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In utero ethanol exposure impairs defenses against experimental Group B Streptococcus in the term guinea pig lung

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Summary

Background—The effects of fetal alcohol exposure on the risks of neonatal lung injury and infection remain under investigation. The resident alveolar macrophage (AM) is the first line of immune defense against pulmonary infections. In utero ethanol (ETOH) exposure deranges the function of both premature and term guinea pig AM. We hypothesized that fetal ETOH exposure would increase the risk of pulmonary infection in vivo.

Methods—We developed a novel in vivo model of group B streptococcus (GBS) pneumonia using our established guinea pig model of fetal ETOH exposure. Timed-pregnant guinea pigs were pair fed ± ETOH and some were supplemented with the glutathione (GSH) precursor S-adenosylmethionine (SAM-e). Term pups were given GBS intratracheally while some were pre-treated with inhaled GSH prior to the experimental GBS. Neonatal Lung and whole blood were evaluated for GBS while isolated AM were evaluated using fluorescent microscopy for GBS phagocytosis.

Results—ETOH-exposed pups demonstrated increased lung infection and sepsis while AM phagocytosis of GBS was deficient compared to control. When SAM-e was added to the maternal diet containing ETOH, neonatal lung and systemic infection from GBS was attenuated and AM phagocytosis was improved. Inhaled GSH therapy prior to GBS similarly protected the ETOH-exposed pup from lung and systemic infection.

Conclusions—In utero ETOH exposure impaired the neonatal lung’s defense against experimental GBS, while maintaining GSH availability protected the ETOH-exposed lung. This study suggested that fetal alcohol exposure deranges the neonatal lung’s defense against bacterial infection, and support further investigations into the potential therapeutic role for exogenous GSH to augment neonatal AM function.

Keywords

fetal alcohol; GBS pneumonia; newborn; alveolar macrophage

Introduction

The use and abuse of alcohol prior to and during pregnancy continues to be a significant problem in our society despite education regarding its dangers, resulting in a significant proportion of both premature and term newborns exposed to alcohol in utero (Albertsen et al., 2004; Drews et al., 2003; Ebrahim and Gfroerer, 2003; Lester et al., 2001). Alcohol has recently been associated with an increased risk of extreme premature delivery at an Odds
Ratio of ~35 (Sokol et al., 2007). However, the majority of alcohol-exposed newborns do not have the recognized phenotypic changes of fetal alcohol syndrome. This results in a clinical under-identification of the alcohol-exposed newborn (Little et al., 1990; Mattson et al., 1997) and an under-estimation of the adverse outcomes in the newborn infant.

We continue to investigate the effects of fetal alcohol exposure on the newborn, with particular interest in the immune function of the developing lung. Chronic exposure to alcohol is well described to increase the risk of pneumonia in adults (Baker and Jerrells, 1993; Szabo, 1999). As a professional phagocyte, the resident immune cell in the lung, the alveolar macrophage (AM), defends the lung by initiating an immune response, participating in the phagocytosis and clearance of infectious particles, and regulating subsequent inflammatory processes within the lung (Fels and Cohn, 1986; Standiford et al., 1995). The adverse effects of chronic alcohol exposure on the adult AM and the risks of pulmonary morbidities continue to be elucidated (Brown et al., 2006; Brown et al., 2007; Joshi et al., 2006; Joshi et al., 2005; Nelson and Kolls, 2002).

However, almost nothing is known about the effects of fetal alcohol exposure on the risks of lung injury and sepsis in the newborn infant. Limited clinical evidence suggests that fetal alcohol exposure increases the risk of neonatal infection in premature (Gauthier et al., 2004) and term newborns (Gauthier et al., 2005a). In previous investigations using a guinea pig model of fetal ethanol exposure, we have demonstrated that, similar to the adult AM exposed to chronic ethanol (ETOH), the neonatal AM exposed to ETOH in utero exhibited significant dysfunction at both premature (Gauthier et al., 2005b) and term gestations (Ping et al., 2007). This AM dysfunction was modulated, in part by the availability of the antioxidant glutathione (GSH). Because of this demonstrated severe ETOH-induced dysfunction in the neonatal AM, we hypothesized that fetal ETOH exposure would increase the risk of infection in the ETOH-exposed term guinea pig lung in vivo. The purpose of this study was to examine the effects of chronic in utero ETOH exposure on the risk of infection in vivo due to experimental group B streptococcus pneumonia. Furthermore, we evaluated whether potential therapeutic interventions, including maternal supplementation with s-adenosyl-methionine (SAM-e) during ETOH exposure or inhaled GSH (iGSH) post natally to the ETOH-exposed pup would protect against experimental GBS.

**Materials and Methods**

**Guinea pig model of fetal ethanol exposure**

Our model of fetal ETOH exposure is based on models of ETOH-induced injury in the timed pregnant guinea pig (Abdollah and Brien, 1995; Kimura and Brien, 1998; Kimura et al., 1996) with modifications as previously reported by our laboratory (Gauthier et al., 2005b; Ping et al., 2007). Timed-pregnant pathogen-free guinea pigs (Elm Hill) were shipped on ~day 32 gestation (d32, term ~71 days) and randomly assigned on ~d35 to ± ETOH in the drinking water with incremental increases up to 4% ETOH (Abdollah and Brien, 1995; Kimura and Brien, 1998; Kimura et al., 1996) (25% calories + 8mg/ 100 ml saccharin) by ~d40 gestation. This experimental mixture was the only access to drinking water. Solid diet was provided ad libitum to the ETOH group, while the other groups were pair fed a solid diet to match the ETOH dam. In some experiments, the GSH precursor S-adenosylmethionine (SAM-e) (10mM Sigma, St. Louis, MO) was added to the drinking water containing the ETOH. The dam’s pregnancy was allowed to proceed until spontaneous delivery at term gestation. In our model, the blood alcohol concentration is approximately 0.05 ± 0.01% in both the ETOH or the ETOH + SAM-e dam and in her pups (Gauthier et al., 2005b). All animals were used in accordance with the NIH Guidelines (Guide for the Care and Use of Laboratory Animals) with protocols reviewed and approved by the Emory University Institutional Animal Care Committee.
Bacterial Culture

Serotype III group *B streptococcus* (*GBS*, ATCC # BAA-22) was aliquoted and stored at −70°C until experimental use. Prior to each experiment, bacteria were grown at 37°C and 5% CO₂ for 24 h in Todd-Hewitt broth and serially washed by centrifugation in 0.9% sterile normal saline at 8500 rpm for 15 min. *GBS* was suspended in normal saline at concentrations of 1×10⁷ colony forming units (CFU)/cc (Herting et al., 1998; Juul et al., 1996). The final CFU concentration of each suspension was confirmed using serial dilutions.

In vivo model of Group B Streptococcus (GBS) Pneumonia

Based on models of *GBS* pneumonia from premature and term rabbits, (Hall and Sherman, 1992; Herting et al., 1999) timed-pregnant guinea pigs were similarly exposed to the experimental diet as described above. The pregnancy was allowed to proceed until spontaneous delivery at term gestation. The pup was sedated with ketamine (20–40 mg/kg) and xylazine (2 mg/kg) intra peritoneally and the trachea surgically identified. Using a tuberculin syringe, the trachea was instilled with 1×10⁷ CFU/kg body weight *GBS* in 0.5cc volume within 72 h after delivery (Herting et al., 1998; Juul et al., 1996). The neck incision was closed with a surgical staple and the pups were allowed to recover from anesthesia for four hours.

In vivo inhaled Glutathione (iGSH)

We hypothesized that inhaled GSH would benefit the ETOH-exposed pup since the ETOH diminished GSH availability in the epithelial lining fluid of the lung, and impaired AM function in the GP model (Gauthier et al., 2005b; Ping et al., 2007). After term delivery, a subset of Control pups and ETOH-exposed pups were given intranasal GSH. Capitalizing on the natural sniffing reflex, iGSH (500 µM diluted in normal saline) was instilled in 2 divided doses (50 µl each) into each nares of the unanaesthetized pup (Ping et al., 2007) 6 hrs prior to administration of *GBS* as described above.

Alveolar Macrophage Isolation

Four hrs after intra tracheal *GBS* instillation, the pups were anesthetized with sodium pentobarbital (40 mg/kg) intra peritoneally and the trachea was re-cannulated for bronchoalveolar lavage and collection of AM. The fetal lungs were lavaged with 1.5 cc of sterile phosphate buffered saline (37°C, pH 7.4). The sample was collected and 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. The supernatant epithelial lining fluid (ELF) was saved for culture (see below). Cells in the lavage fluid were evaluated by Diffquik (Dade Behring, Newark, DE) and cell viability assessed with the calcein/ethidium iodide “live-dead” stain.

Lung Histology

The right upper lung of the term guinea pig lung was isolated and perfusion fixed in 4% paraformaldehyde and embedded in paraffin. Thin sections (5µ) were obtained and stained with haematoxylin and eosin (H&E) for light microscopic analysis of the lung at 40X magnification (1960;Herting et al., 1998).

GBS growth

To quantify *GBS* in the term pup, the fetal ELF, the blood, and lung homogenate samples were collected and cultured for bacterial growth. ELF samples were collected as described above. Whole blood was collected via sterile cardiac puncture. Fluid samples were plated in 100 µl serial dilutions onto sheep blood agar (ATCC) for 18 h of culture. Left lung tissue was homogenized and serial dilutions of the tissue slurry plated for culture. Lung tissue
protein was determined via the Bradford assay. GBS growth is presented in CFU/100 µl fluid and CFU/mg of protein of lung tissue (Herting et al., 1998).

Confocal Microscopic Analysis of Alveolar Macrophage

The in vivo phagocytosis of GBS by the guinea pig AM was evaluated as previously described using confocal fluorescent microscopy (Olympus, Melville, NY) (Ping et al., 2007). After cell isolation via bronchoalveolar lavage, the AM were fixed with 4% paraformaldehyde and non-specific binding blocked with bovine serum albumin. The cells were incubated with the primary antibody to GBS (Fitzgerald Ind, Concord, MA) and then with a FITC fluorescently tagged secondary antibody (Sigma, St. Louis, MO). Using three dimensional confocal analyses, we evaluated the cell at 50% cell depth in the z plane to localize the internal fluorescence of phagocytosed GBS (within the cell and not within the outer 10 µm plasma membrane). Background fluorescence of macrophages not stained with the FITC-labeled antibody was used to distinguish autofluorescence from engulfed bacteria (Brown et al., 2007; Ping et al., 2007). Fluorescence was determined via quantitative digital analysis via FluoView. Values for phagocytosis (defined as internalization of the GBS) are presented the mean percentage of cells with internalized fluorescence ± SEM as tallied from at least 10 experimental fields/pup.

Statistical Analyses

Sigma Stat for Windows was used for statistical calculations. Analysis of Variance (ANOVA) was used to detect overall treatment differences. Statistical differences were detected by post-hoc analysis (Holm-Sidak) and a p < 0.05 was considered significant. Values presented represent mean ± SEM. Each litter of pups was considered a separate n = 1.

Results

There was no difference in the weight of the pups after the experimental diets (Control- 107 ± 7 grams vs ETOH- 98 ± 15 grams vs ETOH + SAM-e- 99 ± 16 grams, p=NS). The pups tolerated the intra tracheal administration of GBS without significant respiratory distress during the experimental observation period. There were no deaths due to the instillation of the GBS. Four hr after the intra tracheal GBS instillation, the pups were sacrificed and the ELF and cell pellet retrieved via bronchoalveolar lavage. There were no significant differences in the retrieved cell count (p=NS) among the experimental groups at this time point (Table 1). Approximately 50% of the retrieved cells were AM while the remaining percentage were neutrophils in all the groups (p=NS) (Table 1).

In utero ETOH exposure increased lung injury in the exposed pup

Representative H&E sections of the right lung tissue 4 hrs after experimental GBS treatment are presented in Figure 1 (40X magnification). In the ETOH-exposed lung, there was an increase in cellular infiltrates and loss of airspaces compared to the Control. With the addition of maternal SAM-e during the ETOH diet, pup lung histology revealed a more normal architecture with open airspace and decreased consolidation (Figure 1). Furthermore, histology from pups exposed to in utero ETOH + iGSH prior to GBS demonstrated decreased cellular infiltrates and consolidation, suggesting that GSH availability via maternal SAM-e or exogenous GSH to the pup via iGSH attenuated the increased sensitivity of the ETOH-exposed lung to acute lung injury.
**GBS in the ELF was similar across experimental groups**

ELF obtained from bronchoalveolar lavage was plated for *GBS* on nutrient agar and incubated at 37 °C for 18 h. There was no significant difference in *GBS* in the ELF across experimental groups (Control - 233 ± 83 CFU vs ETOH- 285 ± 60 CFU vs ETOH + SAM-e- 735 ± 277 CFU, p=NS, n= at least 6 litters in each group). The addition of iGSH did not alter ELF *GBS* in either the ETOH exposed pups (ETOH + iGSH- 325 ± 140 CFU (n=6), or the Control (Control + iGSH- 60 ± 36 CFU, n=4).

**In utero ETOH exposure increased *GBS* in the neonatal lung**

After sacrifice, the left lung was homogenized and 100 µl of the tissue slurry plated for bacterial culture as described above. In Control pups, minimal *GBS* was present in the lung (Figure 2). However, the *GBS* in the lung was exponentially increased after *in utero* ETOH exposure (Figure 2 *p<0.05 vs Control). Lung *GBS* was significantly attenuated in the ETOH + SAM-e pups compared to ETOH exposure alone (Figure 2 **p<0.05 vs ETOH). The addition of iGSH to the Control pup did not significantly change the minimal *GBS* found in the lung (p= NS vs Control). However, the addition of iGSH prior to GBS administration significantly diminished *GBS* in the ETOH-exposed lung to control levels (Figure 2 **p<0.05 vs ETOH).

**In utero ETOH increased systemic sepsis after experimental *GBS* pneumonia**

After sacrifice of the pup, whole blood (100 µl) was obtained via sterile cardiac puncture and was similarly plated for bacterial culture on nutrient agar and incubated at 37 °C for 18 h. Minimal *GBS* was found in the blood of either Control or Control + iGSH pups (Control 60 ± 44 CFU/100 µl vs 100 ± 54 CFU/100 µl, p=NS). However, *GBS* in the ETOH-exposed pup was exponentially increased compared to Control pups (Figure 3, *p<0.05 vs Control). The ETOH-induced increase in *GBS* in the blood was significantly decreased in the ETOH + SAM-e pups (Figure 3, **p<0.05 vs ETOH). Furthermore, the addition of iGSH prior to *GBS* exposure also significantly attenuated the increased systemic sepsis seen in the ETOH-exposed pup (Figure 3, **p<0.05 vs ETOH).

**In utero ETOH impaired phagocytosis of *GBS* in vivo**

Bronchoalveolar lavage was performed 4h after intratracheal instillation of *GBS* and the AM obtained by centrifugation for analysis of phagocytosis. The cells were plated and fixed with 4 % paraformaldehyde. AM were identified by Diffquik staining and morpholopy. Internalized *GBS* within the isolated AM was localized via fluorescent confocal microscopy. In Control pups, internalized phagocytosis of *GBS* was present in the majority (90 ± 2 %) of the cells. The addition of iGSH to Control prior to bacterial instillation did not change internalization of *GBS*, remaining present in the majority of the cells (85 ± 4 %, p=NS). However, *in vivo* phagocytosis of *GBS* in the ETOH-exposed cells was dramatically reduced and internalized bacteria was present in only 63 ± 4 % of the AM (Figure 4, *p<0.05 vs Control). The addition of maternal SAM-e during the ETOH ingestion significantly increased phagocytosis of *GBS* in the AM, with 83 ± 1 % of the ETOH + SAM-e cells positive for internalized bacteria (Figure 4, **p<0.05 vs ETOH). Finally, ETOH pups receiving iGSH prior to *GBS* administration demonstrated increased internalization of 75 ± 3.7% compared to the ETOH alone ( **p<0.05 vs ETOH, n=6).

**Discussion**

Alcohol abuse is well described to diminish immune defenses in the adult population, particularly by impairing the function of the AM, the resident immune cell in the lung (Brown et al., 2006). ETOH exposure diminishes the adult AM’s ability to phagocytose and
clear infectious organisms (Baughman and Roselle, 1987; Greenberg et al., 1999; Nelson and Kolls, 2002; Omidvari et al., 1998; Zhang et al., 1998). Immune paralysis of the AM, with increased apoptosis, deficient phagocytosis and increased oxidant stress with depletion of the critical antioxidant GSH is characteristic of the adult AM exposed to chronic ETOH (Brown et al., 2007).

Alcohol use during pregnancy remains a significantly under-recognized problem in society, particularly when recent data suggests a staggering 35-fold increased risk of extreme premature delivery with maternal alcohol use (Sokol et al., 2007). Despite well recognized alcohol-related risks to adult immune functioning in the lung, the effects of in utero alcohol exposure on the immune defenses of the developing baby, particularly the developing AM remain under-recognized and poorly understood. In experimental animal models, in utero ETOH impairs the developing immune system resulting in cellular deficits in B cell, T cell and lymphocyte subsets which continues even when those animals are examined as juveniles or adults (Biber et al., 1998; Giberson et al., 1997; Jerrells and Weinberg, 1998; Redei et al., 1989). We hypothesized that the resident AM in the neonatal lung would be similarly impaired by in utero ETOH. Our laboratory was the first to demonstrate that in utero ETOH exposure increased oxidant stress in the developing lung, causing derangements in the function and viability of the premature (Gauthier et al., 2005b) and term AM (Ping et al., 2007). Hallmarked by decreased GSH availability in the ETOH-exposed fetal lung, impaired AM phagocytosis of inactivated staph aureus after in utero ETOH exposure was demonstrated both in vitro and in vivo. Furthermore, maternal ingestion of the GSH donor SAM-e maintained GSH in the ELF of the fetal lung and in the fetal AM, improving the function of the AM both in vitro and in vivo (Gauthier et al., 2005b; Ping et al., 2007).

The goal of the current study was to extend our investigations of in utero ETOH’s infection risk for the neonate into a novel guinea pig model of in vivo group B streptococcus pneumonia. The current study demonstrates that after in utero exposure, ETOH pups exhibited increased lung infection, increased systemic sepsis, and impaired phagocytosis of GBS by the term ETOH-exposed resident AM compared to Control pups. Because of diminished phagocytosis, AM dysfunction contributed to this increased risk of pulmonary and systemic GBS in vivo in the ETOH-exposed pup. Interestingly, as we have previously demonstrated with inactivated staph aureus in the term guinea pig (Ping et al., 2007), the ETOH-exposed AM demonstrated an inability to internalize GBS in vivo, similar to that demonstrated in the adult AM exposed to chronic ETOH (Brown et al., 2007). The increase in systemic sepsis demonstrated in the ETOH-exposed pups in this 4 hour experimental period also suggests that the ETOH-induced defects in immune response extend beyond the role of the resident AM into the epithelial barrier of the lung, as has been demonstrated in adult studies of the “alcoholic lung” (Bechara et al., 2004; Guidot et al., 2000). Furthermore, investigations into in utero ETOH’s effects on the peripheral circulating monocyte, the precursor of the alveolar macrophage, are warranted.

Maintaining GSH availability through maternal ingestion of SAM-e protected the ETOH-exposed pup from experimental GBS in both the lung and the blood, while significantly attenuating the ETOH-induced AM inability to internalize the GBS. Maternal SAM-e to Control litters did not significantly alter GBS in the blood or the lungs of the exposed pups (data not shown). The current study suggests that although exogenous iGSH did not alter GBS in the Control group, exogenous GSH via iGSH holds promise as a therapy to protect the alcoholexposed newborn. iGSH, when given to the ETOH pup prior to the GBS exposure, dramatically decreased both pulmonary and systemic GBS and improved AM phagocytosis. We chose the 500 µM GSH concentration for the iGSH for the current study since this approximates the GSH concentration observed in the normal adult ELF (Yeh et al., 2007). Aerosolized GSH has been proposed for adult disease states hallmarked by
increased oxidant stress and infectious complications such as cystic fibrosis (Day et al., 2004; Roun et al., 1999). Furthermore, in initial studies of premature newborns, inhaled GSH reduced markers of oxidant stress in the airway (Cooke and Drury, 2005). Since minimal GBS was found in the blood or lungs of the Control pups, we can not exclude the possibility that iGSH would be beneficial to Control pups exposed to a higher concentration of infectious organisms. Furthermore, it remains to be determined whether “rescue” therapy with iGSH given after the bacterial exposure would prove beneficial for the neonatal lung. The current study supports further investigations into exogenous GSH as either a protective strategy or a rescue therapy for the at-risk newborn under exaggerated oxidant stress, particularly the premature newborn exposed to alcohol in utero.

In the newborn infant, particularly the premature newborn, the baseline function of the resident AM is impaired (Bellanti and Zeligs, 1995; Hall and Sherman, 1992; Sherman et al., 1992). Defenses against organisms in the lung such as GBS are compromised due to reduced number of AM, deficient phagocytosis, decreased chemotaxis and impaired bacterial killing (Kurland et al., 1988). Indeed, pneumonia and sepsis continued to cause significant morbidity and mortality, particularly in the premature newborn, (Bizzarro et al., 2005) despite maternal screening and prophylactic antepartum antibiotic administration (Puopolo et al., 2005).

Clinical data addressing the risk of infection in the alcohol-exposed human offspring remain limited (Johnson et al., 1981; Oleson et al., 1998). Retrospective analyses suggest that fetal alcohol exposure increases the risk of infection in both the premature (Gauthier et al., 2004) and term newborns (Gauthier et al., 2005a), but larger investigations are necessary. The current study demonstrates that exposure to alcohol in utero could exacerbate the already dysfunctional neonatal AM, increasing the risk of systemic sepsis for the exposed newborn challenged to clear pulmonary bacteria.

Since the resident AM is the conductor of the inflammatory milieu within the newborn airway, it is responsible not only for the initiation of the immune response but also the appropriate termination of this response. With inflammatory states, chronic disease, infection, and lung injury, the AM population in the lung contribute to the severity of the local disease state in the adult (Calhoun and Salisbury, 1989; Hance et al., 1985; Meyer et al., 1993; Rosseau et al., 2000). The neonatal AM’s role in this regard remains unknown. Adverse outcomes of prematurity, such as chronic lung disease, have strongly been associated with an inability to terminate the pro-inflammatory environment within the developing lung (Bustani and Kotecha, 2003; De Dooy et al., 2001; Jobe and Ikegami, 1998; Jobe and Ikegami, 2001; Lyon, 2000). Therefore, future investigations evaluating the risk of lung injury and respiratory infection for the alcohol-exposed newborn are warranted to determine clinically whether the results of the current study are translated into the newborn intensive care unit.

In summary, in the current investigation, we have demonstrated that in utero ETOH exposure increased the severity of experimental GBS in the term guinea pig. Deficient AM phagocytosis contributed to an increased GBS in the lung and increased systemic sepsis in the ETOH-exposed pup. Maternal supplementation with SAM-e protected the ETOH-exposed pup from these derangements while neonatal treatment with iGSH to the ETOH-exposed pup also protected against increase lung infection and sepsis, suggesting that GSH availability to the pup modulated in vivo AM defense against experimental GBS pneumonia.

The results of this study note the vital importance for increased surveillance and identification of the alcohol-exposed neonate in order to develop a more comprehensive
understanding of alcohol’s effects on the newborn’s host immune system. Furthermore, investigations into the mechanisms of ETOH’s impact on AM functioning as well as potential clinical interventions, such as GSH replacement to augment immune function remain under investigation. Such clinical interventions to augment the immune function of the ill newborn under exaggerated oxidant stress, such as that seen with in utero alcohol exposure will advance the care of our smallest at-risk patients.

Acknowledgments

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References


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Figure 1. *In utero* ETOH increases lung injury from experimental *GBS*

Term guinea pig pups were given with $10^7$ CFU/kg body weight of *GBS* intra tracheally. Representative hematoxylin and eosin sections at 40X magnification 4hr after *GBS* instillation are presented. The ETOH-exposed lung is consolidated with loss of airspaces and increased cellular infiltrates compared to the Control lung. After maternal ingestion of ETOH + SAM-e, the pup lung demonstrates diminished cellular infiltrates and less consolidation after experimental *GBS* compared to the lung exposed to ETOH alone. With *iGSH*, given to the pup after *in utero* ETOH exposure but prior to experimental *GBS*, the lung also demonstrated less infiltrates and decreased airway consolidation.
Figure 2. GBS in the neonatal guinea pig lung
Term guinea pig pups were inoculated with $10^7$ CFU/kg body weight of GBS intra tracheally. After 4hr, lung samples were homogenized and plated (100 µl) onto sheep blood agar. CFU were determined after 18 hr culture normalized to tissue protein. Bar height represents mean ± SEM of CFU/mg protein. * p<0.05 vs Control, ** p<0.05 vs ETOH; n=at least 6 separate litters for Control, ETOH, ETOH + SAM-e, or ETOH + iGSH.
Figure 3. *GBS in the blood of the neonatal guinea pig*
Term guinea pig pups were inoculated with $10^7$ CFU/kg body weight of *GBS* intra tracheally. After 4 hr, blood samples were obtained via sterile cardiac puncture and plated (100 µl) onto sheep blood agar. CFU were determined after 18 hr culture. Bar height represents mean ± SEM of CFU/100 µl. * p<0.05 vs Control, ** p<0.05 vs ETOH; n=at least 6 separate litters for Control, ETOH, or ETOH + SAM-e or ETOH + iGSH.
Figure 4. *In vivo* phagocytosis of *GBS* by the guinea pig AM
After inoculation with $10^7$ CFU/kg body weight of *GBS* intra tracheally, cells were isolated by bronchoalveolar lavage and the location of *GBS* within the AM assessed at 50% cell depth in the z plane by fluorescent confocal microscopy. Bar height represents mean ± SEM of the percent of cells positive for internalized *GBS*. *p<0.05 vs Control, **p<0.05 vs ETOH; n=at least 3 separate litters for Control, ETOH, or ETOH + SAM-e or ETOH + iGSH.*
Table 1
Composition of bronchoalveolar lavage fluid after experimental GBS

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Cell Count (total)</th>
<th>Alveolar Macrophage (%)</th>
<th>Neutrophils (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.8 ± 2.4 million</td>
<td>61 ± 9</td>
<td>39 ± 9</td>
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<tr>
<td>ETOH</td>
<td>5.2 ± 1.2 million</td>
<td>54 ± 16</td>
<td>46 ± 16</td>
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<tr>
<td>ETOH + SAM-e</td>
<td>3.3 ± 0.3 million</td>
<td>62 ± 13</td>
<td>42 ± 16</td>
</tr>
<tr>
<td>ETOH + iGSH</td>
<td>6.6 ± 0.3 million</td>
<td>47 ± 3</td>
<td>50 ± 16</td>
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</table>