

Time Course of Physiologic Variables in Response to Ventilator-Induced Lung Injury

David J Dries MSE MD, Dana A Simonson, Alexander B Adams MPH RRT FAARC
and John J Marini MD

BACKGROUND: The time course of the physiological derangements that result from ventilator-induced lung injury has not been adequately described. Similarly, the regional topographies of pleural pressure and tissue edema have not been carefully mapped for this injury process. **METHODS:** Lung injury was induced in 9 normal pigs by ventilating for 6 hours at a transpulmonary pressure of 35 cm H₂O, with the animals in the supine position. Eight additional normal pigs received right thoracotomy to place pleural-surface-pressure sensors prior to an identical period and intensity of injurious ventilation. Gas exchange and lung mechanics were tracked in all the animals. Cytokines (tumor necrosis factor alpha, interleukin 6, and interleukin 8) in peripheral blood were assayed at 2 hour intervals, beginning at the onset of mechanical ventilation, from all the animals. **RESULTS:** After a brief "induction" period, P_{aO₂} and tidal volume declined steadily in the animals that were ventilated to induce lung injury. The rate of decline was greater in the animals that received thoracotomy. The pleural pressure gradient steadily increased from ventral to dorsal. The serum cytokine levels did not evolve with developing injury, but cytokines were elevated at the onset of ventilation. Tissue edema, as assessed by the ratio of wet weight to dry weight, was greater in the thoracotomized animals than in the nonthoracotomized animals, and tissue edema tended to be greater in the caudal lung regions than in the cephalad lung regions. **CONCLUSIONS:** Following the induction period, the development of ventilator-induced lung injury progressed steadily and then plateaued, as assessed by quantitative physiology variables during 6 hours of ventilation at a transpulmonary pressure of 35 cm H₂O. Greater injury developed in animals that had a coexisting potential insult (thoracotomy). Injury development was not paralleled by bloodborne inflammatory cytokines. *Key words:* ventilator-induced lung injury, thoracotomy, acute respiratory failure, mechanical ventilation, animal studies. [Respir Care 2007;52(1):31-37. © 2007 Daedalus Enterprises]

Introduction

Mechanical ventilation provides essential life support for patients with various forms of acute respiratory failure.

David J Dries MSE MD, Dana A Simonson, Alexander B Adams MPH RRT FAARC, and John J Marini MD are affiliated with the Pulmonary Research Laboratory, Regions Hospital, St Paul, Minnesota.

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Correspondence: E-mail: alex.b.adams@healthpartners.com.

However, animal experiments and clinical studies suggest that adverse patterns of mechanical ventilation can worsen pre-existing damage and produce ventilator-induced lung injury (VILI), even in previously intact lungs.¹⁻⁵ VILI can produce air leaks, increase endothelial and epithelial permeability, and elicit inflammatory mediators, which have been reported to enter the bloodstream, where they may cause adverse reactions in systemic tissues. The importance of VILI was recently highlighted in clinical trials that found that lung-protective ventilation was associated with lower serum cytokine and chemokine levels, less organ dysfunction, and lower mortality in patients with acute respiratory distress syndrome.^{1,4,5} However, the temporal sequence of associated physiological and pathological pulmonary alterations is incompletely understood.

It is known that functional and structural changes can emerge over the span of a few high-stress ventilatory cycles and reverse within a few cycles of stress relief.^{6,7} It

remains unclear whether injury develops as a consistently escalating process after an induction phase (which suggests materials fatigue or sequential failure of linked structural elements) or whether injury progresses (increases) linearly over time. The overwhelming majority of reported experiments have focused on changes that occur at a specified observational end point taken at some arbitrary time after initiation of mechanical ventilation.^{2,3,8-17} In this study we repeatedly measured physiologic variables of interest during the injury process.

Despite a large number of experiments to investigate VILI, in diverse animal models of acute lung injury,^{2,3,7-28} the topography of the pleural and transalveolar pressures and tissue edema in the various lung regions has not been carefully mapped, nor has the time course of lung dysfunction been adequately described. In this study we used parallel assessment of physiologic changes and systemic inflammatory response, together with direct regional measurements of tissue edema, to address these issues in a large-animal model of lung injury induced in healthy lungs only by mechanical forces.

Methods

We studied 17 juvenile pigs of either sex (mean weight 23.6 kg) in a protocol designed to induce lung injury via elevated pressure during mechanical ventilation. We used 9 normal pigs and another 8 normal pigs that underwent thoracotomy. An additional 6 reference pigs were also studied: 3 with a noninjurious peak pressure (20/3 cm H₂O), and 3 with a lung-protective positive end-expiratory pressure (PEEP) setting of 15 cm H₂O (35/15 cm H₂O) to verify the stability of the preparation under noninjurious conditions. An inflammatory response was not anticipated in the 6 reference animals, so cytokine assays were not conducted on these animals.

The study was approved by our institution's animal care and use committee. Each pig was anesthetized with 30 mg/kg sodium pentobarbital, and deep anesthesia without spontaneous breathing effort was maintained by continuous infusion of pentobarbital (12 mg/kg/h) throughout the experiment. The airway was intubated via tracheotomy with an 8-mm inner-diameter cuffed endotracheal tube. Mechanical ventilation was delivered in the constant-flow, volume-cycled mode (model 840, Nellcor Puritan Bennett, Carlsbad, California), with tidal volume (V_T) of 10 mL/kg, respiratory rate of 20 breaths/min, PEEP of 3 cm H₂O, inspiratory-expiratory ratio of 1:2, and fraction of inspired oxygen (F_{IO_2}) of 0.60. Arterial, venous, and pulmonary artery lines were placed to continuously monitor cardiovascular variables and gas exchange (Paratrend, Diametrics, Roseville, Minnesota). An esophageal catheter was placed in a standard manner, to monitor esophageal pressure and to titrate transpulmonary pressure.²⁹

Lateral thoracotomy was performed at the 5th and 6th intercostal spaces, without rib resection, in 8 animals. The right lung was allowed to transiently collapse, but otherwise was not manipulated, and 2 flat, flexible, air-filled, pressure-sensing wafers were secured to the pleural surface with silk sutures in high ventral and low dorsal positions, to continually monitor local pleural surface pressure. These silastic wafers were vertically separated by approximately 10 cm, and each could be emptied and refilled (via externalized conduits) after chest closure, to maintain optimal sensing characteristics. A "towel clip" technique, borrowed from approaches to emergency thoracotomy for trauma, allowed intermittent assessment of wafer position and function throughout the experiment. This technique can be employed without standard chest drains, with the animal under positive-pressure ventilation. The pleural pressures used in data analysis were those recorded at end-expiration.³⁰

Following the preparatory period, an injury-inducing ventilation protocol was applied, with the animals in the supine position, over a 6-hour period. Pressure-control ventilation was set at 10 breaths/min, inspiratory-expiratory ratio 1:2, PEEP 3 cm H₂O, and F_{IO_2} 0.6, with peak inspiratory pressure set at 41–48 cm H₂O, to establish a transpulmonary pressure (calculated as peak inspiratory pressure minus esophageal pressure) of 35 cm H₂O.

Because we employed large V_T , CO₂ was insufflated into the airway circuit to maintain eucapnia throughout the study period. No adjustments in ventilation were made after the protocol was initiated. V_T , P_{aO_2} , cardiac output, blood pressure, saline infusion, and airway pressure were recorded each hour. Beginning at the onset of ventilation, in all the experimental animals, but not in the 6 reference animals, peripheral blood was collected at 2-hour intervals, for subsequent assay of tumor necrosis factor alpha (TNF- α), interleukin 6 (IL-6), and IL-8. At the conclusion of the study period, the animals were sacrificed and completely examined. The left lungs were extracted to evaluate the wet-weight-to-dry-weight (WW/DW) ratio. Cytokine analysis via enzyme-linked immunosorbent assay (ELISA, R and D Systems, Minneapolis, Minnesota) was performed in duplicate by a blinded technician on coded samples that were randomized for both time and animal subject.

Two-way analysis of variance was employed to evaluate serial changes in P_{aO_2} , V_T , and pleural pressure. Fisher's test for least significant difference was used to identify regional differences in the WW/DW ratios in the explanted lungs. Calculations were made with statistics software (SPSS, SPSS, Chicago, Illinois).

Results

To achieve a transpulmonary pressure of 35 cm H₂O, a mean peak inspiratory pressure of 45.5 cm H₂O was re-

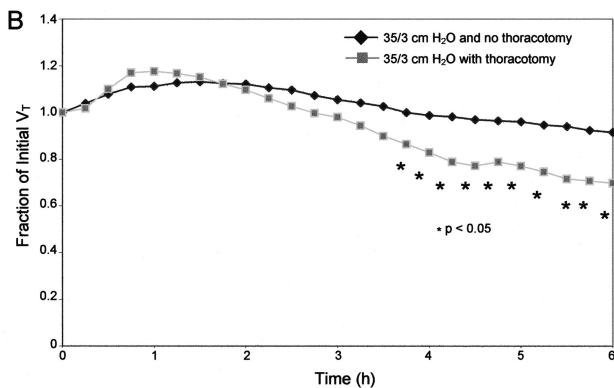
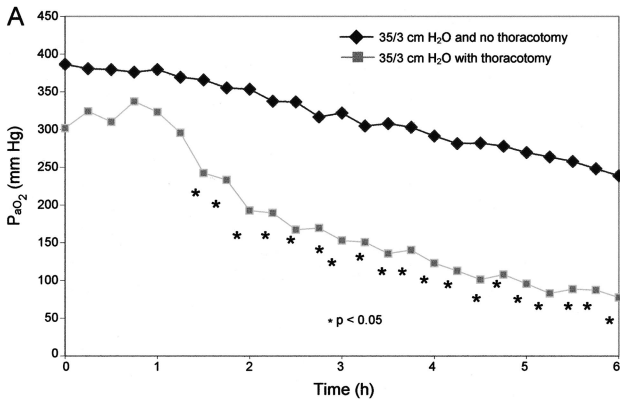


Fig. 1. Physiological changes during 6 hours of mechanical ventilation at 35/3 cm H₂O in 9 normal, nonoperated pigs and 8 normal pigs that underwent thoracotomy for placement of pleural-pressure sensors. A: P_{aO₂} in the nonthoracotomy animals steadily declined, while the thoracotomy animals underwent an accelerated P_{aO₂} decline, which differed from that of the nonoperated animals at 90 min. B: Baseline tidal volume (V_T) at 35/3 cm H₂O was elevated because of the high driving pressure (nonthoracotomy group V_T 1,018 ± 255 mL vs thoracotomy group 1,232 ± 186 mL, *p* = 0.15). The V_T changes are shown as a fraction of the initial V_T. The nonthoracotomy animals experienced insignificant changes over the study period, while the thoracotomy group experienced an initial increase in V_T, followed by a steady decline that differed significantly (* *p* < 0.05) from the nonthoracotomy animals after 210 min.

quired in the nonthoracotomy group, and 42.9 cm H₂O in the thoracotomy group. P_{aO₂} changes indicated that injury developed in all the experimental animals over the 6-hour protocol period (Fig. 1A). P_{aO₂} decreased at an average rate of 0.385 mm Hg/min in the nonthoracotomy group, and at 1.019 mm Hg/min in the thoracotomy group, with an impressive difference between the groups emerging only after the second hour of high-pressure ventilation (see Fig. 1A). The pace of declining oxygenation tended to equalize between the groups after the third hour.

V_T, which is a dependent variable inversely related to impedance and possibly an indicator of evolving injury,

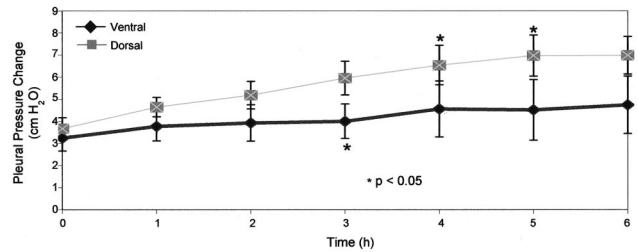


Fig. 2. Pleural pressure changes measured by the pleural-pressure-sensing wafers, which were placed via thoracotomy. The dorsal and ventral pleural pressures increased during ventilation at 35/3 cm H₂O during the 6-hour study period. The mean dorsal pressure increased earlier and to a greater extent than did the mean ventral pressure, which created a significantly widened pleural-pressure gradient after 3 hours (* *p* < 0.05).

changed nonlinearly over time (see Fig. 1B). A similar pattern of V_T response developed earlier in the thoracotomy group. In the first hour of the experiment a V_T increase was observed in response to the high but fixed airway pressure. The V_T effect on the nonthoracotomy group was larger than the effect on the thoracotomy group. After this initial rise, however, V_T began to decrease; the thoracotomy group experienced a significantly faster decline. A significant V_T difference between the 2 groups emerged after the third hour of injurious ventilation.

The thoracotomy group displayed increases in both dorsal and ventral pleural pressure at end-expiration, with a gradually widening vertical gradient of pleural pressure that paralleled other indicators of injury (P_{aO₂} and V_T) (Fig. 2). While overall pleural pressure increased over time, ventral pleural pressure increased at a significantly slower rate than did dorsal pressure. After 3 hours, dorsal pleural pressure was significantly greater than ventral pleural pressure.

The WW/DW measurements displayed regional differences (Fig. 3). Injury (as assessed by WW/DW) tended to develop in the gravity-dependent caudal region, in a pattern that paralleled the downhill slope orientation of the supine porcine lung in situ. The 6 reference animals had less injury from lower-pressure ventilation (20/3 cm H₂O) and high-pressure ventilation with elevated PEEP (15 cm H₂O), compared to the experimental animals (see Fig. 3A). When PEEP was elevated (35/15 cm H₂O) or peak pressure was set at 20/3 cm H₂O, there were no changes in P_{aO₂} or V_T throughout the 6-hour study period, and the WW/DW ratio was not significantly elevated, nor was atelectasis identifiable in the reference animals' lungs.

To test whether mechanical ventilation significantly increases the overall WW/DW ratio, both group WW/DW ratio means were compared to the WW/DW ratio 5.5, which is the laboratory mean for undamaged lungs that we determined in previous studies in our laboratory. Against this standard, the reference animals that received reduced

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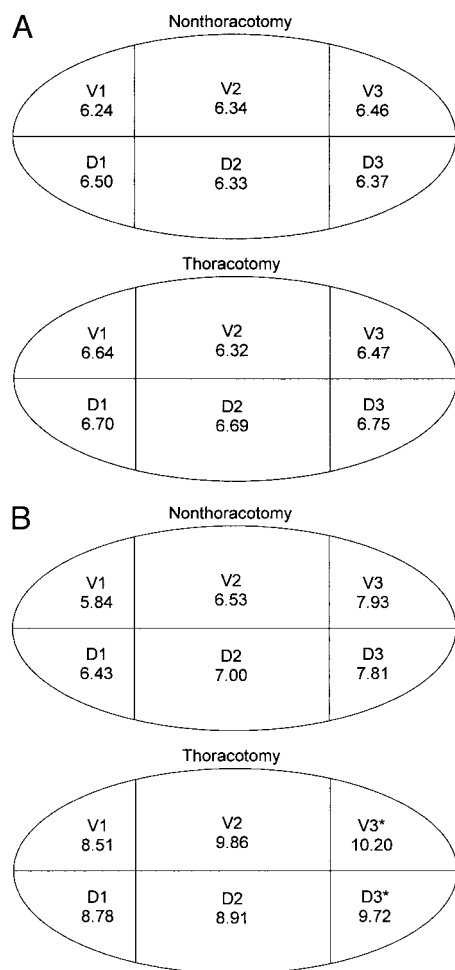


Fig. 3. Wet-weight-to-dry-weight (WW/DW) ratios determined at autopsy for each region of a 6 compartment template (ventral 1–3, and dorsal 1–3) in the experimental thoracotomy versus the nonthoracotomy groups. A: WW/DW ratios from nonthoracotomy/reference animals (20/3 cm H₂O, *n* = 2, 35/15 cm H₂O, *n* = 2) and thoracotomized reference animals (20/3 cm H₂O, *n* = 1, 35/15 cm H₂O, *n* = 1). The reference animals were prepared identically to the experimental animals, but received lung-protective ventilation. B: Both experimental groups experienced marked elevations in edema index, compared to the average WW/DW of healthy lungs (5.5) previously established in our laboratory. * *p* < 0.05 compared to the V1 and V2 nonthoracotomy sections.

pressure or elevated PEEP had small increases in the WW/DW ratio (see Fig. 3A). At the $\alpha = 0.05$ level, the WW/DW ratios were significantly greater than 5.5 in both VILI groups (with and without thoracotomy). In addition, the unoperated left lungs of the thoracotomy group had a significantly higher WW/DW ratio than the left lungs of the nonthoracotomy group (*p* = 0.05). Fisher's protected least significant difference test was used to identify significant WW/DW differences between different lung regions. At the $\alpha = 0.05$ level, the sections labeled V3 and D3 (ventral and dorsal caudal regions, respectively) exhibited significantly greater WW/DW ratios than did sec-

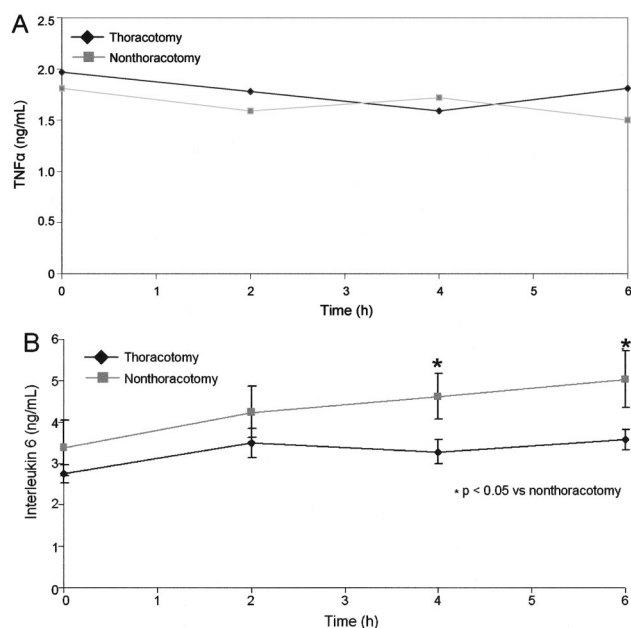


Fig. 4. Serum levels of (A) tumor necrosis factor alpha (TNF- α) and (B) interleukin 6 (IL-6) during the 6-hour study period, with ventilation pressure of 35/3 cm H₂O. IL-6 levels diverged at 4 hours and 6 hours (* *p* < 0.05) for the thoracotomy group versus the nonthoracotomy group. Note the high IL-6 levels at all time points in all the animals.

tions V1 and D1 (ventral and dorsal cephalad regions, respectively) in the nonthoracotomy group (see Fig. 3B).

Systemic inflammation was assessed via serial measurement of the cytokines TNF- α , IL-6, and IL-8 in blood samples. Elevated TNF- α was found in animals with and without thoracotomy from the initial sampling time point and initiation of ventilation. Although the animals that received thoracotomy had a higher baseline TNF- α , there was no statistically significant difference between the groups. Considerable between-subject differences in cytokine concentrations were observed, but the sequential values for each animal were remarkably consistent. A gentle trend toward divergence of IL-6 values was detected, based on thoracotomy category, but the relative magnitude of this difference was modest in comparison to the elevated baseline values of both categories (Fig. 4). Bloodstream IL-8 was not identified in either group.

Discussion

To isolate the effects of mechanical forces on VILI, our model of lung injury was intended to inflict injury solely via tissue strain in previously healthy lungs. Indicators of thoracic mechanics (V_T) and gas exchange (P_{aO_2}) deteriorated progressively, while dorsal pleural pressure increased in the subset of animals in which it was measured. As in previous studies of VILI, damage did not occur with a

uniform spatial distribution, as reflected by the gross post-mortem appearance and the regional WW/DW measurements. During the course of injurious ventilation, dorsal pleural pressure increased to a significantly greater extent than did ventral pressure, but the dorsal region did not sustain proportionally greater injury. Because end-inspiratory “stretch” is greater in the spared ventral areas, this observation suggests the relative importance of avoiding lung-unit collapse when high inflation pressure is employed. Overstretching may disrupt mechanically heterogeneous injured tissue more readily than normal lung tissue. Thus, injury could propagate from an initial point of injury as local stretching and shearing forces progress along the margin of injury.

After the 1-hour “induction” period, during which no detectable P_{aO_2} change occurred, the thoracotomized animals underwent a period of significantly faster decay in both V_T and P_{aO_2} , which led to statistically significant differences at the 60-min and 225-min time points. Pneumothorax as a cause of this differential response was ruled out during postmortem examination of the pleural space in all the animals. The “towel clip” thoracotomy closure technique was employed to avoid local distortion of surface pressure by pleural drains and suction.

Only modest further release of cytokines into the blood was detected above the elevated initial readings, despite the progressive injury manifested in physiological variables and visually evident in the postmortem lungs. TNF- α and IL-6 changes from their high initial values were unimpressive, and the bloodstream IL-8 (a chemokine thought highly relevant to developing injury) was not detectable.

The great majority of studies that have investigated VILI mechanisms have assessed dysfunction and/or tissue alterations only after the completion of the experimental protocol.^{2,3,7-28} Important questions remain regarding the nature of VILI itself. Do small wounds to the gas-blood barrier develop in response to excessive strain, thereby calling forth an inflammatory response? Conversely, do excessive and repeated tissue strains signal an inflammatory cascade? Does the ventilation process invoke cumulative damage as the offending tidal cycles are repeated, or do mechanical accommodations and anti-inflammatory and repair processes that activate in response to the initial injury cycles slow, halt, or even reverse the damaging process?

An elegant series of observations made at the cellular level demonstrated that mechanical signaling from excessive stretching forces triggers a complex pro-inflammatory response that is at least partially countered by simultaneous activation of anti-inflammatory mediators.^{6,7} Although very high stretching forces applied with low PEEP clearly can produce severe damage, overt VILI may occur under conditions of lower mechanical stress if one or more potentially noxious stimuli coexist (the “two-hit” hypoth-

esis).³¹ Moreover, repair of permeability defects and even of overt breaks in the cellular membrane may occur with astonishing rapidity, once the excessive strain is alleviated.^{6,7} Under the specific conditions of this experiment, physiologic derangements continued to progress over its 6-hour duration. Whether the physiological deterioration associated with VILI would have eventually slowed or further intensified cannot be determined from our data.

The deleterious effect of thoracotomy was consistently observed. Why the contour of the oxygenation profile differed for animals that underwent thoracotomy and pressure-sensor placement cannot be answered with the data available. The acceleration of change did not occur until the second hour of aggressive ventilation, which argues strongly against an immediate effect on atelectasis, edema, or pneumothorax from the surgery itself. Moreover, no consistent or noticeable differences were observed between the left and right lungs of animals that received thoracotomy, and the left lungs (contralateral to the incision) formed more edema than did the left lungs of the nonoperated pigs. Although it is tempting to attribute the increased edema and impairment of oxygen exchange to VILI, and the VILI to a “second hit” amplification of mechanical strain caused by the invasive monitoring, neither premise can be proven. Indeed, how such amplification was mediated remains unclear.

Our own results indicate an elevation of 2 pro-inflammatory cytokines (TNF- α and IL-6) associated with animal instrumentation and a damaging ventilation strategy in animals that did not have an initial illness or evidence of prior gas-exchange abnormalities. But neither TNF- α nor IL-6 paralleled the evolving physiologic damage or correlated with the severity of the derangements. The animal preparation/instrumentation may have been the primary cause of cytokine elevation in peripheral blood. Although these measurements from peripheral blood may not reflect cytokine concentrations of pathogenetic importance in situ, it is logical to conclude that endothelial exposure to these circulating mediators did not assume primacy in this setting, and that resuscitation strategies and efforts to block inappropriate inflammatory responses must explore multiple elements in the inflammatory cascade. We chose not to collect alveolar lavage fluid to measure cytokines, because repeated collection of lavage fluid would interrupt the stress applied by the elevated airway pressure and/or induce “surfactant washout” injury of its own accord. However, with our experimental results in mind, sequential measurements of local mediator release would have been of unquestioned interest. Given the high baseline levels of TNF- α and IL-6, we were unable to detect convincing superimposed cytokine signals attributable to VILI. We do not attribute our data to technical error, as the measured cytokine levels were relatively high (nanogram as opposed to picogram amounts) and were quite consistent for each

individual pig over time, despite considerable variation observed among the animals. Moreover, using exactly analogous assay methodology, IL-8 was virtually undetectable in any of the animals.

Observers who place cytokines at the center of a bio-trauma (inflammatory) model for VILI will be impressed that TNF- α and IL-6 were present in these animals exposed to adverse-ventilation risk. Critics of this central role for cytokine-mediated injury who favor a mechanical model for VILI would point to the absence of IL-8 and the failure of blood-borne TNF- α and IL-6 to rise as physiologic changes and lung damage occurred.^{32–38}

Failure of regional edema to correlate closely with the magnitude of transpulmonary pressure exposure calls attention to potential roles for “stretch” co-factors such as vascular pressure and mechanical heterogeneity in the injury process.

Manifestations of VILI emerge more readily when previously healthy experimental preparations are simultaneously given sub-pathological doses of such stressors as lipopolysaccharides.^{39,40} To our knowledge, amplification of VILI by surgical insult has not been reported. Although our intent was simply to place pleural pressure sensors without invoking injury, the thoracotomized animals experienced greater alterations of function and more tissue edema than those not operated upon. While the thoracotomy disrupted the chest wall and thus changed the chest wall dynamics, the collapse, manipulation, and re-expansion of the lung was temporary and minimal. Injury in the thoracotomy animals could not be explained by differences in supportive care, such as drugs, depth of anesthesia, or volume of fluids administered, or by the pro-inflammatory cytokines measured in peripheral blood. In addition, even though we cannot entirely exclude roles for atelectasis development or surfactant depletion, our 6 reference animals remained uninjured, and our 2 experimental groups were ventilated in a similar manner. We speculate that inflammatory mediators that were localized and/or not measured by our cytokine assays of peripheral blood played a role in injury development.

The disparity in injury severity between animals that did and did not undergo thoracotomy may have clinical relevance, as ventilated patients are frequently “primed” by pre-existing surgical or medical problems or interventions, and placement of foreign objects (such as indwelling vascular catheters, chest tubes, and drains) is virtually routine. Conceivably, such factors could amplify vulnerability to injury from excessive ventilating stress.

Limitations

Our discussion suggests limitations of this study. While VILI evolved nonlinearly over time, our protocol was limited to 6 hours and began in animals with no known pre-

existing pulmonary or extrapulmonary insult, and these conditions do not correspond to the usual clinical situation. We also note that the V_T we used was intentionally excessive, although the pressures applied were not unusual for ventilation of patients with acute respiratory failure. Moreover, Gajic and co-workers recently found that tissue injury in patients with uninjured lungs depends on the ventilator settings.⁴¹ In our study, the driving pressures and V_T levels that differed (although not significantly) between the 2 experimental groups could account for the differences in injury development. In the thoracotomy group the greater V_T would favor injury while the lesser driving pressure would reduce vulnerability to injury. Finally, a limited number of mediator systems were assessed, and we did not explore alternative injury pathways.^{42–44}

Conclusions

The time-series data from this purely mechanical model of VILI document the time course of key physiological alterations, describe the lung-region topography of ventilator-inflicted edema, and strongly suggest that measurements of de-compartmentalized inflammatory cytokines in peripheral blood are not sufficiently sensitive to mirror injury development in this experimental setting.

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