Brief, Large Tidal Volume Ventilation Initiates Lung Injury and a Systemic Response in Fetal Sheep

Noah H. Hillman¹, Timothy J. M. Moss², Suhas G. Kallapur¹, Cindy Bachurski¹, J. Jane Pillow², Graeme R. Polglase², Ilias Nitsos², Boris W. Kramer³, and Alan H. Jobe¹

¹Division of Pulmonary Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio; ²School of Women's and Infants' Health, The University of Western Australia, Perth, Australia; and ³Department of Pediatrics/Neonatology, Academisch Ziekenhuis Maastricht, Maastricht, The Netherlands

Rationale: Premature infants are exposed to potentially injurious ventilation in the delivery room. Assessments of lung injury are confounded by effects of subsequent ventilatory support.

Objectives: To evaluate the injury response to a brief period of large tidal volume (V_T) ventilation, simulating neonatal resuscitation in preterm neonates.

Methods: Preterm lambs (129 d gestation; term is 150 d) were ventilated (V_T = 15 ml/kg, no positive end-expiratory pressure) for 15 minutes to simulate delivery room resuscitation, either with the placental circulation intact (fetal resuscitation [FR]) or after delivery (neonatal resuscitation [NR]). After the initial 15 minutes, lambs received surfactant and were maintained with either ventilatory support (FR-VS and NR-VS) or placental support (FR-PS) for 2 hours, 45 minutes. A control group received no resuscitation and was maintained with placental support. Samples of bronchoalveolar lavage fluid, lung, and liver were analyzed.

Measurements and Main Results: Inflammatory cells and protein in bronchoalveolar lavage fluid, heat shock protein-70 immunostaining, IL-1 β , IL-6, IL-8, monocyte chemoattractant protein-1, serum amyloid A (SAA)-3, Toll-like receptor (TLR)-2, and TLR4 mRNA in the lungs were increased in the FR-PS group compared with control animals. There were further elevations in neutrophils, IL-6, and IL-8 mRNA in the FR-VS and NR-VS groups compared with FR-PS. SAA3, TLR2, and TLR4 mRNA increased in the liver in all resuscitation groups relative to control animals.

Conclusions: Ventilation for 15 minutes with a V_T of 15 ml/kg initiates an injurious process in the preterm lung and a hepatic acute-phase response. Subsequent ventilatory support causes further increases in some injury indicators.

Keywords: resuscitation; premature; bronchopulmonary dysplasia; positive end-expiratory pressure; volutrauma

Interventions during the birth transition from fetus to newborn may affect long-term outcomes, particularly for the preterm infant. During resuscitation and stabilization, a depressed infant is exposed to a variety of potentially harmful interventions, such as oxygen, barotrauma or volutrauma, and poor temperature control. Acute injury of the adult lung results from mechanical

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

The preterm lung is easily injured by high V_T, which may occur during neonatal resuscitation. Assessment of the injury is confounded by the need to continue ventilatory support after preterm delivery.

What This Study Adds to the Field

A brief period of high V_T ventilation injures the fetal lung and postdelivery ventilation selectively amplifies the indicators of injury. The initial brief ventilation also initiates a systemic response in the preterm lamb.

ventilation-induced volutrauma and biotrauma (1). In the preterm infant, large tidal volumes (V_Ts) can cause lung injury and may contribute to the progression of acute lung injury to bronchopulmonary dysplasia (2). Large V_T ventilation followed by routine ventilatory care causes epithelial damage, which allows proteins to leak into the airspaces and to inhibit surfactant function (3–5). Excessive stretch of the preterm lung also induces proinflammatory cytokines and an influx of inflammatory cells into the airspaces (6, 7). Although the damage may occur with resuscitation at birth, indicators of lung and systemic injury take hours to develop. In contrast to the adult lung (8), there is very little information about how a preterm newborn lung develops an injury response to high V_T ventilation.

Current guidelines for resuscitation of the preterm infant have no sound experimental basis and have not been tested in randomized clinical trials (9). In the clinical environment, it is not feasible to measure the effects of the elements of the ventilation maneuvers, such as V_T, positive end-expiratory pressure (PEEP), and inspiratory time, on the newborn lung. The elements of the ventilation maneuvers are used simultaneously to stabilize the infant. Because the preterm lung is so easily injured (10), the continued ventilatory support that follows the initial ventilatory stabilization may further increase the injury. We describe a fetal sheep model for the evaluation of the elements of the initiation of ventilation separately from the need for continued ventilatory support. By maintaining placental support after the initial ventilation maneuver, indicators of injury can be evaluated without the need for sustained ventilation. By comparing the fetal intervention with similar interventions in newborn lambs, we have characterized the initial injury and measured the effects of continued ventilation on that initial injury.

METHODS

The online supplement includes additional details on methods used.

The animal studies were performed in Western Australia; the Cincinnati Children's Hospital and the Western Australia Department

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Correspondence and requests for reprints should be addressed to Alan Jobe, M.D., Ph.D., Cincinnati Children's Hospital Medical Center, Division of Pulmonary Biology, 3333 Burnet Avenue, Cincinnati, OH 45229-3039. E-mail: alan.jobe@cchmc.org

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of Agriculture approved the animal use procedures. Time-mated Merino ewes with singletons or twins at 129 days gestation (term, 150 d) were randomized into four groups (Table 1).

Fetal Resuscitation and Placental Support

After maternal hysterotomy, the head and chest of fetus were exteriorized, the fetus was intubated, and lung fluid removed. The fetus was ventilated (rate, 30 breaths/min; inspiratory time, 1 s) with heated and humidified air using escalating V_T and no PEEP, while the placental circulation remained intact. The target V_T was 15 ml/kg at 15 minutes. The fetus was treated with 100 mg/kg surfactant (Survanta; Ross Laboratories, Columbus, OH) and was returned to the uterus. The lamb was delivered for tissue collection 2 hours and 45 minutes after the end of the ventilation period.

Fetal Resuscitation and Ventilatory Support

The fetal resuscitation and ventilatory support (FR-VS) group was used to assess the effects of continued ventilation on the resuscitation injury to the fetal lung. Fetuses received the same resuscitation as the fetal resuscitation and placental support (FR-PS) group, but after the initial 15-minute ventilatory period they were treated with surfactant, delivered, and ventilated (40 breaths/min; PEEP, 5 cm H_2O ; inspiratory time, 0.7 s) with a heated and humidified oxygen and air mixture for 2 hours, 45 minutes (11). The ventilation support period used V_T s similar to those achieved by preterm lambs spontaneously breathing on continuous positive airway pressure (CPAP) (12). The peak inspiratory pressure only was adjusted to target a P_{aCO_2} of 50 mm Hg. The F_{IO_2} was adjusted to minimize oxygen exposure. Tissues were collected from FR-VS lambs 2 hours, 45 minutes, after the end of the initial ventilation period.

Neonatal Resuscitation and Subsequent Ventilatory Support

The neonatal resuscitation and subsequent ventilatory support (NR-VS) lambs were used to evaluate if the fetal resuscitation differed from resuscitation in the newborn. Lambs were delivered, intubated, and lung fluid was removed. Newborn lambs were resuscitated for 15 minutes, as with the FR-PS and FR-VS groups, treated with surfactant, and then ventilated similarly to the FR-VS group. Tissues were collected 2 hours, 45 minutes, after the end of the initial resuscitation period.

Control Animals

Nonmanipulated twins of lambs of the FR-PS or FR-VS groups had tissue collected after delivery of the twin.

Lung Processing and Bronchoalveolar Lavage Fluid Analysis

The lungs were weighed, and bronchoalveolar lavage fluid (BALF) of the left lung was used for cell counts and protein measurements (13, 14). Tissues from the right lung and liver were snap frozen for RNA analysis. The right upper lobe of the lung was inflation fixed with 10% formalin (15). Heat shock protein (HSP)-70 was detected by immunohistochemistry. Total RNA isolated from frozen lung and liver was used

for RNase protection assays using sheep-specific riboprobes for IL-1 β , IL-6, IL-8, monocyte chemotactic protein (MCP)-1, Toll-like receptor (TLR)-2, TLR4, serum amyloid A (SAA)-3, and the RNA for ribosomal protein L32 (16–18).

Statistics

All values are expressed as means $\pm$ SE, and comparisons between intervention groups were made with two-tailed Mann-Whitney non-parametric tests or Welch's *t* tests. Significance was accepted at $P < 0.05$.

RESULTS

Description of Lambs

The lambs' body weight at delivery was not different between groups (Table 2). The V_T s achieved at 5 and 10 minutes into the initial ventilation period were similar for the three ventilated groups. The 15-ml/kg target was achieved for the FR-PS and FR-VS groups but was somewhat lower for the NR-VS group. The pressures required to achieve those V_T s were close to 50 cm H_2O , and the three groups had similarly low compliances at 15 minutes, indicative of lung immaturity.

The cord blood of the FR-VS lambs had a pH of 7.26 ± 0.01 , a P_{aCO_2} of 60 ± 1 mm Hg, and a P_{aO_2} of 22 ± 1 mm Hg at the end of the 15-minute V_T intervention, which indicates the lambs had not been oxygenated or hyperventilated as a result of the fetal ventilation. The arterial blood gas values for the NR-VS group at the end of the initial 15-minute ventilation period were pH 7.36 ± 0.03 , P_{aCO_2} 42 ± 3 and P_{aO_2} 35 ± 4 mm Hg. The cord blood gas values at delivery for the FR-PS group were pH 7.17 ± 0.03 , P_{aCO_2} 73 ± 4 mm Hg, and P_{aO_2} 6 ± 1 mm Hg. The respiratory acidosis resulted from anesthesia and surgery before delivery. The ventilated lambs had similar V_T at 3 hours (Table 3), but the FR-VS group required lower peak inspiratory pressures to achieve a higher compliance and had better gas exchange than the NR-VS group. Lung function at a V_T of 15 ml/kg was better in FR-PS and FR-VS groups than in the NR-VS group. The ventilatory pressures and blood gas values for the 2 hours and 45 minutes of ventilation are presented and discussed in the online supplement.

Protein and Inflammatory Cells in BALF

An increase in protein in BALF is a global indicator of lung injury. Total protein in the BALF was fivefold higher in the FR-PS group than in the control group (Figure 1A), and was 11- and 14-fold higher than the control group in the FR-VS and NR-VS groups, respectively. Inflammatory cells in BALF also

TABLE 1. DESCRIPTION OF STUDY GROUPS

Groups	Target for 15-min Intervention	Post-Ventilation Period (2 h, 45 min)
FR-PS	Fetal: V_T = 15 ml/kg	Surfactant before return to uterus
FR-VS	Fetal: V_T = 15 ml/kg	Surfactant treatment Lamb delivered Newborn ventilated
NR-VS	Newborn: V_T = 15 ml/kg	Surfactant treatment Newborn ventilated
Control	Fetal: anesthesia with twin No ventilation	Remained <i>in utero</i> after anesthesia

Definition of abbreviations: FR-PS = fetal resuscitation with placental support; FR-VS = fetal resuscitation with ventilatory support; NR-VS = newborn resuscitation with ventilatory support.

TABLE 2. PHYSIOLOGIC AND VENTILATION VARIABLES DURING INITIAL 15-MINUTE VENTILATION PERIOD

	Surgical Controls	FR-PS	FR-VS	NR-VS
n	8	6	7	7
Body weight, kg	2.7 ± 0.2	2.6 ± 0.2	3.0 ± 0.3	2.8 ± 0.1
V_T , ml/kg				
At 5 min	—	9.5 ± 1.6	8.5 ± 1.9	8.1 ± 1.4
At 10 min	—	13.0 ± 1.6	11.4 ± 1.8	9.8 ± 1.0
At 15 min	—	15.0 ± 2.4	14.9 ± 2.8	12.3 ± 1.1
PIP at 15 min, cm H_2O	—	49 ± 0.9	50 ± 1.0	48 ± 2.0
Compliance, ml/cm H_2O /kg	—	0.30 ± 0.05	0.31 ± 0.06	0.28 ± 0.07

Definition of abbreviations: FR-PS = fetal resuscitation with placental support; FR-VS = fetal resuscitation with ventilatory support; NR-VS = newborn resuscitation with ventilatory support; PIP = peak inspiratory pressure.

TABLE 3. PHYSIOLOGIC AND VENTILATION VARIABLES

Values after 2 h, 45 min, of Ventilation	FR-VS	NR-VS
FiO ₂	40 ± 0.9	41 ± 1.4
V _T , ml/kg	8.5 ± 1.4	6.6 ± 0.2
PIP, cm H ₂ O	29.3 ± 2.0	36.4 ± 1.6*
Compliance, ml/cm H ₂ O/kg	0.30 ± 0.07	0.18 ± 0.01*
pH	7.22 ± 0.03	7.00 ± 0.07*
PaCO ₂ , mm Hg	57.7 ± 4.9	70.7 ± 7.9
PaO ₂ , mm Hg	30.5 ± 3.4	22.4 ± 5.0

Definition of abbreviations: FR-VS = fetal resuscitation with ventilatory support; NR-VS = newborn resuscitation with ventilatory support; PIP = peak inspiratory pressure.

Data are mean ± SEM.

* $P < 0.05$ NR-VS versus FR-VS.

were greatly increased in FR-PS, FR-VS, and NR-VS groups (Figures 1B and 1C). BALF from control animals contained very few neutrophils. Neutrophil counts were 300-fold higher in the FR-PS group compared with the control group, and were elevated further in the FR-VS and NR-VS groups. Monocyte numbers in BALF were higher in the FR-PS, FR-VS, and NR-VS groups compared with the control group (Figure 1C). In contrast to neutrophil counts, monocyte counts were not higher after ventilatory support than after placental support.

Figure 1. Total protein and cell count. (A) Total protein increased in bronchoalveolar lavage fluid in the fetal intervention group (fetal resuscitation and placental support [FR-PS]) and the newborn ventilation groups (fetal resuscitation and ventilatory support [FR-VS] and neonatal resuscitation and subsequent ventilatory support [NR-VS]) compared with control animals. (B) Neutrophils increased in the FR-PS group, and increased further in the FR-VS and NR-VS groups. Cell count is expressed as cells/kg on a log scale. (C) Monocytes increased similarly for the FR-PS and the newborn ventilation groups. * $P < 0.01$ versus controls; [†] $P < 0.05$ versus FR-PS group.

Cytokines and Chemokines in the Lungs

IL-1 β mRNA increased 30-fold relative to control animals in the FR-PS group (Figure 2A) and was elevated further in the FR-VS and NR-VS groups, but IL-1 β mRNA was not significantly higher in FR-VS and NR-VS groups than in the FR-PS group. A similar pattern of mRNA response was seen for MCP-1, although MCP-1 mRNA was significantly higher in the NR-VS group (115-fold higher than in the control group) compared with the FR-PS group (30-fold higher than the control group; Figure 2B). In contrast to IL-1 β and MCP-1, IL-6 and IL-8 mRNA was induced only about threefold in the FR-PS group relative to the control group but was 20- to 100-fold higher in the FR-VS and NR-VS groups compared with the control group (Figures 2C and 2D).

Acute-Phase Responses in Lung

HSP-70 was higher in the FR-PS group than in the control group, and was further elevated in the lung parenchyma of the NR-VS group (Figure 3). Messenger RNA for SAA is an acute-phase reactant that is expressed in the fetal sheep lung (19). SAA mRNA increased in the lung after the resuscitation maneuver in all groups (Figure 4). The mRNA for TLR4 and TLR2 also was higher in FR-PS, FR-VS, and NR-VS groups compared with the control group (Figures 5A and 5B), and was higher in FR-VS and NR-VS groups compared with the FR-PS group.

Expression of SAA and TLR mRNA in Liver

The inducible form of liver SAA was fivefold higher in the FR-PS, FR-VS, and NR-VS groups than in the control group (Figure 4B). TLR4 and TLR2 mRNA in the liver was higher than in the control group in all resuscitation groups (Figures 5C and 5D). TLR4 mRNA was higher in the FR-VS and NR-VS groups than in the FR-PS group.

DISCUSSION

These translational experiments were targeted at the poorly studied clinical problem of how to optimize initiation of ventilatory support for the preterm infant. The International Guidelines for Neonatal Resuscitation (ILCOR) give essentially no guidance for initial ventilation of the preterm infant. The guidelines simply state that ventilation should be sufficient to achieve a clinical response and that CPAP may be helpful (9). Preterm lungs are easily injured, because the lungs are fluid-filled, an FRC needs to be established, initial inflation is nonuniform and may require long and variable time constants to achieve inflation, and the total lung capacity is low relative to the adult lung (10). Surfactant deficiency compromises the establishment of an FRC and results in the need for higher ventilatory pressures (20). Mechanical ventilation is also complicated by the following: masks or tracheal tubes that frequently leak (21); no practical way to measure V_Ts; and a high level of anxiety of the resuscitation team, which frequently results in hyperventilation of the preterm infant (22). In one report, skilled clinicians, instructed to follow ILCOR guidelines for resuscitation, used mean V_Ts of 18 ml/kg and pressures of approximately 40 cm H₂O to ventilate preterm lambs (23).

Large V_Ts injure the preterm lung, as demonstrated experimentally using preterm sheep and rabbits (3, 5, 24), but the mechanisms causing the injury have not been ascertained to the same degree as in the adult lung. Uhlig (25) described the types of injuries caused by lung stretch, such as necrosis, stress failure of epithelial and endothelial barriers, overdistension without tissue injury, and effects on the vasculature. These injuries can

Figure 2. Cytokine mRNA in lung tissue. (A) IL-1 β mRNA increased with fetal intervention (fetal resuscitation and placental support [FR-PS]) and newborn ventilation groups (fetal resuscitation and ventilatory support [FR-VS] and neonatal resuscitation and subsequent ventilatory support [NR-VS]). (B) Monocyte chemotactic protein (MCP)-1 mRNA increased in all groups relative to control, with a further increase with ventilation in the NR-VS group. (C) IL-6 and (D) IL-8 increased with the fetal V_T intervention in the FR-PS group and increased more with additional ventilation in the FR-VS and NR-VS groups. Cytokine mRNA was initially normalized to L32 mRNA (loading control). All values are reported as fold increase compared with control animals, normalized to 1. * $P < 0.05$ versus fetal controls; † $P < 0.05$ versus FR-PS group; ** $P < 0.05$ versus all other groups.

result in biotrauma (cytokine release, inflammatory cell recruitment) and pulmonary edema over several hours in the adult lung (26–28). Large V_T s delivered to the preterm lung without PEEP cause structural injury to the airspaces and epithelial leaks that result in interference with surfactant function (5). Proinflammatory cytokine mRNA and inflammatory cells appear in the lung if ventilation without PEEP continues for 2 hours (7). These effects are similar to those observed in adult lungs, but it is risky to assume that the injury responses of the preterm lung will parallel those in the adult lung. For example, the sentinel cell for many types of injury in the adult lung is the alveolar macrophage (29). The preterm lung and airspaces contain a few immature monocytes and essentially no mature macrophages (30). Also, the preterm sheep lung does not respond to tumor necrosis factor- α (31), the primary early-response cytokine in adult mice and rabbits (32, 33). A significant experimental problem is that the preterm lung is easily injured even with gentle ventilation (2); this effect can be minimized with surfactant treatment (5).

We developed this model of fetal resuscitation followed by recovery *in utero* to separate the injury caused by the initiation of ventilation from subsequent mechanical ventilation required to support the preterm animal. The recovery period of 2 hours, 45 minutes, *in utero* was enough time for markers of lung injury to increase. The initial resuscitation maneuver was compared between groups that received the same maneuver, either as fetuses or newborns, and then were supported by the placenta or mechanical ventilation for the same period of time, to assess the effects of postnatal ventilation on the initial resuscitation maneuver. The maneuver was intended to be sufficient to achieve lung injury. In previous experiments by our group, preterm fetal lambs ventilated with V_T s of about 7 ml/kg for 15 minutes followed by a period of placental support did not demonstrate much injury (34). Although we used higher pressures in these experiments and targeted a V_T of 15 ml/kg by 15 minutes, the volumes were less and the pressures were similar to injury models in adult and young animals (1, 35, 36). The fetuses

Figure 3. Heat shock protein (HSP)-70 immunostaining in lung. (A) Control animals showed no staining in the lung parenchyma. (B) Fetal resuscitation with placental support (FR-PS) had increased HSP-70 staining of the airways. (C) The neonatal resuscitation maneuver after delivery followed by ventilatory support (NR-VS) resulted in increased HSP-70 staining in the airways and lung parenchyma. (D) HSP-70 staining intensity increased when graded using blinded criteria in the FR-PS and NR-VS groups. * $P < 0.05$ versus fetal controls.

Figure 4. Serum amyloid A (SAA) mRNA in lung and liver. (A) The mRNA for SAA increased in the lungs of the fetal resuscitation and placental support group (FR-PS) and similarly in the fetal resuscitation and ventilatory support group (FR-VS). The neonatal resuscitation and ventilatory support (NR-VS) groups had more expression of SAA than did the FR-PS group. (B) Liver SAA mRNA from all groups increased relative to control animals. All values are reported as fold increases compared with control animals, normalized to 1. * $P < 0.05$ versus fetal controls; [†] $P < 0.05$ versus FR-PS group.

tolerated the 15 minutes of partial exteriorization well. In pilot experiments, we found other fetuses to be healthy at delivery at 24 hours after the fetal ventilation procedure, suggesting that the model may also be good for studying the evolution and resolution of lung injury in the preterm over extended time periods.

A target V_T of 15 ml/kg is high relative to the 4 to 6 ml/kg used to ventilate the preterm human. Preterm lambs differ from preterm infants because the preterm lambs breathe spontaneously on CPAP with a V_T of about 8 ml/kg, and a V_T in this range is needed to normalize Pa_{CO_2} values in mechanically ventilated preterm lambs (12). Therefore, the injurious V_T was only twice the optimal V_T for preterm sheep. A doubling of the normal V_T would cause minimal injury in the healthy adult lung or in a postnatal rodent with incomplete alveolarization (35). A V_T as large as 50 ml/kg for 4 hours did not cause lung injury in healthy pigs (37).

Ventilation of the preterm lungs with a V_T of 15 ml/kg and no PEEP caused a large recruitment of monocytes and neutrophils, and an increase in total protein, in the lungs. Expression of the IL-1 β and the chemokine MCP-1 was strikingly increased in the lung. Therefore, the initial resuscitation period caused some responses typical of biotrauma. However, expression of IL-6 and IL-8 was only modestly increased relative to that caused by the resuscitation maneuver followed by mechanical ventilation. This result suggests that most of the epithelial leak and inflammatory cell recruitment occurs by signaling downstream from IL-1, MCP-1, or other unmeasured mediators. IL-6 and IL-8 may not be critical to this initial injury response. This result is in contrast to potent effects of inhibition of IL-8 signaling in hyperoxic injury in rats (29, 38).

The confounding effects of continued ventilation are demonstrated by the further increase in markers of lung inflammation, even after surfactant treatment and normal V_T ventilation. The increase in some injury mediators and markers in the FR-VS group relative to the FR-PS group demonstrates the additive effect of subsequent ventilation to resuscitation injury of the preterm lung. The differences in compliance and some cytokines between the FR-VS and NR-VS groups indicate increased injury and suggest that routine clinical management has the potential to be very injurious. The difference between the NR-VS and FR-VS groups, with respect to decreased arterial pH, decreased lung compliance, and increases in IL-6 and IL-8 mRNA after 2 hours and 45 minutes of ventilation, suggests a role of the fetal circulation or placental blood flow in modulating the initial injury. The fetal resuscitation maneuver did not cause increased oxygenation of the cord arterial blood, indicating that pulmonary blood flow remained low. In contrast, oxygenation increased and Pa_{CO_2} decreased with the neonatal ventilation maneuver, indicating increased pulmonary blood flow. This increased blood flow may have contributed to the increased injury.

The recruitment of neutrophils to the BALF was rapid in these experiments. Neutrophils are the major mediators of the acute inflammatory process in most models of acute lung injury (39). Depletion of systemic neutrophils decreased ventilation- or endotoxin-induced lung injury in the adult lung (40, 41). Similarly, depletion of neutrophils decreased lung vascular injury and edema in preterm lambs (42). Ultimately, it will be important to learn how stretch signals neutrophil recruitment into the fetal lung. In the adult mouse lung, stretch and endotoxin have distinct signaling pathways that each result in neutrophil recruitment (43). The neutrophil sequestration caused by ventilation-induced lung injury is L-selectin dependent but CD-18 independent (44). In contrast, inhibition of intraamniotic endotoxin-mediated

Figure 5. Toll-like receptor (TLR)-2 and 4 (TLR4) mRNA induction in lung and liver. (A) TLR4 mRNA increased in lung for the fetal resuscitation and placental support (FR-PS), and increased more for the fetal resuscitation followed by ventilation (FR-VS) and for the newborn resuscitation followed by ventilation (NR-VS) groups. (B) TLR2 mRNA also increased in lung after the fetal resuscitation with further increases with continued ventilation. (C) TLR4 and (D) TLR2 mRNA increased in liver in response to the high V_T ventilation and TLR4 mRNA increased further with mechanical ventilation. All values are reported as fold increases compared with control animals, normalized to 1. * $P < 0.05$ versus fetal controls; [†] $P < 0.05$ versus FR-PS group.

neutrophil recruitment to the lungs of fetal sheep with an anti-CD-18 antibody prevents fetal lung inflammation and injury, whereas IL-1-mediated neutrophil recruitment to the fetal sheep lung is CD-18 independent (45). Further research may identify a specific target to minimize neutrophil recruitment caused by stretch to the preterm lung. Together with neutrophil influx, similar increases in BALF monocytes were seen in all groups given the initial resuscitation maneuver. The similar monocyte influx, despite the large increase in MCP-1 in the NR-VS group, suggests that these monocytes are from the lung tissue and not enough time has elapsed for recruitment of systemic monocytes to the airspace.

A provocative new result is the induction of mRNA for the acute-phase reactants serum amyloid and TLR2 and TLR4 receptors in both the lung and liver. We previously reported that 6 hours of mechanical ventilation increased SAA expression in the preterm and term lung and liver (16). Intratracheal endotoxin also induced serum amyloid to very high levels in the lung and liver of preterm lambs (16). In this experiment, just 15 minutes of high V_T ventilation increased lung and liver serum amyloid expression, and subsequent ventilation did not further increase the response, indicating there is an immediate systemic response to perinatal lung injury. We recently cloned and characterized TLR2 and TLR4 expression in fetal sheep (46). Intraamniotic endotoxin induced TLR2 and TLR4 mRNA in the fetal lung. We now show that the fetal ventilation maneuver also induces these two TLRs in both the lung and liver. Although the TLR signaling pathway is complex, up-regulation of TLR mRNA could result in an increased inflammatory response.

Mechanical ventilation that is not normally injurious can amplify mild lung injury caused by endotoxin in sepsis models (47, 48). Ventilation-mediated lung injury also releases proinflammatory mediators and endotoxin into the systemic circulation of adult and newborn animals and causes shock and multisystem organ failure (49–51). This possibility is a real risk for preterm infants because 50% or more of preterm infants born before 30 weeks' gestation have been exposed *in utero* to infection and/or inflammation (52), which results in inflammation of the fetal lung (53). Our results suggest a new mechanism for a synergistic interaction between mechanical ventilation and a systemic inflammatory response. Mechanical ventilation can simultaneously result in a systemic inflammatory response, as indicated by activation of acute-phase responses in the liver and increases in TLR mRNA, and deliver the mediators to those receptors. These relationships need to be further explored, and our novel fetal sheep model may be ideal for such studies.

Our results demonstrate that brief high V_T ventilation, similar to what may occur during resuscitation in the delivery room, can injure the preterm lung. Limitations to application of the results to the clinical environment result primarily from the differences between the healthy sheep and the preterm infant. Most preterm infants have been exposed to antenatal corticosteroids, which may inhibit inflammatory responses to ventilation-mediated injury (25). Many of these infants also have been exposed to chorioamnionitis, which could increase the injury response to mechanical ventilation (54). The style of ventilation we used included no PEEP because PEEP levels are poorly controlled in the delivery room and there is no standard for the use of PEEP in the delivery room (55). An inspiratory time of 0.7 seconds was used because there is also no standardized inspiratory time for neonatal resuscitation. The relatively long inspiratory time was used because of flow limitations in this large animal model. Surfactant was given after the initial ventilation period because it is not routine practice to give surfactant to preterm infants before ventilation is established (56). These clinically relevant variables can be tested using this animal model to evaluate the

components of resuscitation and better inform the ILCOR guidelines for neonatal resuscitation.

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