Lung Lavage of Newborns with Respiratory Distress Syndrome*

Prolonged Neutrophil Influx Is Associated with Bronchopulmonary Dysplasia

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Bronchopulmonary dysplasia (BPD) is a chronic lung disease of neonates which usually follows exposure of the premature lung to high concentrations of oxygen and mechanical ventilation delivered by endotracheal intubation as therapy for severe respiratory distress (RDS). Pulmonary inflammation is thought to play a role in oxygen toxicity* and in the development of an imbalance between elastase and elastase inhibitors within the lung,* both of which may lead to chronic lung disease in the adult. While pulmonary inflammatory elements have been associated with many adult lung conditions, the role of pulmonary inflammation in neonatal lung disease, including BPD, has not previously been studied. This prospective study was designed to explore pulmonary inflammatory processes in neonates with RDS and to determine if inflammation might play a role in the development of BPD.

MATERIALS AND METHODS

Patient Population

After receiving approval for the study from the University of New Mexico Human Subjects Committee and having obtained written, informed parental consent, bronchoalveolar lavage (BAL) was performed on 11 neonates who subsequently developed BPD, 20 neonates with RDS alone, and 10 normal newborns requiring endotracheal intubation for surgery or indications other than lung disease. The diagnosis of BPD was made prospectively when an infant enrolled in the study fulfilled all of the following criteria: 1) required mechanical ventilation for longer than 3 days, 2) had respiratory distress requiring supplemental oxygen for more than 28 days, and 3) developed chest x-ray film changes consistent with the diagnosis of BPD.

Bronchoalveolar Lavage

A standardized technique for performing lavage in the newborn was developed. The infant is placed right side down with the head turned toward the left. In this position, 1.0 ml of sterile, preservative-free saline solution is instilled endotracheally. 3 breaths are delivered through the ventilator, and deep endotracheal suctioning is performed using a 6 Fr catheter. This procedure is repeated 5 times over the space of less than 15 minutes such that a total of 5.0 ml is instilled per lavage. Three to 4 ml of lavage fluid is routinely recovered and collected in a sputum trap. Lavage total white cell counts were performed using a hemacytometer. A cytocentrifuge preparation stained using a modified Wright-Giemsa procedure was made for differential cell counts. Lavage fluids were centrifuged and cell-free supernatants were frozen at −70°C for later assays. Normal newborns were lavaged only once at ≤24 hours; RDS and BPD infants were lavaged at ≤24 hours, 48 hours, 96 hours, 1 week, and

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Figure 2. Newborn lung lavage alveolar macrophage count in human neonates. At 96 hours, the lavage alveolar macrophage count is elevated in RDS. No significant elevation in alveolar macrophage count is seen in BPD; at 4 and 5 weeks of life BPD lavage alveolar macrophage count is significantly depressed. Statistical analysis by ANOVA/Duncan evaluation. *Signifies a significant difference from normal subjects at p<.05.

then weekly through 5 weeks of life. No RDS infant remained intubated at 2 weeks of life, and as such, lavage samples were not available for RDS infants beyond 1 week. Normal newborns at <24 hours of life had lavage chemistry and cellular profiles similar to that seen in lavage of healthy children and adults; therefore, lavage variables from RDS and BPD at all times of lavage were compared to those of normals at <24 hours.

Albumin and Alpha1-proteinase Inhibitor

Albumin and alpha1-proteinase inhibitor (1Pi) were measured by nephelometry using specific antibodies, commercial reagents, and the standard technique (New Mexico Medical Reference Laboratory, Albuquerque, New Mexico).

BAL Elastase Activity

Evaluation of newborn lavage elastase activity was performed using a colorimetric assay for the enzymolysis of a substrate consisting of horseradish peroxidase bound to bovine elastin. Porcine pancreatic elastase (Sigma) standards were used. Since it takes approximately 2 mg of 1Pi to neutralize each mg of elastase, the elastase to 1Pi ratio was evaluated and expressed in these proportions.

Statistical Analysis

Evaluation of data was performed using analysis of variance followed by application of the Duncan test.

Results

A direct correlation was found between lung lavage neutrophil (PMN) count and the birth weights of the infants (r = 0.75, p<.0003). Because of this correlation, the lavage neutrophil count was divided by the birth weight to eliminate variability due solely to differences in weight. BAL neutrophil counts per kg birth weight at the various times of lavage are shown in Figure 1. In RDS, lung neutrophil influx is maximal at 48 and 96 hours and returns to normal at 1 week of life. In BPD, while lavage neutrophil influx paralleled that seen in RDS at 48 and 96 hours, lavage neutrophil counts remained elevated for the entire 5 weeks of the study.

Newborn BAL alveolar macrophage (AM) counts (Fig 2) were significantly elevated in RDS at 96 hours. No significant difference in BAL AM counts were seen in BPD until weeks 4 and 5 at which point the AM counts were significantly depressed compared to control values.

In order to determine if an imbalance between elastase and 1Pi existed in the lungs of newborns with RDS, these elements were measured in BAL fluids. Since it takes approximately 2 mg of 1Pi to neutralize each mg of elastase (ie, a 1:1 molar ratio), the elastase:1Pi ratio was evaluated in these proportions (Fig 3). In RDS, the BAL elastase:1Pi ratio did not differ from normals at any point in time. In contrast, this ratio was markedly elevated in BPD during the 1 to 4 week period. In addition, this ratio in BPD was significantly higher than the ratio in RDS at 48 hours, 96 hours, and at 1 week of life.

Discussion

Extensive characterization of inflammatory elements in many adult lung diseases has been carried out by bronchoalveolar lavage. Evaluation of inflammation in the lungs of newborns with RDS utilizing bronchoalveolar lavage had not previously been undertaken, and was the purpose of this prospective study.

Both RDS and BPD BAL inflammatory elements at <24 hours of life are similar to those seen in normal subjects despite the presence of respiratory distress syndrome. By 48 and 96 hours, BAL from both RDS and BPD show a marked influx of neutrophils with no significant difference between RDS and BPD. At 1 week of life, a time when most neonates with RDS alone are recovering, there is an associated return of the BAL neutrophil count to normal level. In contrast, neonates who subsequently develop BPD have BAL neutrophil counts that remain significantly elevated throughout the entire 5-week study period. The prolonged neutrophil influx seen in BPD may place these infants at risk for neutrophil related oxygen radical and protease lung damage.
for a minimum of 5 weeks.

Alveolar macrophages are known to produce and release anti-oxidant and anti-protease elements; AM have also been shown to phagocytize neutrophil produced elastase. Under certain conditions, the AM plays a protective role in lung inflammation by neutralizing harmful neutrophil products. BAL AM counts in RDS were significantly elevated at 96 hours. In BPD, AM counts were not elevated at any point, but were depressed at 4 and 5 weeks of life, a time when a significant neutrophil influx persisted in the lungs.

An imbalance between elastase and anti-elastase elements within the lung has been proposed as one mechanism for the development of adult chronic lung disease. Infants with RDS had no imbalance in elastase:1Pi; however, a significant and prolonged elevation in the elastase:1Pi ratio was found in the neonates who developed BPD.

The prolonged inflammatory response found in the lungs of neonates with respiratory distress may play a role in the development of neonatal chronic lung disease.

REFERENCES

Hyperoxia Injures Endothelial Cells in Culture and Causes Increased Neutrophil Adherence*

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Pulmonary vascular injury, with endothelial cell damage, edema formation and polymorphonuclear leukocyte (PMN) accumulation, is a prominent feature of the adult respiratory distress syndrome (ARDS), but the pathogenetic mechanisms causing these changes are unknown. Lung injury from hyperoxia is a good experimental model of ARDS. Lungs from animals exposed to hyperoxia show edema, reduced capillary surface area and endothelial cell abnormalities such as vacuolization, swelling, degeneration and necrosis. Furthermore, in most species, endothelial abnormalities appear before damage to other lung cell types and increase with increasing exposure to hyperoxia. These findings imply that lung endothelial cells may be more sensitive to hyperoxic injury than other lung cell types and that direct endothelial injury with capillary leak may account in part for lung dysfunction seen in animals exposed to hyperoxia. In addition to endothelial cell injury, lungs from animals exposed to hyperoxia accumulate large numbers of PMNs, often adjacent to damaged endothelial cells. Previous studies from our laboratory and others have suggested the importance of PMN in the development of ARDS and lung injury from hyperoxia, but their exact role and interactions with injured endothelium remain unclear.

Because of the importance of endothelial damage in lung injury from hyperoxia, we have proposed the hypothesis shown in Figure 1. To test this premise, we grew endothelial cells in tissue culture and evaluated their susceptibility to injury by hyperoxia. This method has the advantage of using a single cell type (endothelium) in a setting where precise evaluation of hyperoxic effects can be made.

We found that hyperoxia is directly toxic to cultured bovine pulmonary artery endothelial cells. Endothelial injury was manifested by decreased growth rate, morphologic changes, release of lactic dehydrogenase (LDH) into the culture medium and increased PMN adherence to endothelial monolayers.

MATERIALS AND METHODS

Endothelial cells were isolated from bovine pulmonary arteries by scraping. Harvested endothelial cells were placed in tissue culture and refed twice weekly with M199 (Gibco) supplemented with 20% fetal calf serum (Biocell Laboratories), 25mM HEPES (pH 7.4), penicillin (100u/ml), streptomycin (100u/ml), amphotericin B (100u/ml) (Gibco), gentamicin (40g/ml) (Schering), and thymidine (10-5 M) (Sigma). Cells were passaged by treatment with 0.05% trypsin, 0.02% EDTA (Gibco) and were identified as endothelium by morphologic characteristics and presence of factor VIII antigen.

In some experiments, freshly fed monolayers were placed in humidified dessicators which were flushed for 15 min with normoxic (80% N2-15% O2-5% CO2) gas at ambient Denver atmospheric pressure (approximately 630 mm Hg). As samples were added or removed each day, dessicators were again flushed with gas for 15 min. For examination by electron microscopy.

Figure 1. Hypothetical mechanism of lung endothelial injury from hyperoxia.