Linking lung function and inflammatory responses in ventilator-induced lung injury

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Cannizzaro V, Hantos Z, Sly PD, Zosky GR. Linking lung function and inflammatory responses in ventilator-induced lung injury. Am J Physiol Lung Cell Mol Physiol 300: L112–L120, 2011. First published October 15, 2010; doi:10.1152/ajplung.00158.2010.—Despite decades of research, the mechanisms of ventilator-induced lung injury are poorly understood. We used strain-dependent responses to mechanical ventilation in mice to identify associations between mechanical and inflammatory responses in the lung. BALB/c, C57BL/6, and 129/Sv mice were ventilated using a protective [low tidal volume and moderate positive end-expiratory pressure (PEEP) and recruitment maneuvers] or injurious (high tidal volume and zero PEEP) ventilation strategy. Lung mechanics and lung volume were monitored using the forced oscillation technique and plethysmography, respectively. Inflammation was assessed by measuring numbers of inflammatory cells, cytokine (IL-6, IL-1β, and TNF-α) levels, and protein content of the BAL. Principal components factor analysis was used to identify independent associations between lung function and inflammation. Mechanical and inflammatory responses in the lung were dependent on ventilation strategy and mouse strain. Three factors were identified linking 1) pulmonary edema, protein leak, and macrophages, 2) atelectasis, IL-6, and TNF-α, and 3) IL-1β and neutrophils, which were independent of responses in lung mechanics. This approach has allowed us to identify specific inflammatory responses that are independently associated with overstretch of the lung parenchyma and loss of lung volume. These data provide critical insight into the mechanical responses in the lung that drive local inflammation in ventilator-induced lung injury and the basis for future mechanistic studies in this field.

MECHANICAL VENTILATION IS known to produce lung damage in otherwise healthy lungs (3, 5) in a process known as ventilator-induced lung injury (VILI). The mechanisms underlying the pathobiology of VILI are poorly understood, and mortality and morbidity due to acute respiratory distress syndrome (ARDS) represent a significant burden to the health system (28).

One of the fundamental problems with mechanical ventilation is the need to ventilate sufficiently to maintain an “open lung” while avoiding the risk of overinflation (1, 6). The benefits of avoiding overinflation were highlighted by the findings of an ARDSNet study, which demonstrated a substantial decrease in mortality with the use of 6 compared with 12 ml/kg tidal volume (6). However, despite the application of these strategies, the morbidity and mortality associated with ARDS remain high, and the use of these low tidal volume ventilation strategies comes with the risk of atelectasis and the inflammatory response associated with derecruitment of the peripheral lung (31). In line with this, we have demonstrated that even so-called “protective” ventilation strategies using low tidal volume ventilation (to avoid overinflation) and periodic recruitment maneuvers (to maintain an open lung) can result in significant loss of lung volume and cytokine production in mice (9, 40).

Although the mechanisms of VILI are not fully understood, a number of basic processes have been implicated including 1) baro/volutrauma (19, 33), 2) atelectasis, and 3) biotrauma (10). However, our knowledge of the association between mechanical trauma (e.g., atelectasis and baro/volutrauma) and the development of biotrauma (e.g., inflammation), which leads to poor outcome in the patient (10), is currently lacking. These mechanisms have been primarily studied in animal models, and the value of such animal models is that they provide an opportunity to conduct mechanistic studies while allowing the use of invasive measurements and ventilation strategies that would otherwise be inappropriate for use on human subjects.

Mouse models of disease are excellent tools for understanding mechanisms of disease as they allow strain-dependent responses to be studied and compared, which provides the opportunity to identify mechanisms linking pathways of disease progression. These genetically determined differences that allow links between mechanical and inflammatory responses in the lung to be identified are yet to be fully explored/exploited in laboratory studies on VILI.

The aim of this study was to monitor changes in lung mechanics and lung volume (as markers of volutrauma/atelectasis) and inflammation (cellular influx and cytokine production as markers of biotrauma) using protective and injurious mechanical ventilation strategies in three commonly used mouse strains. By using strains of mouse with varying inflammatory responses, we aimed to identify associations between these responses and changes in lung mechanics with a view to understanding some of the critical processes leading to local biotrauma in VILI.

MATERIALS AND METHODS

Animals

Eight-week-old female BALB/c and C57BL/6 mice were purchased from the Animal Resources Centre (ARC; Murdoch, Western Australia, Australia). Eight- to twelve-week-old female 129/Sv mice were obtained from a breeding colony at the Telethon Institute for...
Child Health Research. All mice were provided food and water ad libitum and housed in a 12:12-h light-dark cycle. All experiments were conducted according to the guidelines of the National Health and Medical Research Council of Australia and were approved by the Telethon Institute for Child Health Research Animal Ethics Committee.

Study Protocol and Ventilation Regime

Mice were prepared as described previously (40). Briefly, mice were anesthetized with a 10 µL/g ip injection of a solution containing 40 mg/ml ketamine (Troy Laboratories) and 2 mg/ml xylazine (Troy Laboratories). Initially, two-thirds of the dose was given to induce a surgical plane of anesthesia before tracheostomy and insertion of a 10-mm polyethylene cannula (0.86-mm inner diameter). Mice were placed inside a custom-made whole body plethysmograph (~180 ml) and attached to a small animal ventilator (flexiVent; SCIREQ, Montréal, Québec, Canada; http://www.scireq.com/products/flexivent) via an external port in the plethysmograph. Once connected to the ventilator, the remaining anesthetic was given. Top-up doses of the anesthetic were given throughout the ventilation period when necessary.

Mice were initially ventilated at 360 breaths/min with a tidal volume of 10 ml/kg and 3 cmH2O positive end-expiratory pressure (PEEP). Once connected to the ventilator, lung volume history was every 5 min up to 20 ml/kg; BALB/c, n = 8; 129/Sv, n = 10; C57BL/6, n = 6; or 2) high tidal volume with zero PEEP (HVZP); 150 breaths/min, 24 ml/kg, zero end-expiratory pressure (ZEEP; BALB/c, n = 10; 129/Sv, n = 10; C57BL/6, n = 6).

Lung volume and lung mechanics were measured (see below for details) before randomization to one of two ventilation protocols: 1) low tidal volume ventilation with PEEP (LVP); 360 breaths/min, 10 ml/kg, 3 cmH2O PEEP with periodic recruitment maneuvers (2 × sigh every 5 min up to 20 ml/kg; BALB/c, n = 8; 129/Sv, n = 10; C57BL/6, n = 6); or 2) high tidal volume with zero PEEP (HVZP); 150 breaths/min, 24 ml/kg, zero end-expiratory pressure (ZEEP; BALB/c, n = 10; 129/Sv, n = 10; C57BL/6, n = 6).

Lung volume and lung mechanics were measured every 10 min for 2 h until the end of the ventilation period. At the end of the ventilation period, a bronchoalveolar lavage (BAL) sample was taken for analysis of inflammatory cells, cytokines, and protein leak. Separate groups of anesthetized and tracheostomized mice from each strain (BALB/c, n = 8; 129/Sv, n = 6; C57BL/6, n = 6) had a BAL taken to serve as nonventilated controls.

Inflammatory Cells

BAL samples were obtained by gently instilling and withdrawing 0.5 ml of pyrogen-free 0.9% saline three times via the tracheal cannula. BAL samples were centrifuged, and the supernatant was removed for later analysis. The cell pellet was resuspended in PBS, 0.5 ml of pyrogen-free 0.9% saline three times via the tracheal cannula at EELV during pauses in ventilation. The signal was delivered by a loudspeaker-in-box setup via a wave tube of known impedance. A four-parameter model with constant-phase tissue impedance (16) was fit to the measured respiratory system impedance (Zrs) spectrum to obtain parameters describing airway resistance (Raw), tissue damping (G), the dissipative properties of the lung), and tissue elastance (H; the elastic properties of the lung).

Statistical Analysis

Between-group (strain and ventilation strategy) comparisons were made using ANOVA (1-way, 2-way with interaction, and repeated-measures). Data were transformed as appropriate to satisfy the assumptions of normality and homoscedasticity. All ANOVA comparisons were conducted in SigmaStat (v3.5; Systat). Principal components factor analysis was used to identify associations between lung mechanical and inflammatory responses. To account for differences in baseline (naïve) levels of inflammation and lung mechanics between strains, maximum G, maximum H (Raw was not included as it was largely unaffected by ventilation), and TGV after 10 min of ventilation (this point was arbitrarily chosen as it reflected the speed at which TGV was lost in each strain) were expressed as a percentage of baseline values, whereas IL-6, protein, IL-1β, TNF-α, number of macrophages, and number of neutrophils in the BAL were expressed as a percentage of control values. The values of these parameters were averaged for each strain, and ventilation strategy and principal components factor analysis was run in Stata (v11; StataCorp). Factors with an eigenvalue >1 were retained and transformed using varimax orthogonal rotation. Data are presented as means (SD).

RESULTS

Inflammatory Cells

Unventilated C57BL/6 mice had substantially higher TCCs [1.46 (0.78) × 105 cells/ml] than the other strains (vs. BALB/c, P < 0.001; vs. 129/Sv, P < 0.001). 129/Sv mice [0.42 (0.11) × 105 cells/ml] also had higher TCCs than BALB/c [0.23 (0.06) × 105 cells/ml] mice (P = 0.01). The bulk of the cells in BALs from unventilated mice were macrophages in all strains (Fig. 1). In contrast, unventilated 129/Sv mice [0.54 (0.31) × 105 cells/ml] had the highest number of neutrophils in the BAL (vs. BALB/c, P < 0.05; C57BL/6, P < 0.05), whereas there were no differences between the other two strains (P > 0.05; Fig. 1).

Mechanical ventilation had no effect on the number of macrophages in the BAL of BALB/c mice (P = 0.50). In contrast, 129/Sv mice had higher numbers of macrophages after the HVZP ventilation regime [P = 0.05; 0.61 (0.19) × 105 cells/ml] but not after LVP ventilation [P = 0.56; 0.37 (0.09) × 105 cells/ml], whereas C57BL/6 mice had decreased numbers of macrophages after both the LVP [P < 0.001; 0.31 (0.10) × 105 cells/ml] and HVZP [P = 0.003; 0.55 (0.15) × 105 cells/ml] ventilation compared with controls [1.46 (0.78) × 105 cells/ml]. The decrease in the number of macrophages in LVP-ventilated C57BL/6 mice was greater than in HVZP-ventilated mice (P = 0.03; Fig. 1).
Mechanical ventilation was associated with an increase in the number of neutrophils in BALB/c mice on both the LVP \( P = 0.04; 1.91 \times 10^3 \) cells/ml and HVZP ventilation strategies \( P = 0.04; 1.45 \times 10^3 \) cells/ml compared with controls \( 0.10 \times 10^3 \) cells/ml. Whereas the number of neutrophils appeared to increase in the HVZP 129/Sv, there was no statistical association between the number of neutrophils and mechanical ventilation in this strain \( P = 0.24 \). Mechanical ventilation was also not associated with a change in neutrophil numbers in C57BL/6 mice \( P = 0.10 \); Fig. 1).

**Total Protein**

For all three strains, BAL protein levels were increased after 2 h of the HVZP ventilation regime (BALB/c, \( P = 0.05 \); 129/Sv, \( P = 0.05 \); C57BL/6, \( P = 0.05 \)) but not after the LVP ventilation strategy (BALB/c, \( P > 0.05 \); 129/Sv, \( P > 0.05 \); C57BL/6, \( P > 0.05 \)) compared with naïve levels (Fig. 1).

**Cytokine Production**

**Naïve mice.** There were substantial differences in baseline levels of BAL cytokines (Fig. 2) between the three strains. IL-6 levels were significantly higher in BALB/c \( 79 \) (22) pg/ml mice compared with the other two strains (vs. 129/Sv, \( P < 0.001 \); vs. C57BL/6, \( P < 0.001 \)), and C57BL/6 \( 31 \) (4) pg/ml mice also had higher levels than 129/Sv \( 20 \) (5) pg/ml mice \( P = 0.005 \). IL-1β levels were the highest in the 129/Sv \( 430 \) (120) pg/ml strain (vs. BALB/c, \( P < 0.001 \); vs. C57BL/6, \( P < 0.001 \)), but there was no difference between BALB/c \( 140 \) (39) pg/ml and C57BL/6 \( 150 \) (24) pg/ml mice \( P = 0.68 \); Fig. 2). Both BALB/c \( P < 0.001; 370 \) (130) pg/ml and 129/Sv mice \( P < 0.001; 340 \) (90) pg/ml had higher levels of TNF-α than C57BL/6 \( 150 \) (20) pg/ml, however, there was no difference between these two strains \( P = 0.79 \); Fig. 2).

**IL-6.** Increased levels of IL-6, compared with their respective controls, were detected in all strains in response to mechanical ventilation (BALB/c: LVP, \( P < 0.001 \); HVZP, \( P < 0.001 \); 129/Sv: LVP, \( P < 0.001 \); HVZP, \( P < 0.001 \); C57BL/6: LVP, \( P < 0.001 \); HVZP, \( P < 0.001 \); Fig. 2). In both 129/Sv \( P = 0.003 \); HVZP, 210 (170) pg/ml; LVP, 80 (40) pg/ml and C57BL/6 \( P = 0.002 \); HVZP, 340 (230) pg/ml; LVP, 80 (30) pg/ml mice, IL-6 in the BAL was significantly higher in those on the HVZP ventilation strategy compared with those on the LVP, whereas there was no difference between the two ventilation strategies in BALB/c mice \( P = 0.21 \); Fig. 2).

**IL-1β.** Whereas there appeared to be a tidal volume-dependent response in both IL-6 and protein, the pattern for IL-1β production in the BAL was less clear cut. For both the 129/Sv \( P = 0.04; 280 \) (110) pg/ml and C57BL/6 \( P = 0.004; 90 \) (40) pg/ml mice, there was no significant difference between the LVP and HVZP groups.

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**Fig. 1.** Box plots (median, interquartile range, 10th to 90th percentile, and circles indicating points outside of this range) of the number of macrophages (A), number of neutrophils (B), and total protein (C) in the bronchoalveolar lavage (BAL) of naïve (white boxes) BALB/c, 129/Sv, and C57BL/6 mice and mice ventilated with protective [low tidal volume ventilation with positive end-expiratory pressure (PEEP) (LVP); light gray boxes] or injurious [high tidal volume with zero PEEP (HVZP); dark gray boxes] ventilation strategies. *Significant difference \( P < 0.05 \) compared with naïve levels; #significant \( P < 0.05 \) difference between the LVP and HVZP groups.

**Fig. 2.** Box plots (median, interquartile range, 10th to 90th percentile, and circles indicating points outside of this range) of cytokines (IL-6, A; IL-1β, B; TNF-α, C) in the BAL of naïve (white boxes) BALB/c, 129/Sv, and C57BL/6 mice and mice ventilated with protective (LVP; light gray boxes) or injurious (HVZP; dark gray boxes) ventilation strategies. *Significant difference \( P < 0.05 \) compared with naïve levels; #significant \( P < 0.05 \) difference between the LVP and HVZP groups.

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pg/ml) strains, the LVP ventilation strategy was associated with a decrease in IL-1β, whereas there was no change in these levels in BALB/c mice (P = 0.25; 170 (50) pg/ml). There was no statistically significant change in IL-1β levels after the HVZP ventilation regime in any strain (BALB/c, P = 0.18; 129/Sv, P = 0.14; C57BL/6, P = 0.48; Fig. 2).

TNF-α. In BALB/c mice, there was a decrease in TNF-α levels in the BAL for both ventilation strategies [LVP, 100 (20) pg/ml, P = 0.01; HVZP, 50 (40) pg/ml, P = 0.04]. In contrast, mechanical ventilation was not associated with changes in TNF-α levels in 129/Sv mice (P = 0.38), whereas in C57BL/6 mice, TNF-α levels were significantly lower in LVP-ventilated mice [P < 0.001; 90 (50) pg/ml] but not in HVZP-ventilated mice [P = 0.68; 160 (20) pg/ml] compared with naïve controls (Fig. 2).

Lung Volume and Lung Mechanics

Baseline. Both baseline TGV and lung mechanics (R_aws, G, and H) varied between strains. TGV in the 129/Sv strain was higher than the BALB/c strain (P = 0.017), whereas TNF-α was higher again in the C57BL/6 strain (vs. BALB/c, P < 0.001; vs. 129/Sv, P = 0.002; Table 1). R_aws was significantly lower in the 129/Sv strain compared with the BALB/c strain (P = 0.02) but not the C57BL/6 strain (P = 0.19), and there was no difference between the latter (P = 0.34). G was highest in the 129/Sv strain (vs. BALB/c, P < 0.001; vs. C57BL/6, P < 0.001) with no difference between BALB/c and C57BL/6 mice (P = 0.67; Table 1). In contrast, H was lowest in the BALB/c mice (vs. 129/Sv, P = 0.02; vs. C57BL/6, P = 0.003), whereas there was no difference between the other strains (P = 0.51).

LVP. TGV decreased in all three strains on the LVP ventilation strategy in an almost linear fashion over time, however, there was no difference between strains (P = 0.80; Fig. 3). In 129/Sv and C57BL/6 mice, R_aws remained stable throughout the ventilation period (Fig. 4). In contrast, R_aws appeared to decrease in the first part of the ventilation period in BALB/c mice, although the magnitude of this change was small, before returning to baseline levels after ~1 h of ventilation (Fig. 4). Both G and H increased in all three strains over the course of the ventilation period on the LVP ventilation strategy. Although all strains appeared to reach the same point for G (~60% increase), this parameter increased more rapidly in BALB/c mice (60–80 min; P < 0.05) and to a greater extent in both the C57BL/6 (90–120 min; P < 0.05) and BALB/c (120 min; P < 0.05) strains compared with 129/Sv mice (Fig. 4). A similar pattern was observed in H with all three strains reaching the same point (~70% increase) with the BALB/c mice reaching a plateau more rapidly (although the difference between strains was only statistically significant at 70 min; Fig. 4).

HVZP. For both the BALB/c and 129/Sv strains, the pattern of decrease in TGV on the HVZP ventilation strategy was similar to that observed in the LVP strategy with a steady, consistent loss of volume over time (Fig. 3). In contrast, the C57BL/6 mice lost TGV more rapidly in the first part of the ventilation period (10, 40, and 50 min; P < 0.05) on the HVZP ventilation strategy, whereas there was no difference between the other strains (Fig. 3). As per the LVP ventilation strategy, R_aws remained stable throughout the ventilation period in 129/Sv and C57BL/6 mice on the HVZP strategy, whereas there was a significant decrease in R_aws in BALB/c mice for the first half of the ventilation period (Fig. 4). Whereas G increased steadily in all three strains on the LVP ventilation strategy, this was not the case for the HVZP strategy. BALB/c had a small increase in G after 10 min of ventilation, which remained stable thereafter (Fig. 2). A similar pattern was observed in 129/Sv mice, however, the increase in G was slightly greater than BALB/c mice such that there was a significant difference between these strains in later parts of the ventilation period (110 min; P = 0.02). In contrast, G increased rapidly in C57BL/6 mice before reaching a plateau after 1 h of ventilation. G was significantly higher in C57BL/6 mice compared with the other strains from 30 min of ventilation and beyond that point (P < 0.001 for all pairwise comparisons). A similar pattern was observed in H, although in this case the response in BALB/c and 129/Sv mice was identical (P > 0.25 for all pairwise comparisons), and C57BL/6 mice had higher H from 40 min of ventilation and beyond (P < 0.04 for all pairwise comparisons).

Factor Analysis

BAL protein content, number of inflammatory cells, cytokine levels, and lung function were all dependent on strain and ventilation strategy. Identification of meaningful correlations between these responses was compromised by the number of variables in this data set (Table 2). To overcome this problem, we used principal component factor analysis to identify independent associations between lung function and inflammatory (cells and cytokines) responses. Principal component factor analysis yielded three factors that explained 93% of the cumulative variance. Factor 1 explained 35.8% of the variance and was primarily loaded negatively on G and H and positively on the number of macrophages and protein content of the BAL (Table 3). Factor 2 explained 35.5% of the variance and was loaded negatively on the magnitude of the drop in TGV in the first 10 min of the ventilation period and positively on TNF-α and IL-6 content in the BAL (Table 3). Factor 3 explained 21.6% of the variance and was loaded on the level of IL-1β and number of neutrophils in the BAL (Table 3).

Table 1. Baseline thoracic gas volume (TGV), airway resistance (R_aws), tissue damping (G), and tissue elastance (H) in the 3 strains studied

<table>
<thead>
<tr>
<th>Strain</th>
<th>TGV, ml</th>
<th>R_aws, hPa·s⁻¹·l⁻¹</th>
<th>G, hPa/l</th>
<th>H, hPa/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>0.23 (0.04)</td>
<td>533.6 (79.2)</td>
<td>7,497 (848)†</td>
<td>41,830 (4,320)</td>
</tr>
<tr>
<td>129/Sv</td>
<td>0.27 (0.05)*</td>
<td>478.2 (73.2)*</td>
<td>9,239 (1,904)‡</td>
<td>46,110 (7,180)*</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>0.33 (0.08)*†</td>
<td>514.2 (60.5)</td>
<td>7,648 (811)‡</td>
<td>47,230 (5,490)*</td>
</tr>
</tbody>
</table>

Values are means (SD). *Significant difference (P < 0.05) compared with the BALB/c strain; †significant difference compared with the 129/Sv strain.

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To investigate the potential origin of different patterns we observed in decreases in TGV, which was more rapid in C57BL/6 mice on the HVZP strategy, and increases in G and H, which were more severe in C57BL/6 mice on the HVZP strategy, additional groups of BALB/c and C57BL/6 mice were exposed to either the LVP or HVZP ventilation strategy. At the end of the ventilation period, the mice were euthanized, and their lungs were removed. The lungs were patted dry and weighed to obtain a “wet” weight. Lungs were then dried in a commercial dehydrator (Sunbeam) for several hours before reweighing to obtain a dry weight. The lung wet-to-dry (W:D) ratio was then calculated as an indicator of pulmonary fluid accumulation. Groups of unventilated BALB/c and C57BL/6 mice also had the W:D ratio measured for comparison with ventilated animals. In C57BL/6 mice, there was no difference in W:D ratio between groups ($P = 0.13$; Fig. 5). In contrast, whereas there was no difference in W:D ratio between unventilated and LVP-ventilated BALB/c mice ($P = 0.73$), the W:D ratio in HVZP-ventilated BALB/c mice was significantly higher than both unventilated ($P = 0.03$) and LVP ($P = 0.04$)-ventilated mice (Fig. 5).

**DISCUSSION**

We have shown here that the outcome of experimental mechanical ventilation is dependent on both the ventilation strategy (injurious vs. protective) and the strain of mouse used. This strain dependence of the response allowed us to link patterns of inflammation, as a marker for biotrauma, to specific mechanical responses in the lung. Specifically, our data indicate that 1) protein leak and macrophage responses are associated with pulmonary edema, 2) IL-6 and TNF-α production are linked to atelectasis, and 3) IL-1β production and neutrophil recruitment are genetically determined (i.e., strain-dependent) and independent of mechanical responses in the lung.

As per studies by us (7–9, 40) and others (1, 2, 4, 36), mechanical ventilation of any kind in mice resulted in an inflammatory response in the lung characterized by cytokine production and/or inflammatory cell influx. We (40) have previously demonstrated that the LVP strategy used here is protective in the sense that it does not exacerbate preexisting viral-induced lung injury. As expected, the intentionally injurious ventilation strategy resulted in a greater inflammatory response than the LVP ventilation strategy, however, the magnitude of these responses varied considerably between strains. In particular, the most overt strain-related discrepancy was in the response in lung mechanics to the HVZP strategy, namely the large change in G and H in the C57BL/6 strain. In contrast, the pattern of change in lung mechanics was consistent between strains for mice on the LVP strategy. It was also clear that the overall decrease in TGV (at the end of the ventilation period) was consistent between strains and ventilation strategies. The volume dependence of the lung mechanics parameters measured in this study (15, 30) predicts that a drop in TGV should be associated with a concomitant rise in G and H. Although a decrease in TGV without a corresponding increase in G and H may suggest altered parenchymal rheology (39), the fact that this observation correlated with the level of pulmonary edema (e.g., increased W:D ratio in the BALB/c but not C57BL/6 mice on the HVZP ventilation strategy) suggests that such a pattern may be indicative of pulmonary edema. This raises the intriguing possibility that a comparison of TGV vs. parenchymal mechanics may provide a diagnostic tool for identifying whether mechanical ventilation is resulting in alveolar collapse (atelectasis) or alveolar flooding (edema). This is an area that requires further investigation.

As far as we are aware, this is the first study to directly compare strain responses in lung mechanics in the context of VILI, which makes comparison with other data difficult. Sim-
ilarly, there is a lack of data regarding strain-related structural and mechanical properties of lung tissue. Unfortunately, studies that have compared lung architecture have focused on comparisons in strains other than those used here. One study has directly compared mechanical properties of the lung parenchyma in BALB/c and C57BL/6 mice and suggested that baseline differences in in vitro $G$ and $H$ (analogous to $G$ and $H$ measured in this study) were related to the higher levels of collagen and elastin in the parenchyma and higher levels of $\alpha$-actin in the terminal bronchioles of the BALB/c strain (12). Thus one explanation may be that increased levels of collagen and elastin in the BALB/c strain leave the lung parenchyma more susceptible to the effects of mechanical ventilation. This observation may have important implications in patients where the lung pathology is associated with increased deposition of these fibers such as that seen in fibrosis (29). However, these data are in contrast to our in vivo measurements, which suggests that whereas the tissue mechanics between strains

Table 2. Correlation coefficients for all outcome variables used in the principal components factor analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>G</th>
<th>H</th>
<th>TGV</th>
<th>TNF-α</th>
<th>IL-6</th>
<th>IL-1β</th>
<th>Protein</th>
<th>Macrophages</th>
<th>Neutrophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>0.84</td>
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<tr>
<td>TGV</td>
<td>-0.46</td>
<td>0.02</td>
<td></td>
<td></td>
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<tr>
<td>TNF-α</td>
<td>0.65</td>
<td>0.20</td>
<td>-0.74</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>IL-6</td>
<td>0.45</td>
<td>-0.01</td>
<td>-0.66</td>
<td>0.95</td>
<td></td>
<td></td>
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<tr>
<td>IL-1β</td>
<td>0.07</td>
<td>-0.10</td>
<td>0.03</td>
<td>-0.09</td>
<td>-0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>-0.37</td>
<td>-0.68</td>
<td>-0.30</td>
<td>0.34</td>
<td>0.61</td>
<td>-0.06</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Macrophages</td>
<td>-0.79</td>
<td>-0.92</td>
<td>0.21</td>
<td>-0.2</td>
<td>0.05</td>
<td>0.27</td>
<td>0.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>-0.32</td>
<td>-0.31</td>
<td>0.29</td>
<td>-0.52</td>
<td>-0.44</td>
<td>0.88</td>
<td>-0.13</td>
<td>0.41</td>
<td></td>
</tr>
</tbody>
</table>
were statistically different, the magnitude of differences in lung mechanics at baseline between strains was small. In contrast, there were substantial differences in lung volumes between strains. In particular, the TGV at baseline in the C57BL/6 was substantially higher (43% compared with BALB/c mice) than the other strains. This may explain their apparent resistance to the HVZP whereby their increased lung volume can cope with the increased tidal volume applied during the injurious ventilation strategy.

Like many other mouse models of disease, there was strong strain dependence in the responses we observed in the present study. Contrary to the suggestion that strain-related differences (34) represent a deficiency of the mouse model of VILI by using consistent ventilation protocols, the strain differences observed in our study could be used to directly assess the link between lung mechanical responses to ventilation and the local inflammatory response.

The use of factor analysis allowed us to identify the relationship between changes in lung mechanics and specific inflammatory responses. The factor that explained the largest percentage of the variance (factor 1; 35.8%) loaded positively on macrophage and protein content of the BAL and negatively on changes in tissue mechanics (G and H). This loading pattern suggested that a smaller change in tissue mechanics was associated with greater recruitment of macrophages to the lung and protein production. This pattern was driven by: 1) a smaller change in tissue mechanics in the BALB/c and 129/Sv mice on the HVZP ventilation strategy, which was associated with increased macrophages and protein levels in the BAL compared with the LVP strategy; and 2) little difference in lung mechanics between ventilation strategies at the end of the ventilation period in C57BL/6 mice, which was the strain with the smallest change in protein levels in the BAL and responded with a decrease in the number of macrophages in the BAL. The mechanisms of this decrease in macrophages in the C57BL/6 strain are unclear but have been seen previously in other studies and may be related to cellular apoptosis (17), although the 2-h ventilation period may argue against this possibility. As discussed earlier, the BALB/c mice appeared to be more susceptible to pulmonary edema on the HVZP strategy with no evidence for edema in the C57BL/6 mice. Thus these results suggest that protein leak and macrophage recruitment are associated with alveolar flooding. It is important to recognize here that the measurement of total protein content of the BAL is an indicator of leakage across (26) the endothelial barrier, however, it is nonspecific and may include proteins from sources other than plasma; in particular, changes in protein content of the BAL may reflect changes in the airway surfactant pool. It is well-known that mechanical stretch of the lung may result in increased surfactant production (22, 23), and, as such, we cannot rule out a role for surfactant in the responses we have observed in our study, although there is a strong correlation between protein content in the BAL and W:D ratio, which is consistent with our study (38) and suggests significant pulmonary edema in the BALB/c mice on the HVZP strategy. We have clearly demonstrated a direct link between protein in the BAL, macrophage influx, and altered parenchymal mechanics that correlates with the presence of pulmonary edema. Thus, by avoiding high tidal volumes in mechanically ventilated patients, lung edema and the recruitment of macrophages to the alveolar spaces may be limited, which is likely to be important given the fact that macrophages are one of the primary sources of inflammatory mediators in VILI in humans (27).

Factor 2 was primarily loaded positively on TNF-α and IL-6 responses and the rate at which TGV was lost. Based on lung W:D ratio data, a rapid decrease in TGV at the beginning of the ventilation period (C57BL/6 mice on the HVZP strategy) was indicative of alveolar collapse as opposed to alveolar flooding. Thus the loading pattern for this factor suggests that atelectasis resulting from alveolar collapse is associated with an inflammatory response that is characterized by the production of TNF-α and IL-6. Both of these cytokines can be produced by the epithelium and are thought to be important in the progression of sepsis (32). Additionally, polymorphisms in TNF-α associated with increased TNF-α production have been linked to increased mortality in ARDS (21). However, the interpretation of this observation is somewhat complicated by the fact that TNF-α levels were decreased in mechanically ventilated mice compared with naïve mice. The decrease in TNF-α levels in all three strains on the LVP strategy compared with naïve levels

Table 3. Factor loadings from principal components factor analysis and orthogonal (varimax) rotation of lung mechanics and inflammatory parameters of mechanically ventilated mice

<table>
<thead>
<tr>
<th>Variable</th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>−0.8361</td>
<td>0.5392</td>
<td>0.0407</td>
</tr>
<tr>
<td>H</td>
<td>−0.9636</td>
<td>0.0232</td>
<td>−0.1146</td>
</tr>
<tr>
<td>TGV</td>
<td>0.0768</td>
<td>−0.8226</td>
<td>0.0314</td>
</tr>
<tr>
<td>TNF-α</td>
<td>−0.1628</td>
<td>0.9556</td>
<td>−0.1482</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.0996</td>
<td>0.9607</td>
<td>−0.1199</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.0117</td>
<td>0.0543</td>
<td>0.9943</td>
</tr>
<tr>
<td>Protein</td>
<td>0.79</td>
<td>0.5108</td>
<td>−0.1175</td>
</tr>
<tr>
<td>Macrophages</td>
<td>0.9449</td>
<td>−0.0497</td>
<td>0.2461</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0.1977</td>
<td>−0.3494</td>
<td>0.9119</td>
</tr>
</tbody>
</table>

Bold indicates significant factor loadings (greater than 0.75 or less than −0.75).
consistent with our previous studies using the same ventilation regime in naïve BALB/c mice (40). The TNF-α response observed in mice to mechanical ventilation varies widely between studies, which seems to be related to differences in strain and protocol (14). In this instance, we have at least controlled for protocol and showed that the decrease in TNF-α is consistent between strains. Additionally, the role of IL-6 in inflammatory processes is controversial as it is thought to have both pro- and anti-inflammatory properties (37). Interestingly, one of the anti-inflammatory actions of IL-6 is thought to work via modulation of levels of TNF-α (37). In contrast, our data show that a smaller decrease in levels of TNF-α is associated with a greater increase in IL-6 production, which suggests that in this instance the local production of IL-6 is not modulating TNF-α in an anti-inflammatory manner. Our analysis has reiterated the important association between TNF-α and IL-6 in the progression of VILI and clearly demonstrated that these responses are linked to atelectasis.

Factor 3 was positively loaded on the remaining parameters, IL-1β and the number of neutrophils in the BAL. The positive correlation between IL-1β levels and neutrophils is perhaps not surprising given that IL-1β is known to induce neutrophil migration (24). Neutrophils are considered to be an important cell in the progression of VILI (17). Importantly, whereas IL-1β (25) is thought to be intimately linked to IL-6- and TNF-α-related pathways, our analysis suggests that these responses are unrelated in the context of VILI in mice. Additionally, the fact that both of these responses appeared to be unrelated to any of the measured lung mechanics parameters suggests that these inflammatory responses were simply due to a genetic predisposition to neutrophil recruitment in response to an injury-inducing stimulus rather than a specific mechanical response in the lung associated with mechanical ventilation. The lack of neutrophilia in the C57BL/6 mice, compared with the other strains, observed in this study is consistent with other VILI studies (35) and studies comparing strain responses with neutrophil-inducing stimuli. For example, Lichtenstein and colleagues (20) showed increased neutrophil responses in BALB/c but not C57BL/6 mice following intratracheal exposure to fungal spores (Stachybotrys chartarum). These authors argued that this genetic susceptibility in neutrophil responses may be analogous to variations in susceptibility observed in humans. Our data are in agreement with this suggestion given that modification of ventilation strategy did not alter neutrophil recruitment. So, although neutrophils are likely to be important in VILI, their recruitment in mechanically ventilated patients may not be influenced by the ventilation strategy per se, and their importance in prognosis in the mechanically ventilated patient may be largely determined by a genetic predisposition to neutrophilia.

Like all models of disease, the mouse model of VILI has its limitations that need to be identified so that the results presented here can be placed in context. In all animal models of VILI, the duration of mechanical ventilation is substantially shorter compared with that for which human patients are usually exposed (13, 34). We recognize that the duration of ventilation used in our study was short, however, in using the mouse model, our experimental design made use of some of the major advantages that mouse models afford. First, we were able to apply otherwise unethical ventilation strategies to push the boundaries of the system. Second, we were able to use invasive measures of lung mechanics, which allowed us an insight into the impacts of ventilation on the mechanics of the lung, and, finally, we were able to make use of the strain dependence of the responses. This approach allowed us to directly link mechanical responses in the lung to local inflammatory responses.

In summary, we have shown here that inflammatory responses to mechanical ventilation are associated with distinct changes in lung mechanics. Specifically, protein leak and macrophage infiltration were associated with responses related to pulmonary edema: alveolar collapse was associated with IL-6 production and modulation of TNF-α; and neutrophil infiltration was associated with changes in IL-1β but not related to specific mechanical responses of the lung to ventilation. These observations suggest that a ventilation strategy should be adopted that prevents edema due to high tidal volumes (e.g., reduces recruitment of macrophages and protein leak) while using adequate tidal volume to prevent loss of lung volume due to atelectasis (e.g., reduces the production of TNF and IL-6). Additionally, the recruitment of neutrophils does not appear to be influenced by mechanical responses of the lung to ventilation and is largely genetically determined. These data provide the foundation for future mechanistic studies, which will allow us to unravel the pathobiology of disease associated with mechanical ventilation.

GRANTS

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DISCLOSURES

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

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