Inflammation and lung maturation from stretch injury in preterm fetal sheep

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Mechanical ventilation with large tidal volumes causes lung injury and poor pulmonary outcomes in adults (1). In preterm infants, mechanical ventilation is associated with the development of bronchopulmonary dysplasia (BPD) (44), and strategies to reduce mechanical ventilation at birth tend to decrease BPD (15, 34). The newborn infant is more susceptible to lung injury from mechanical ventilation at birth because the lung is transitioning from fluid to air filled (24). Although clinicians attempt to provide low tidal volumes in the delivery room, the tidal volumes delivered are not measured and are likely high (40, 42). Clinicians using a T-piece respirator to ventilate preterm infants (<32 wk) provided tidal volume breaths between 0 and 31 ml/kg (42). As few as six large-tidal volume breaths at birth can eliminate the response to surfactant treatment in preterm sheep (8). Since the majority of preterm infants with birth weights <1,500 g receive some ventilatory support at birth (14), understanding the mechanisms and progression of injury should suggest strategies to decrease that injury.

We have used a fetal sheep model to explore the effects of large-tidal volume ventilation at birth (19). Fifteen minutes of ventilation with escalating tidal volumes to 15 ml/kg caused lung inflammation and increased serum amyloid A3 mRNA in the liver within 3 h. The lung injury was most evident in the large and small airways (17). Changes in the smooth muscle heat shock protein 70 (HSP70) surrounding cartilaginous airways suggested repetitive distension of airways during resuscitation and recruitment of functional residual capacity. Large airways also had epithelial sloughing with localization of IL-1β mRNA in epithelial cells (17). The small airways had early growth response protein 1 (Egr-1) activation and subsequent production of proinflammatory cytokines [monocyte chemoattractant protein-1 (MCP-1) and IL-6] in the mesenchyme surrounding smaller airways (17). Since fetal sheep do not have mature alveolar macrophages (31), the proinflammatory cytokines are likely produced by parenchymal cells. In utero ventilation of very immature sheep for 6 h with lower tidal volumes created a BPD model with decreased secondary septal crests and increased smooth muscle staining (2, 38).

We previously found that fetal exposure to chorioamnionitis and inflammation induced lung maturation and maturation of monocytes to macrophages in the fetal lung (31). We hypothesized that a stretch-induced inflammatory response in the fetal lung might also induce lung maturation. We now have used the fetal ventilation model to evaluate the dynamic progression of inflammation and induction of maturation caused by a defined stretch injury. By maintaining the placental circulation and returning the fetus to the uterus after resuscitation, we report the progression of injury without the confounding effects of oxygen exposure or continued ventilation (19).

METHODS

The investigations were approved by the Animal Ethics Committees of the University of Western Australia and Cincinnati Children’s Hospital Medical Center.

Fetal ventilation procedure. Date-mated Merino ewes at 129 ± 1 days of gestational age were premedicated with ketamine (20 mg/kg im) and xylazine (0.5 mg/kg im) before induction of maternal anesthesia with inhaled isoflurane. Each ewe was mechanically ventilated in a supine position. The fetal head and chest were exteriorized through a midline hysterotomy while placental blood flow was maintained (19). The fetus was orally intubated, and airway fluid (~30 ml) was passively removed before mechanical ventilation (Babylog 8000; Dräger, Lübeck, Germany). The fetuses randomized to the high-tidal volume (VT) ventilation had VT targets of 5 ml/kg at 5 min, 10 ml/kg at 10 min, and 15 ml/kg by 15 min, with a peak inspiratory pressure limit of 55 cmH2O. An escalating VT was used to allow for...
some clearance of lung fluid and to minimize severe injury. The lambs were ventilated at 40 breaths/min, with a PEEP of 0 cmH₂O and an inspiratory time of 0.7 s using heated, humidified 100% nitrogen. Control lambs received no ventilation and a PEEP of 2 cmH₂O for 15 min. After the intervention, the fetus was returned to the uterus, and the uterus and maternal abdomen were closed. The ewe recovered from general anesthesia, and fetal tissues were collected at 1 (n = 7), 6 (n = 7), or 24 h (n = 8) after the 15-min intervention. Tissues from control lambs (n = 2 at 1 h, n = 2 at 6 h, and n = 3 at 24 h) were also collected.

Lung processing and bronchoalveolar lavage analysis. At autopsy, a deflation pressure-volume curve was measured from a gas inflation to 40 cmH₂O pressure (23). Bronchoalveolar lavage fluid (BALF) of the left lung was used for measurements of total protein (33), MCP-1 by ELISA, IL-1β by ELISA, IL-6 by ELISA, and differential cell counts from cytopsins. Tissue from the left lung was snap frozen for RNA isolation. The entire right lung was inflation fixed with 10% formalin for 1 h at 30 cmH₂O (32). The lung was then segmented into right upper lobe, nondependent right middle lobe, dependent right middle lobe, and dependent right lower lobe, fixed in 10% formalin, and paraffin embedded. The dependent region was defined as a ventral region of lung as the lamb was ventilated in a supine position. Injury scoring was done on segmental regions of lungs of 1-h animals by a blinded scorer (18).

Quantitative RT-PCR. mRNA was extracted from left lung tissue and thymus with TRIzol (Invitrogen) and treated with DNase. cDNA was produced from 1 mg of mRNA using the Verso cDNA kit (Thermoscientific). Custom TaqMan gene primers (Applied Biosystems) were designed from ovine sequences for IL-1β, IL-1 receptor agonist (IL-1ra), IL-6, MCP-1, MCP-2, Egr-1, HSP70, granulocyte/macrophage colony-stimulating factor (GM-CSF), a smooth muscle actin-γ (SMA-γ), and surfactant proteins A, B, and C. Quantitative RT-PCR was performed with 25 ng of cDNA using TaqMan Master mix in a 25-μl reaction on a 7300 RT-PCR machine and software (Applied Biosystems). 18S primers (Applied Biosystems) were used for internal loading control, and results are reported as relative increase over the mean for control animals.

Immunohistochemistry/in situ hybridization. Immunostaining protocols used paraffin sections (5 μm) of formalin-fixed tissues that were pretreated with 3% hydrogen peroxide to inactivate endogenous peroxidases (28, 29). The sections were incubated with anti-human Egr-1 (1:250 dilution; Santa Cruz Biotechnology), inducible nitric oxide synthase (iNOS) anti-mouse (1:250 dilution; BD Transduction), anti-mouse Pu.1 (1:250 dilution; Santa Cruz Biotechnology), anti-mouse α-SMA (1:10,000 dilution; Sigma), or rabbit anti-ovine MCP-1 (1:1,000 dilution; Seven Hills Bioreagents) in 4% normal goat serum overnight, followed by biotin-labeled secondary antibody. Immunostaining was visualized using a Vectastain ABC Peroxidase Elite kit to detect the antigen-antibody complexes (Vector Laboratories). The antigen detection was enhanced with nickel-diaminobenzidine followed by Tris-cobalt, and the nuclei were counterstained with nuclear fast red or eosin (Egr-1) (29).

In situ localization of mRNA was performed with digoxigenin-labeled antisense sheep riboprobes for HSP70, SMA-γ, or GM-CSF (Roche). Briefly, digoxigenin-labeled riboprobes (sense and antisense) were synthesized from cDNA templates using DIG RNA labeling kits (Roche) and diluted in hybridization buffer to a final concentration of 1 μg/ml. The sections were pretreated with 4% paraformaldehyde, treated with proteinase K, and hybridized with the probe overnight at 49–62°C, based on the GC content of probe. Sections were washed with formamide, treated with RNase A (100 μg/ml), and then blocked with 10% horse serum. After incubation overnight at 4°C with anti-digoxigenin antibody (Roche), the slides were developed with NBT-BCIP (Roche) in dark cases. The slides were monitored for color development and then stopped with Tris-EDTA buffer. Controls for specificity of riboprobe binding included use of the homologous (sense) probe. Percent staining of α-SMA and SMA-γ were quantified using Metamorph 3.5 (Universal Imaging) on random selected images.

Blood analysis. Fetal blood was collected at the end of the 15-min ventilation procedure and at delivery. Complete blood cell counts were done on delivery blood, and plasma was snap frozen. A MCP-1 ELISA was measured with anti-ovine MCP-1 antibodies on plasma and BALF (43). Plasma cortisol was extracted with ether acetate and quantified by competitive binding ELISA (Oxford Biomedical Research).

Data analysis and statistics. Results are means ± SE. Statistics were analyzed with InStat (GraphPad) using Student’s t-test, the Mann-Whitney nonparametric test, or ANOVA when appropriate. Significance was accepted as *P < 0.05.

RESULTS

All the lambs survived the fetal ventilation, return to the uterus, and the interval to delivery. There were no differences in birth weights between groups, and all ventilated animals received similar escalating Vt by the end of the 15-min procedure (Table 1). Control lambs, who received only a PEEP of 2 cmH₂O and no Vt, had an increased venous PCO₂ after the 15-min treatment interval (Table 1). This may represent some placental insufficiency due to maternal anesthesia. The ventilated lambs may have had partial correction of hypercapnia. The peak pressures needed for the Vt were the maximum value of 55 cmH₂O in many animals, and thus the values for tidal volume per kilogram at 15 min were somewhat lower than the target of 15 ml/kg. Since the entire fetal chest was exteriorized, lung expansion was limited by the low compliance of the lungs and not chest compression. The preterm fetuses did not receive antenatal steroids or surfactant treatment, and low compliance would be expected.

Lung inflammation. Proinflammatory cytokine mRNAs for IL-1β, IL-6, MCP-1, and MCP-2 were increased 1 h after the 15-min stretch injury (Fig. 1). These mRNA levels decreased by 6 h and returned to near baseline by 24 h after the high-Vt injury. Interestingly, mRNA for the anti-inflammatory IL-1ra was also induced by the 15-min stretch injury and remained

Table 1. Characteristics and ventilation parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW, kg</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>PIP</th>
<th>pH</th>
<th>PvcO₂</th>
<th>Pvo₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>7</td>
<td>3.6 ± 0.1</td>
<td>No VT</td>
<td>PEEP 2 cmH₂O</td>
<td>12.4 ± 0.4</td>
<td>53 ± 1</td>
<td>7.16 ± 0.04</td>
<td>81 ± 7</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>1 h</td>
<td>7</td>
<td>3.4 ± 0.1</td>
<td>5.0 ± 0.2</td>
<td>9.3 ± 0.6</td>
<td>13.1 ± 0.7</td>
<td>52 ± 2</td>
<td>7.25 ± 0.03</td>
<td>60 ± 4 *</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>6 h</td>
<td>7</td>
<td>3.5 ± 0.1</td>
<td>5.6 ± 0.4</td>
<td>9.4 ± 0.3</td>
<td>13.0 ± 0.5</td>
<td>52 ± 2</td>
<td>7.26 ± 0.02 *</td>
<td>60 ± 3 *</td>
<td>18 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of fetal lambs. BW, body weight; VT, tidal volume, determined per kilogram of lung tissue; PIP, peak inspiratory pressure, determined at 15 min of ventilation; PvcO₂ and Pvo₂, venous partial pressures of carbon dioxide and oxygen, respectively, determined at 15 min of ventilation. Controls had no VT and a positive end-expiratory pressure (PEEP) of 2 cmH₂O. *P < 0.05 vs. controls.
elevated at 24 h. The high-Vt ventilation caused alveolar wall thickening, less alveolar expansion, and inflammatory cell infiltrates (Fig. 2, A–C). Egr-1 mRNA increased at the 1-h time point, and prominent Egr-1 protein was localized to the cells surrounding the airways in all 1-h animals (Fig. 2E). Egr-1 protein decreased by 6 h after the stretch injury (Fig. 2F). MCP-1 protein was localized to the regions surrounding the small airways by 1 h after the stretch injury (Fig. 2G) and to the inflammatory cells by 6 h (Fig. 2I). MCP-1 protein was not identified at 24 h. BALF MCP-1 protein increased from very low levels (0.01 ± 0.01 ng/ml) in controls to 52 ± 8 ng/ml at 1 h and 37 ± 5 ng/ml at 6 h and remained high at 12 ± 5 ng/ml at 24 h after ventilation ($P < 0.05$ vs. control at all times). IL-1β and IL-6 protein in the BALF did not significantly increase at 1, 6, or 24 h.

**Lung maturation.** Inflammatory cells in the BALF increased, with initial increases in neutrophils and monocytes by 1 h and a transition to foamy macrophages by 24 h after the high-Vt injury (Table 2 and Fig. 3A). The monocyte-to-alveolar macrophage maturation was likely driven by GM-CSF, since the mRNA levels increased in lungs in the 1-h group (Fig. 3B).

GM-CSF mRNA was localized by in situ to occasional cells within the lung parenchyma in 1-h animals (data not shown). Pu.1 staining increased in the lung parenchyma over time (Fig. 3D) compared with controls (Fig. 3C), demonstrating maturation of lung inflammatory cells. Myeloperoxidase was localized to inflammatory cells and increased from 1 to 24 h, whereas iNOS was not induced by ventilation (data not shown). High-Vt ventilation also increased the mRNA for surfactant proteins A, B, and C mRNA (Table 2). Increased total protein in BALF also indicated injury at 6 and 24 h (Table 2).

**Heat shock protein and smooth muscle actin.** The chaperone protein HSP70 and smooth muscle actin also changed dynamically after ventilation injury. HSP70 mRNA was localized to bronchial epithelium of the lung in control animals (Fig. 4A). Ventilation decreased the mRNA expression in bronchial epithelium and increased the signal within the airway smooth muscle in the 1-h animals (Fig. 4B). By 6 h, the HSP70 mRNA had returned to bronchial epithelium and was qualitatively increased compared with controls (Fig. 4C). HSP70 mRNA localization was similar in the 24-h animals and control animals. HSP70 protein had a similar pattern in bronchial epitel-
Lium but was not well visualized in smooth muscle (data not shown). Similar in situ localization of HSP70 mRNA occurred in the tracheal epithelium lining, but there were no increases in tracheal smooth muscle (Fig. 4F). Tracheal sections also did not show inflammation, Egr-1, or MCP-1 protein increases compared with controls. Small increases in α-SMA in the alveolar ducts were measured at 6 h (16.9 ± 2.0% tissue staining) relative to controls (10.8 ± 0.9% tissue staining, \(P < 0.05\)), but α-SMA returned to baseline at 24 h (11.9 ± 1.9% tissue staining) (Fig. 5, A–D). SMAγ is an actin isoform only found in smooth muscle. The mRNA signal increased in the parenchyma surrounding smaller airways in the peripheral lung at 1 h (6.7 ± 0.8% staining, \(P < 0.05\)) vs. controls (3.5 ± 0.9) (Fig. 5, E and F), although no overall increase was seen on RT-PCR using lung tissue.

Table 2. Inflammation in BAL fluid and lung tissue surfactant protein mRNA

<table>
<thead>
<tr>
<th>Group</th>
<th>BAL Protein, mg/kg</th>
<th>BAL cells/kg, (\times 10^6)</th>
<th>Lung mRNA Fold Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Neutrophils</td>
<td>Monocytes</td>
</tr>
<tr>
<td>Control</td>
<td>41 ± 15</td>
<td>0.5 ± 0.5</td>
<td>0.9 ± 1.4</td>
</tr>
<tr>
<td>1 h</td>
<td>73 ± 19</td>
<td>40 ± 28*</td>
<td>10.2 ± 7.9*</td>
</tr>
<tr>
<td>6 h</td>
<td>94 ± 15*</td>
<td>35 ± 8*</td>
<td>2.6 ± 1.9†</td>
</tr>
<tr>
<td>24 h</td>
<td>80 ± 13*</td>
<td>16 ± 10*</td>
<td>3.1 ± 3.3†</td>
</tr>
</tbody>
</table>

Values are means ± SE. BAL, bronchoalveolar lavage; SP, surfactant protein; NT, not tested. *\(P < 0.05\) vs. controls. †\(P < 0.05\) vs. animals at 1 h.
staining between the lung regions 1 h after injury (Fig. 6, A–D). Inflammatory cells were seen in airways of all sections. Egr-1 protein was localized to the cells surrounding the small airways in all regions, with no differences seen (Fig. 6, E–H). MCP-1 protein was localized to airways and some inflammatory cells in all regions (Fig. 6, I–L). HSP70 mRNA was lost from bronchial epithelium and induced in smooth muscles surrounding airways in all lung regions (data not shown). Therefore, there was no regional difference in the localization of injury with the fetal ventilation.

Systemic responses to ventilation. There were no differences between the controls and 1-, 6-, or 24-h ventilated animals for the peripheral white blood cells counts and differentials. Although inflammatory cells and MCP-1 protein increased in the BALF, plasma MCP-1 levels did not change. Plasma cortisol values after 15-min ventilation were very low and not elevated in the ventilated lambs (1.6 ± 0.3 μg/dl) compared with the control animals (1.2 ± 0.2 μg/dl). There were also no changes in the thymus for mRNA for the cytokines GM-CSF, IL-1β, or IL-6.

DISCUSSION

We demonstrated the progression of lung injury in response to a brief, high-Vt ventilation in these preterm fetal lambs. We avoided oxidative stress, by using nitrogen as the gas for ventilation, and the confounding effects of continued ventilation, by maintaining placental circulation (19). A cascade of both pro- and anti-inflammatory signals was initiated by the 15-min intervention and triggered maturation pathways that were independent of plasma cortisol. This fetal model permits an evaluation of a brief period of standardized volutrauma and identifies inflammatory pathways that may contribute to development of bronchopulmonary dysplasia in premature infants.

Stretch injury to the fetal lamb leads to lung inflammation characterized by induction of multiple classes of cytokines and...
chemokines. Stretch injury causes Egr-1 activation through MAP kinase phosphorylation (36). Egr-1 signaling, as seen in these animals, can then activate a variety of pathways, including proinflammatory cytokines (37). Initiation of ventilation in preterm sheep also increases multiple early response genes, including CYR61 and CTGF (46). Similar activation of multiple early response genes occurs in adult rats ventilated with high VT (11). The simultaneous induction of both IL-1β and its endogenous regulatory molecule, IL-1ra, demonstrates the ability of the fetal lung to modulate inflammation. IL-1ra mRNA induction within 1 h could be either an initial activation by the high-Vt ventilation or an anti-inflammatory response, which continues to 24 h. The cells initially recruited to the lungs are neutrophils and monocytes, with progressive recruitment or maturation of monocytes into macrophages. There are very few alveolar macrophages in the lungs of preterm sheep, but macrophages increase rapidly after birth in sheep and other mammals (30). By 24 h after the 15-min high-Vt ventilation, the lung had increased Pu.1 staining, which is likely due to the increase in GM-CSF (7). Preterm fetal sheep exposed to intra-amniotic lipopolysaccharide (LPS) have increased lung GM-CSF and Pu.1 signaling and increased alveolar macrophages (31). Stretch injury induces many pathways that overlap with LPS-induced inflammation, and the multiple types of pathways may make selective blockade of inflammatory pathways difficult. We have attempted to decrease the inflammatory response to stretch injury with postnatal steroids (dexamethasone, cortisol) and inhibitors of NF-κB, IL-1, and IL-8 without success (16, 20). Antenatal betamethasone decreased, but did not eliminate, the inflammatory process (20).

The increase in surfactant protein mRNA synthesis could be a response to 1) a stretch injury to type II cells, 2) the proinflammatory cytokines or GM-CSF released in response to stretch, or 3) the inflammatory cells recruited to the lung. When stimulated by stretch in vitro, fetal rat type II cells increase surfactant protein mRNA by activation of the MAP kinases ERK1/2 (41). Activation of these kinases also induces Egr-1 (36). The 15-min stretch injury also leads to increased GM-CSF mRNA production within 1 h. GM-CSF receptors are present on the type II cells (9), and GM-CSF modulates surfactant homeostasis (13), suggesting the production of GM-CSF could lead to maturation. GM-CSF, when given to pregnant rats, resulted in fetal lung maturation and alveolar septal thinning similar to antenatal betamethasone (4). GM-CSF is also increased in the BALF of very low birth weight infants who develop BPD compared with infants without BPD (5). The induction of the surfactant proteins could result from the inflammatory cells recruited to the lung. Fetal sheep exposed to intra-amniotic LPS require an inflammatory cell influx into the lungs for increases in surfactant protein mRNA (27). The increases in surfactant protein mRNA measured 24 h after fetal resuscitation were similar to the increases that occurred 24 h after exposure to intra-amniotic LPS (3). Similar to LPS-induced lung maturation (26), the maturational effects of the brief fetal ventilation injury were not mediated through increased plasma cortisol.

The epithelium of the fetal airway modulates the acute response protein HSP70 in response to a stretch injury. HSP70 is an endogenous ligand for Toll-like receptor 4 and can trigger an inflammatory response (47). We reported a loss of HSP70 protein and mRNA from the bronchial epithelium and an
induction of mRNA in the bronchial smooth muscle with 3 h of ventilation (19). Although HSP70 is released into airways with stretch (10, 17), the bronchial and tracheal airway epithelium responded with increased mRNA expression at 6 h. A similar airway response has been seen in rats ventilated with high VT (11). Chong et al. (10) demonstrated that minimal distention of isolated sheep tracheas leads to appearance of HSP70 into lung fluid, suggesting that our control animals, receiving a distending pressure of 2 cmH2O, may have had some HSP70 release. We choose to use a distending pressure for 15 min because the fetal lung is normally distended by lung fluid to a pressure of 2–3 cmH2O (35). Other investigators have demonstrated the ability of fetal airway epithelium to recover from injury. In utero ventilation of very immature sheep for 12 h increased mucin-producing cells in the airways, and these changes had resolved by 7 days (38). Airway injury and epithelial disruption also seen in these animals at 6 and 12 h after ventilation had resolved by 7 days (38). Prolonged ventilation (6–12 h) of very immature lambs, with a lower VT (4–6 ml/kg) than used in this study, increased staining of smooth muscle actin within the lungs (2). We have demonstrated subtle changes in smooth muscle actin in these animals, although the percent staining was lower than that in younger control lambs (11%) (2). The changes in the airway bronchial epithelium and smooth muscle demonstrate an airway response to stretch injury in the fetal lamb. This progression of injury, and possible repair, demonstrates how ventilation initiates prolonged responses in the preterm.

Evaluation of the lung regions did not demonstrate differential injury between the nondependent and dependent regions of the lung. We expected to find increased markers of injury in the nondependent regions, where higher local VT might have caused more injury. With prolonged ventilation after high-VT resuscitation, we often find larger areas of hemorrhage and plural blebs along the nondependent regions of the preterm lamb lung (unpublished observations). The upper pole of the sheep lung develops surfactant earlier than lower regions of the lungs, as do human lungs (25). Rabbit pups also demonstrate different regions of airspace recruitment during initiation of ventilation at birth, with upper lobes opening earlier than the remainder of the lung (21). Ventilation of saline-lavaged rabbits demonstrated differences in the type of cytokine activated between dependent and nondependent regions of the lungs, with IL-8 increased in dependent lung and MCP-1 increased in nondependent lung (39). The damage caused by the ventilation of fluid-filled, surfactant-deficient lungs with large VT may have reached a threshold for inflammation in all regions and masked the potential to detect regional differences. Because protein staining for Egr-1 and MCP-1 did not differ between...
regions for the 1 h group, no evaluation was done on later time points.

A limitation of our study, as with many large animal studies, is the inability to prove causality between the associations of airway stretch and maturation of the fetal lung. Since the first time point in this study is 1 h after completion of ventilation, the initial signaling that increased cytokine and chemokine production was not evaluated. In vitro models of both airway epithelial and endothelial cell lines demonstrate that stretch leads to production of IL-8 (22, 45). Multiple pathways are activated by stretch, with Egr-1 and IL-6 likely activated through MAP kinases and HSP70 and IL-1β being activated through NF-κB (12). Future studies examining earlier time points after stretch injury may lead to a better understanding of molecular pathways that can result in injury of the preterm lung at birth.

The initiation of ventilation with large tidal volume caused lung inflammation within 1 h and maturation of inflammatory cells within 24 h. This brief stretch injury to the fetal sheep lung also causes changes in the airway epithelium and increases in surfactant proteins. These maturational changes occur in absence of an increase in plasma cortisol and are likely due to the local production of chemokines, such as GM-CSF, or direct effects on type II cells. Premature infants exposed to ventilation at birth have an increased risk of developing bronchopulmonary dysplasia. Our current study demonstrates the ability of isolated stretch to activate both maturational chemokines and pro- and anti-inflammatory cytokines. The study stresses the complexity of pathways activated by ventilation and the need to avoid excessive ventilation of extremely premature infants at birth.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
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