, we evaluated the effects of *in vivo* and *in vitro* GSH availability on ethanol-exposed macrophage function. Using a guinea pig model of chronic *in utero* ethanol exposure, fetal epithelial lining fluid (ELF) and alveolar macrophage GSH were decreased with increased markers of oxidative stress. Ethanol-exposed macrophage exhibited impaired phagocytosis and increased apoptosis compared with gestational control. When the GSH precursor S-adenosyl-methionine (SAM) was added to the maternal drinking water containing ethanol, fetal ELF and macrophage GSH were maintained and ELF oxidative stress diminished. *In vivo* maternal SAM therapy maintained macrophage phagocytosis and decreased apoptosis. *In vitro* GSH supplements also improved phagocytosis and viability in both premature and ethanol-exposed macrophage. This suggested that *in utero* ethanol impaired premature macrophage function and viability *via* decreased GSH availability. Furthermore, GSH supplementation during and after ethanol exposure improved fetal macrophage function and viability. These results add a new dimension to the detrimental effects of fetal alcohol exposure on the developing alveolar macrophage, raising the possibility of GSH therapy to augment premature alveolar macrophage function. **(*Pediatr Res* 57: 76–81, 2005)**

Abbreviations

ELF, epithelial lining fluid
GSH, glutathione
GSSG, oxidized glutathione
HNE, 4-hydroxynonenal
MDA, malonyldialdehyde
PARP, poly (ADP-ribose) polymerase
SAM, S-adenosyl-methionine

Fetal alcohol exposure remains a significant problem in our society. Alcohol abuse and binge drinking by pregnant women has been estimated at an alarming 35% of pregnancies (1–3). Fetal alcohol syndrome or alcohol-related neurodevelopmental disorder has been estimated to occur in ~1/1000 pregnancies (4). Because there is a strong relationship between cocaine abuse and concurrent alcohol ingestion (5–7), and both substances may increase the risk of premature delivery (7,8), a significant population of premature infants is exposed to alcohol *in utero*.

In animal models of fetal alcohol exposure, alcohol increases systemic oxidative stress in the developing fetus (9–14). A decrease in the antioxidant GSH has been demonstrated, particularly in the alcohol-exposed fetal liver (9, 15, 16). As an essential antioxidant in the body, GSH is normally present in high concentrations in the ELF of the lung (17). The fetal lung is at risk for increased oxidative stress during development, because maturation of antioxidant systems, including GSH, is gestationally dependent (18,19). A reduction in alveolar GSH, as seen in the premature infant, leaves the preterm lung susceptible to increased pulmonary oxidative injury (20,21) and chronic lung disease (18,22). The impact of the oxidative stress of fetal alcohol exposure superimposed on the low GSH environment of the developing lung has received little attention.

The immature alveolar macrophage's functions contribute to an increased risk of infections in the premature lung. When compared with the adult, the newborn macrophage functions of chemotaxis, phagocytosis, and bacterial killing are diminished (23–25). GSH is essential for optimal functioning of phagocytic cells, such as the alveolar macrophage. For neutrophils, phagocytosis is impaired in many clinical states associated with decreased GSH (26), and the essential role for GSH is supported by the ability of *in vitro* GSH to normalize intracel-

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lular GSH pools and phagocytosis (27). Thus, we hypothesized that the exaggerated oxidative stress associated with *in utero* alcohol (ethanol) exposure would decrease GSH availability for the alveolar macrophage in the developing lung. Furthermore, we postulated that diminished GSH availability and the corresponding oxidant stress would further impair the function of ethanol-exposed premature alveolar macrophage.

By using a guinea pig model of fetal alcohol exposure, the purpose of this study was to examine the effects of chronic *in utero* ethanol exposure on GSH availability in the premature lung and the resulting impact on fetal alveolar macrophage function. The second goal was to determine whether GSH supplements, *in vitro* or *in vivo*, would maintain or restore fetal alveolar macrophage function.

MATERIALS AND METHODS

Guinea pig model of fetal ethanol exposure. Timed-pregnant pathogen-free guinea pigs (Harlan Bioproducts for Science, Indianapolis, IN) were shipped on ~d 35 gestation (term, ~71 d) and randomly assigned to ethanol or no ethanol in the drinking water with incremental increases up to 4% ethanol (28–30) (25% calories + 8 mg/100 mL saccharin) by ~d 40 gestation. The ethanol was increased in increments to avoid decreased food intake or loss of pregnancy secondary to inadequate caloric intake. The only access to drinking water was the experimental mixture. Solid diet was provided *ad libitum* to the ethanol group, whereas the control dams were pair-fed solid diet to match the ethanol dam. In initial studies using this guinea pig model, we found that the solid food intake was significantly less in the ethanol-fed dams (28–30 g/d) compared with the *ad libitum* control dams (45–50 g/d). Therefore, we initiated this pair-fed model of fetal ethanol exposure in attempts to eliminate nutrition as a confounding factor in the experimental design and present data only from pair-fed controls. Where appropriate, the GSH precursor SAM (P-toluenesulfonate salt, 1.0 mg/mL; Sigma Chemical Co., St. Louis, MO) was added to the drinking water containing the ethanol. The experimental drinking water was replenished every other day and the SAM solution prepared twice a week. The fetuses were then removed by cesarean section on d 55 of gestation, corresponding to ~30 wk human gestation. Serum alcohol levels drawn at approximately 0900 h were ~0.05 ± 0.01% in both the mother and the pup. This level approaches legal intoxication in the state of Georgia (0.07%). All animals were used in accordance with the National Institutes of Health Guidelines (Guide for the Care and Use of Laboratory Animals), with protocols reviewed and approved by the Emory University Institutional Animal Care Committee.

Alveolar macrophage isolation. After delivery, the pups were anesthetized with sodium pentobarbital intraperitoneally and the trachea was cannulated for lavage and collection of alveolar macrophage. The fetal lungs were lavaged with 1.5 mL sterile PBS (37°C, pH 7.4). The sample was centrifuged at 1200 rpm for 8 min and the cell pellet resuspended in Dulbecco's modified Eagle's medium (DMEM)-F-12 media with 2% fetal bovine serum, with penicillin and streptomycin (100 u/L each). The supernatant of the ELF was saved for further analysis (see below). Cells in the lavage fluid were predominantly macrophage (>95%), with viability of >95% as determined by calcein/ethidium iodide "live-dead" stain. This method routinely obtained 10^6 pooled macrophage/litter, with each litter representing $n = 1$.

Glutathione analysis. ELF and macrophage GSH and GSSG were determined by HPLC analysis as previously described by this laboratory (31–33). Briefly, after isolation the sample was immediately acidified with perchloric acid (5% total) and 50 mM (final) γ-glutamyl-glutamate (an internal standard) added. Fractions were derivatized with iodoacetic acid and dansyl chloride and then the GSH and GSSG fractions separated by HPLC on an amino μBondaPak column (Waters, Milford, MA). To control for dilution by the lavage procedure, the GSH and GSSG content of the ELF was corrected by the urea method (31–33). For alveolar macrophage measurements, GSH and GSSG values were normalized to cell number. Fluorescent detection was used to separate and quantitate the dansyl derivatives relative to the fluorescence of the γ-glutamyl-glutamate standard.

ELF oxidative stress. To further evaluate the ELF for signs of oxidative stress, we measured the lipid peroxidation product HNE *via* commercially available ELISA (Research Diagnostics, Flanders, NJ). Values for HNE were normalized to ELF IgA, a secretory product not altered by injury (34–36). The ELF IgA was measured by ELISA with the primary antibody obtained from Sigma Chemical Co.

Phagocytosis. After isolation, macrophage were plated at 10^6 cell/mL in DMEM-F-12 media with 2% fetal bovine serum, penicillin plus streptomycin and cultured at 37°C, 5% CO_2. Supplemental GSH (200 μM) was added when appropriate to the culture media. After 2 h, alveolar macrophages were washed, FITC-labeled inactivated *Staphylococcus aureus* (Molecular Probes, Eugene, OR) was added in a 1:1 ratio (alveolar macrophage:bacteria), and the cells were cultured for an additional 2 h (37). After the incubation period, cells were washed and phagocytosis of *S. aureus* was determined *via* quantitative digital analysis of fluorescence (QImaging, Burnaby, BC, Canada) with data analysis *via* Image-Pro Plus for Windows. Values are presented as mean relative fluorescent units (RFU)/cell ± SEM and the mean percentage of cells fluorescently positive ± SEM as tallied from at least 10 experimental fields per set.

Apoptosis. After culture for 4 h, macrophages were fixed with 3.7% paraformaldehyde and nonspecific binding blocked with BSA. Apoptosis was determined by staining for the cleaved PARP, and for DNA fragmentation *via* terminal dUTP nick-end labeling (TUNEL). The primary antibody for cleaved PARP (Oncogene, Cambridge, MA) was added in a 1:100 dilution and the sample incubated for 2 h. Cells were serially rinsed with PBS and the secondary antibody (anti-rabbit IgG, Alexa Fluor, Molecular Probes, Eugene, OR) added in a 1:200 dilution for 1 h. For the TUNEL analysis, fixed cells were permeabilized with 0.1% Triton ×100 and 0.1% sodium citrate and then incubated for 1 h with TMR-Red (*in situ* cell death detection kit, Roche Molecular Biochemicals, Indianapolis, IN). Cellular fluorescence was determined *via* quantitative digital analysis *via* Image-Pro Plus for Windows. Values are presented as the mean percentage of cells fluorescently positive ± SEM as tallied from at least 10 experimental fields per set.

Macrophage oxidative stress. After culture for 4 h, macrophages were fixed with 3.7% paraformaldehyde and nonspecific binding blocked with BSA. Oxidative stress was evaluated by determining the presence of the fatty acid oxidation products HNE and MDA (12) *via* immunohistochemistry. The primary antibodies were added in a 1:100 dilution (MDA, Academy Biomedical, Houston, TX; HNE, Alexis, San Diego, CA) and the sample incubated for 2 h. Cells were serially rinsed with PBS and the secondary fluorescent antibody (or peroxidase antibody) (Sigma Chemical Co.) added in a 1:200 dilution for 1 h. Macrophage staining was determined *via* quantitative digital analysis *via* Image-Pro Plus for Windows. Values are presented as mean RFU/cell ± SEM for MDA and mean relative units (RU)/cell ± SEM for HNE as tallied from at least 10 experimental fields per set.

Statistical analysis. SigmaStat for Windows (Systat Software, Inc., Point Richmond, CA) was used for statistical calculations. ANOVA was used to detect overall differences. Statistical differences between groups were detected by posthoc analysis (Student-Newman-Keuls) and a $p \leq 0.05$ was considered significant.

RESULTS

In utero *ethanol exposure decreased ELF GSH availability.* Fetal ELF GSH was significantly decreased after *in utero* ethanol exposure ($p < 0.05$, Fig. 1*A*), with a corresponding increase in the oxidized portion of GSSG when compared with gestational controls ($p < 0.05$, Fig. 1*B*). Therefore, *in utero* ethanol increased the oxidative stress of the fetal airway as indicated by decreased GSH and increased GSSG/(GSSG + GSH). The addition of the GSH precursor SAM to the maternal diet during ethanol ingestion maintained fetal ELF GSH and decreased oxidized GSSG ($p < 0.05$ *versus* ethanol, Fig. 1, *A* and *B*, respectively).

In utero *ethanol increased products of oxidative stress in the ELF.* The lipid peroxidation product HNE was significantly increased in the fetal ELF after *in utero* ethanol expo-

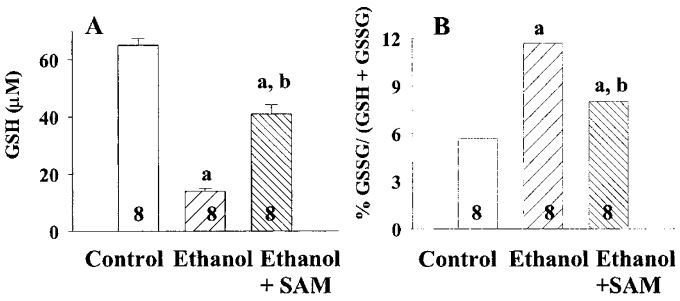

Figure 1. ELF GSH (*A*) and percentage GSSG (*B*). Timed-pregnant guinea pigs were randomly assigned to ethanol or no ethanol in the drinking water (4%) and the groups pair-fed to match the ethanol dam. Where appropriate, SAM (1.0 mg/mL) was added to the drinking water. Pups were delivered by cesarean section on d 55 and ELF was collected by bronchoalveolar lavage. ELF GSH and GSSG were determined *via* HPLC analysis. Bar heights represent mean values ± SEM of eight separate litters. [a]$p < 0.05$ compared with control, [b]$p < 0.05$ compared with ethanol.

sure ($p < 0.05$ *versus* control, Fig. 2). When dams were given ethanol + SAM, the expected increase in free HNE in the fetal ELF due to *in utero* ethanol exposure was significantly attenuated ($p < 0.05$ *versus* ethanol, Fig. 2).

Decreased ELF GSH corresponded with altered alveolar macrophage GSH. Because *in utero* ethanol exposure decreased GSH availability and increased oxidative stress in the fetal ELF, we investigated whether this was accompanied by decreased GSH within the fetal alveolar macrophage. Fetal alveolar macrophage were isolated and GSH and GSSG measured *via* HPLC (33). *In utero* ethanol exposure decreased the fetal macrophage GSH/GSSG ratio by ~73% ($p < 0.05$ *versus* control, Fig. 3). The addition of SAM to the maternal diet during ethanol ingestion maintained macrophage GSH/GSSG at control levels ($p < 0.05$ *versus* ethanol, Fig. 3).

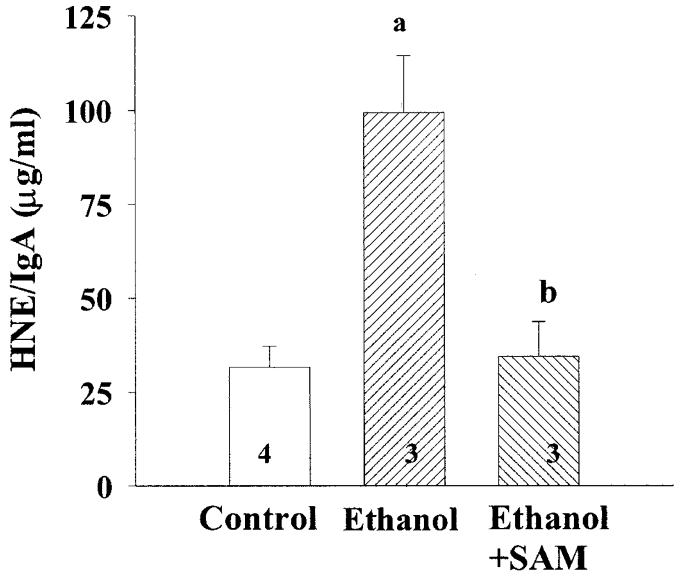

Figure 2. Lipid peroxidation product HNE in the ELF. Pups were delivered by cesarean section on d 55 after exposure to the experimental diet, and ELF was collected by bronchoalveolar lavage. Free HNE was measured *via* ELISA with normalization to ELF IgA. Bar heights represent mean values (μg/mL) ± SEM of *n* separate litters. [a]$p < 0.05$ compared with control, [b]$p < 0.05$ compared with ethanol.

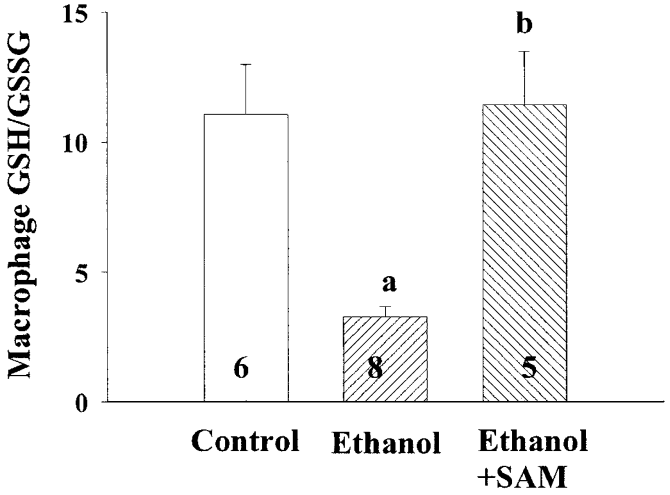

Figure 3. Fetal macrophage GSH/GSSG ratios. After cesarean section on d 55 gestation, fetal macrophage were isolated by bronchoalveolar lavage. Macrophage GSH and GSSG were determined *via* HPLC. Bar heights represent mean values ± SEM of *n* litters. [a]$p < 0.05$ compared with control, [b]$p < 0.05$ compared with ethanol.

In utero ***ethanol increased lipid peroxidation in the alveolar macrophage.*** To further verify that *in utero* ethanol exposure increased oxidative stress in the fetal alveolar macrophage, the cells were evaluated for the presence of HNE and MDA, lipid peroxidation by-products. *In utero* ethanol exposure increased MDA staining on the macrophage by >150% ($p < 0.05$ *versus* control, Fig. 4*A*), whereas HNE staining was increased by >80% ($p < 0.05$ *versus* control, Fig. 4*B*). Concurrent treatment with the glutathione precursor SAM during the ethanol ingestion normalized both MDA staining per cell and HNE staining per cell to control levels ($p < 0.05$ *versus* ethanol, Fig. 4, *A* and *B*, respectively).

Fetal ethanol exposure impaired premature macrophage phagocytosis. When compared with the macrophages from pair-fed gestational controls, the mean phagocytosis per cell was decreased by ~30% after *in utero* ethanol exposure ($p < 0.05$ *versus* control, Fig. 5*A*). Ethanol exposure also reduced the percentage of cells positive for ingested *S. aureus* by

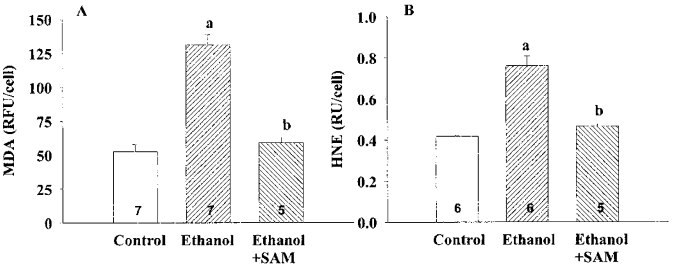

Figure 4. Lipid peroxidation products in the fetal macrophage. Fetal macrophage were isolated by bronchoalveolar lavage and cultured for 4 h. Cells were fixed with 3.7% paraformaldehyde and then incubated with the primary antibody for MDA (*A*) and HNE (*B*). Cells were then stained with a secondary fluorescent antibody or a secondary peroxidase antibody. For MDA staining (*A*), bar heights represent mean relative fluorescent units (RFU/cell) ± SEM of *n* litters. For HNE staining (*B*), bar heights represent mean relative staining (RU/cell) ± SEM of *n* litters. [a]$p < 0.05$ compared with control, [b]$p < 0.05$ compared with ethanol.

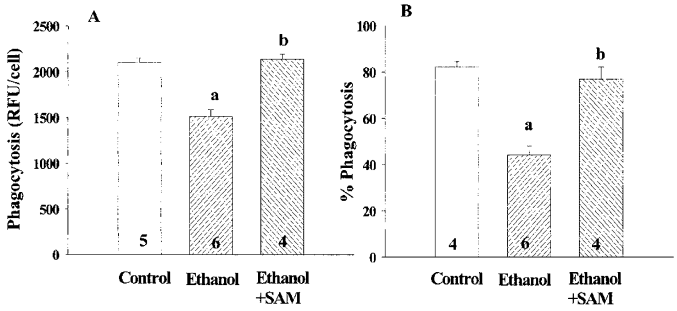

Figure 5. Baseline fetal macrophage phagocytosis. Fetal macrophages were isolated on d 55 by bronchoalveolar lavage and cultured for 2 h. Cells were incubated with FITC-labeled inactivated *S. aureus* (1:1 ratio) for an additional 2 h. Fluorescence was determined by quantitative digital analysis (Q imaging). Bar heights represent (*A*) mean relative fluorescent units (RFU/cell) ± SEM and (*B*) percentage phagocytosis ± SEM of *n* litters. $^{a}p < 0.05$ compared with control, $^{b}p < 0.05$ compared with ethanol.

~50% ($p < 0.05$ *versus* control, Fig. 5*B*). The GSH precursor SAM maintained both the mean phagocytosis per cell at control values ($p < 0.05$ *versus* ethanol, p = NS *versus* control, Fig. 5*A*) as well as the percentage of macrophage positive for phagocytosis ($p < 0.05$ *versus* ethanol, p = NS *versus* control, Fig. 5*B*).

In utero *ethanol exposure decreased fetal alveolar macrophage survival.* Because of the decreased GSH availability for the ethanol-exposed macrophage and the dramatic reduction in cell phagocytosis, we postulated that the dysfunction of the ethanol-exposed macrophage was due to increased apoptotic cell death. To determine apoptosis, cells were stained after 4 h culture for the cleavage of PARP by caspase-3, an early indicator of the apoptosis pathway (38), and for DNA fragmentation *via* the TUNEL assay. Fetal ethanol exposure significantly increased the percentage of cells staining positive for PARP by more than 2-fold ($p < 0.05$ *versus* control, Fig. 6*A*). Similarly, TUNEL-positive cells were significantly increased in the ethanol group compared with control ($p < 0.05$ *versus* control, Fig. 6*B*). The addition of SAM normalized the percentage of cells with any staining for PARP to control values and significantly diminished TUNEL staining ($p < 0.05$ *versus* ethanol, respectively, Fig. 6, *A* and *B*). These results suggested

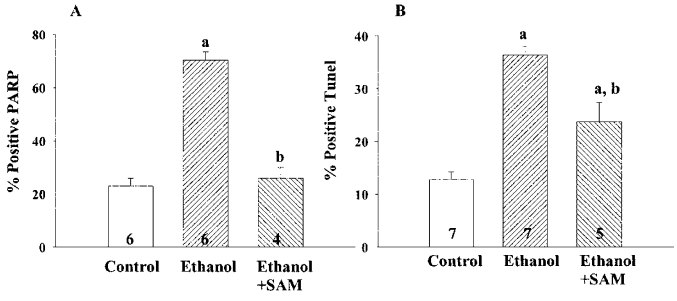

Figure 6. Baseline apoptosis in fetal macrophage. Fetal macrophage were isolated by bronchoalveolar lavage and cultured for 4 h. Cells were fixed with 3.7% paraformaldehyde and then evaluated for apoptosis by staining for cleaved PARP (*A*) and DNA fragmentation (TUNEL, *B*). Bar heights represent the percentage of positive cells ± SEM of n litters. $^{a}p < 0.05$ compared with control, $^{b}p < 0.05$ compared with ethanol.

that GSH availability in the ELF protected the fetal macrophage from ethanol-induced apoptosis.

In vitro *GSH attenuated macrophage oxidative stress and improved function and viability.* Given the role of extracellular GSH availability in macrophage oxidative stress, function, and viability, the possibility of macrophage rescue by *in vitro* GSH supplements was addressed. After isolation, supplemental GSH (200 μM) was added to the culture media and macrophage oxidative stress, as determined by HNE and MDA staining, phagocytosis, and apoptosis were evaluated. *In vitro* GSH supplementation significantly diminished MDA per cell in the ethanol-exposed macrophage ($p < 0.05$ compared with ethanol without GSH, Fig. 7*A*). Similarly, HNE staining was significantly attenuated by *in vitro* GSH supplementation in the ethanol macrophage ($p < 0.05$ compared with ethanol without GSH, Fig. 7*B*).

Furthermore, the addition of GSH *in vitro* improved the mean phagocytosis per cell (Fig. 8*A*) and the percentage phagocytosis (Fig. 8*B*) in both the premature control macrophages and the ethanol-exposed macrophages ($p < 0.05$ compared with control without GSH, $p < 0.05$ compared with ethanol without GSH, Fig. 8, *A* and *B*).

Finally, *in vitro* GSH significantly reduced PARP-positive cells in control premature cells ($p < 0.05$ compared with control without GSH, Fig. 9*A*). For the macrophages exposed to ethanol *in utero*, *in vitro* GSH significantly reduced the percentage of apoptotic cells as determined by both PARP staining ($p < 0.05$ compared with ethanol without GSH, Fig. 9*A*) and TUNEL staining ($p < 0.05$ compared with ethanol without GSH, Fig. 9*B*).

DISCUSSION

A growing body of clinical and experimental evidence has demonstrated that the chronic oxidative stress of alcohol exposure decreases the availability of the antioxidant GSH in the adult lung (39) and independently increases the risk and severity of acute respiratory distress syndrome (40). Indeed, experimental studies from our laboratories investigating the adult

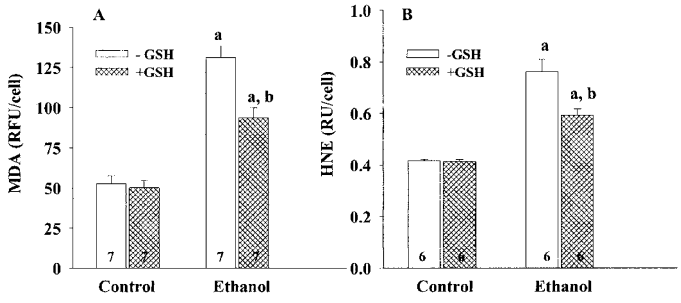

Figure 7. Macrophage oxidative stress with *in vitro* GSH supplements. Fetal macrophage were isolated by bronchoalveolar lavage and cultured with ± 200 μM GSH for 2 h. Cells were fixed with 3.7% paraformaldehyde and then incubated with the primary antibody for MDA (*A*) and HNE (*B*). Cells were then stained with a secondary fluorescent antibody or a secondary peroxidase antibody. For MDA staining (*A*), bar heights represent mean relative fluorescent units (RFU/cell) ± SEM of *n* litters. For HNE staining (*B*), bar heights represent mean relative staining (RU/cell) ± SEM of *n* litters. $^{a}p < 0.05$ compared with control without GSH, $^{b}p < 0.05$ compared with ethanol without GSH.

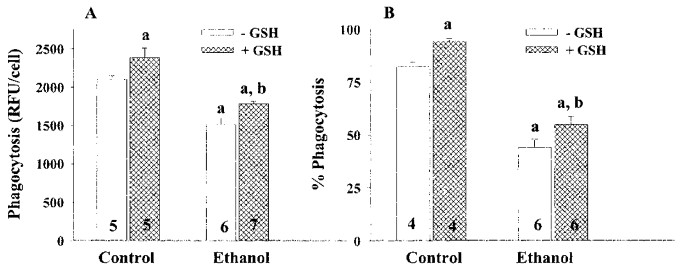

Figure 8. Macrophage phagocytosis with *in vitro* GSH supplementation. Fetal macrophage were isolated by bronchoalveolar lavage and cultured with ± 200 μM GSH for 2 h. FITC-labeled inactivated *S. aureus* was added to the media for an additional 2 h. Fluorescence was determined by quantitative digital analysis. Bar heights represent (*A*) mean relative fluorescent units (RFU/cell) ± SEM and (*B*) percentage phagocytosis ± SEM of *n* litters. [a]$p < 0.05$ compared with control without GSH, [b]$p < 0.05$ compared with ethanol without GSH.

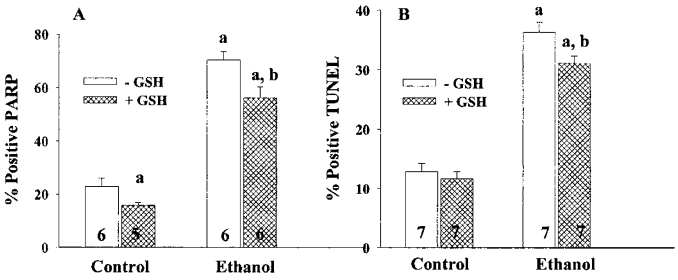

Figure 9. Apoptosis of the fetal macrophage after *in vitro* GSH supplementation. Fetal macrophage were cultured with ± 200 μM GSH for 4 h. To determine apoptosis, cells were fixed with 3.7% paraformaldehyde and then evaluated for cleaved PARP and DNA fragmentation (TUNEL). Fluorescence was determined *via* quantitative digital analysis. Bar heights represent (*A*) percentage positive PARP ± SEM and (*B*) percentage positive TUNEL ± SEM of *n* litters. [a]$p < 0.05$ compared with control without GSH, [b]$p < 0.05$ compared with ethanol without GSH.

"alcoholic lung" have demonstrated that GSH homeostasis is impaired, with decreased GSH and increased oxidized GSSG in the ELF (41,42). This state of chronic oxidative stress results in increased acute lung injury with sepsis, impaired type II epithelial cell function (42), and increased sensitivity to oxidant-induced apoptosis (33).

Prematurity is associated with decreased GSH availability in the ELF and decreased alveolar macrophage phagocytosis (22,23). The current study shows that, similar to the adult, the fetus exposed to ethanol *in utero* demonstrated decreased GSH availability in the ELF compared with the pair-fed control. This was accompanied by increased oxidant stress in the ELF as assessed by diminished GSH/GSSG and increased lipid peroxidation. This ethanol-induced chronic oxidative stress in the ELF was coupled with oxidative stress in the alveolar macrophages as assessed by decreased GSH/GSSG and increased lipid peroxidation (MDA and HNE). Such chronic oxidative stress had physiologic ramifications for the alveolar macrophage, including decreased function such as phagocytosis and diminished cellular viability, when compared with cells from gestationally matched pair-fed control pups.

The increased macrophage apoptosis demonstrated after chronic *in utero* ethanol parallels the oxidant-induced injury and apoptosis described in other organs models of fetal alcohol syndrome, such as the developing brain (13) and liver (43). Because the entire fetus is exposed to the oxidative stress of *in utero* ethanol exposure, our current results suggest that the developing resident alveolar macrophage within the lung is similarly vulnerable to the toxicity of *in utero* ethanol.

Antioxidant replacement has been described as beneficial to other cell systems exposed to the chronic oxidative stress of fetal alcohol exposure. Replacement of GSH or vitamin E in the developing ethanol-exposed fetus has restored fetal growth (44), blunted neurologic injury (45), and preserved hepatic cell function (46,47). In the current study, maintaining GSH availability *in vivo via* the maternal GSH donor SAM during ethanol ingestion protected the fetal lung and alveolar macrophage from oxidative stress. This was evident by attenuation of lipid peroxidation, as well as increased GSH/GSSG in the both the ELF and the alveolar macrophages. Maintenance of GSH status corresponded with protection of the developing macrophage from ethanol-induced dysfunction, with increased phagocytosis and decreased apoptosis. These results suggested that ethanol-induced decreases in GSH availability to the fetal lung were a central feature in the ethanol toxicity to the alveolar macrophages. Furthermore, increasing GSH availability *in vitro* improved control pair-fed fetal macrophage phagocytosis and blunted ethanol-induced dysfunction, in part by reducing apoptosis in the cell.

We chose to examine macrophage function at one point in gestation after chronic ethanol exposure. At this point (~30 wk of human gestation), GSH remains deficient in the developing fetus, as levels normally increase with gestation, with a surge at the time of term delivery (18). In our guinea pig model, the pair-fed control premature macrophage demonstrated cellular vulnerability, as evidenced by the presence of oxidative stress and apoptosis. Pair-feeding and diminished maternal food intake in the control dams may have contributed to the cellular vulnerability seen in the control premature macrophage. The additional oxidative stress of ethanol exposure augmented this vulnerability. Premature newborns are often exposed to increased oxidative stress in the lung due to the need for oxygen and/or ventilator therapy. Additional studies are necessary to evaluate whether the detrimental effects of ethanol-induced oxidative stress on the developing alveolar macrophage persist throughout gestation, especially at term when the GSH system approaches maturation.

In summary, this study demonstrates a novel deleterious effect of fetal ethanol exposure on the function and viability of the developing alveolar macrophage. *In utero* ethanol impaired macrophage function in part *via* decreased GSH availability in the lung. Maternal GSH supplementation during ethanol exposure and *in vitro* GSH supplementation after ethanol exposure protected the developing macrophage from apoptosis and dysfunction. The clinical implications and significance of these findings for the premature newborn's risk of infection and infectious complications remain under investigation. We hypothesize that the need for GSH availability in alveolar macrophage functioning can be applicable to other conditions in pregnancy that increase oxidative stress for the developing fetus. These include diabetes (48,49), maternal smoking (50), pregnancy-induced hypertension (50–52), intrauterine growth retardation (53), and preterm premature rupture of membranes (54). Therefore, the potential implica-

tions of these results have a broader therapeutic potential than maternal alcohol abuse alone. Moreover, this study provides important evidence for the need to identify the alcohol-exposed premature newborn. Future studies are necessary to explore a potential role of GSH availability to augment macrophage function in premature newborns under exaggerated oxidative stress, such as those exposed to alcohol *in utero*.

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