

Relation between Respiratory Mechanics, Inflammation, and Survival in Experimental Mechanical Ventilation

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Abstract

Low-tidal volume (V_T) ventilation might protect healthy lungs from volutrauma but lead to inflammation resulting from other mechanisms, namely alveolar derecruitment and the ensuing alveolar collapse and tidal reexpansion. We hypothesized that the different mechanisms of low- and high-volume injury would be reflected in different mechanical properties being associated with development of pulmonary inflammation and mortality: an increase of hysteresis, reflecting progressive alveolar derecruitment, at low V_T ; an increase of elastance, as a result of overdistension, at higher V_T . Mice were allocated to “protective” (6 ml/kg) or “injurious” (15–20 ml/kg) V_T groups and ventilated for 16 hours or until death. We measured elastance and hysteresis; pulmonary IL-6, IL-1 β , and MIP-2 (macrophage inflammatory protein 2); wet-to-dry ratio; and blood gases. Survival was greater in the protective group (60%) than in

the injurious group (25%). Nonsurvivors showed increased pulmonary cytokines, particularly in the injurious group, with the increase of elastance reflecting IL-6 concentration. Survivors instead showed only modest increases of cytokines, independent of V_T and unrelated to the increase of elastance. No single lung strain threshold could discriminate survivors from nonsurvivors. Hysteresis increased faster in the protective group, but, contrary to our hypothesis, its change was inversely related to the concentration of cytokines. In this model, significant mortality associated with pulmonary inflammation occurred even for strain values as low as about 0.8. Low V_T improved survival. The accompanying increase of hysteresis was not associated with greater inflammation.

Keywords: artificial respiration; ventilator-induced lung injury; acute lung injury; respiratory mechanics

Low-tidal volume (V_T) ventilation reduces mortality of acute respiratory distress syndrome (ARDS) (1) and may also be beneficial for patients with initially healthy lungs (2–4). This has significant importance, considering that more than 230 million patients per year require mechanical ventilation for major surgery

under general anesthesia (5) and that 35% of all patients in intensive care units (ICUs) worldwide receive mechanical ventilation even if they do not have ARDS (6). However, it is still uncertain whether low- V_T mechanical ventilation in healthy lungs has detrimental effects of its own. This “low-volume” injury (7) would

originate from two main mechanisms. One mechanism consists of increased mechanical stress at the boundary of derecruited lung regions (8, 9). This mechanism does not require intratidal recruitment, because it originates from local amplification of distending pressures in parenchyma abutting derecruited regions,

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Clinical Relevance

Low-tidal volume (V_T) ventilation is standard of care for acute respiratory distress syndrome and may also be beneficial in healthy lungs. However, it can lead to alveolar derecruitment and repeated end-expiratory alveolar collapse and reexpansion, which might promote inflammation. Consequently, there is a need to understand the interplay among ventilation strategy, respiratory mechanics, inflammation, and outcome in animal models over time scales and V_T values comparable to those of patients undergoing general anesthesia or receiving ventilation for days even if they do not have acute respiratory distress syndrome. In a 16-hour murine mechanical ventilation model, mortality was 1) associated with development of pulmonary inflammation even for lung strain values of approximately 0.8, much lower than the threshold of 2 above which mortality has been attributed to ventilator-induced lung injury in a variety of species; and 2) reduced by lower V_T . Survivors developed only modest pulmonary inflammation, regardless of V_T and despite an increase of hysteresis consistent with progressive alveolar derecruitment. The relative magnitude of the hysteresis increase was inversely related to the expression of pulmonary cytokines.

even in purely static conditions. A second mechanism results from the shear forces that develop during reopening of derecruited airspaces with tidal breathing (10–12). Consequently, for both these mechanisms of low-volume injury to be at play, part of the lung must be in a derecruited state. A consequence of alveolar derecruitment is an increase of hysteresis. In fact, magnetic resonance imaging has shown that hysteresis during mechanical ventilation of uninjured rodent lungs predominantly reflects alveolar derecruitment–recruitment (i.e., reeration [“opening”] of previously nonaerated airspaces) (13).

Part of the uncertainty concerning the significance of low-volume injury stems from the fact that most of the knowledge of

ventilator-induced lung injury (VILI) has been obtained from experimental models employing high V_T s (“high-volume” injury [14–16]). Because the pathophysiologic mechanisms of low-volume injury are expected to exert their detrimental effects *in vivo* on time scales much longer (17) than the volutrauma induced by marked overdistension, there is a need for animal models of ventilation over time scales and V_T ranges comparable to those of patients undergoing general anesthesia or patients in the ICU who require mechanical ventilation for days even if they do not have ARDS (18–20). Although large-animal models of ARDS and VILI have the advantage of replicating gravitational forces on a scale similar to the human, murine models allow for more convenient and mechanistically advanced assessment of molecular and genetic pathways. In addition, because the biologic time scale is compressed in mice relative to larger mammals with longer life-spans, it should be possible to study processes that are expected to unfold over several days in the latter within more manageable experimental durations in the former. Consequently, we developed a long-term murine model of mechanical ventilation that specifically avoids marked overdistension.

The aim of this study was to compare the alterations of respiratory mechanics, their time course, and their relationship to the expression of pulmonary cytokines in a group exposed to the mechanisms of low-volume VILI (V_T 6 ml/kg; “protective” group) and a group with mild to moderate overdistension (V_T 15–20 ml/kg; “injurious” group). We hypothesized the following:

1. The main mechanical alteration in the protective group would be an increase of hysteresis, reflecting progressive alveolar derecruitment at lower V_T (13). In the injurious group, we would expect an increase of elastance as a result of injury induced by tidal overdistension, but not of hysteresis, because the higher V_T would keep the initially healthy lung in a recruited state, thus minimizing the amount of cyclic recruitment–derecruitment.
2. These mechanical alterations would become manifest only over periods longer than the experimental durations common to murine VILI, given the lower V_T range employed.

3. Given the different pathophysiologic mechanisms of high- and low-volume injury, the pulmonary expression of cytokines would mainly parallel the increase of elastance in the injurious group and that of hysteresis in the protective group.

Some results of this study were previously reported in the form of abstracts (21, 22).

Methods

The methods are described in detail in the data supplement.

Experimental Design

C57BL/6 mice were anesthetized with intraperitoneal ketamine (120 mg/kg bolus followed by 30 mg/kg/h infusion) and fentanyl (0.05 mg/kg). After tracheotomy, the animals were mechanically ventilated with V_T 8 ml/kg, respiratory rate 160/min, and positive end-expiratory pressure (PEEP) 2 cm H_2O . The carotid artery was cannulated to monitor blood pressure and administer muscle relaxant (vecuronium, 1.5–2 mg/kg/h) and heparin (4 U/kg/h) in lactated Ringer’s with 5% dextrose solution. After surgical preparation, mice were connected to a flexiVent ventilator (SCIREQ) and assigned to two main groups according to ventilator settings: the low- V_T (“protective”) group (V_T , 6 ml/kg; respiratory rate, 180/min; $n = 10$) and the “injurious” group. The latter was composed of two subgroups: *inj-15* (V_T , 15 ml/kg; respiratory rate, 80/min; $n = 7$) and *inj-20* (V_T , 20 ml/kg; respiratory rate, 52/min; $n = 5$). All groups had fraction of inspired oxygen of 50%, PEEP of 2 cm H_2O , and inspiratory-to-expiratory time ratio of 1:2. Lung volume history was standardized with two recruitment maneuvers (30 cm H_2O , 5-s ramp and 1-s plateau) upon connection to the flexiVent. One maneuver was performed every 5 minutes during the experiment to recruit atelectatic lung. Frequency and pressure of the maneuver were based on studies showing that these settings maintained respiratory mechanics stable in mice ventilated for 2 to 6 hours with V_T and PEEP similar to those in our study (23, 24). Ventilation was continued for 16 hours or until the mouse died. Mice that died within the first 3 hours were excluded from the study because, at the V_T employed, death within this time window is not expected to result from VILI (Figure 1B in Reference 25).

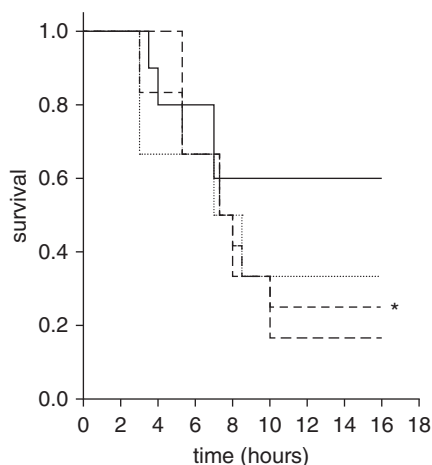


Figure 1. A significant difference was found between the survival rates of the “injurious” (*inj*) and “protective” groups at the end of the study ($*P < 0.05$). Solid line = protective group (tidal volume [V_T], 6 ml/kg; respiratory rate [RR], 180/min; $n = 10$); dotted line = *inj-15* subgroup (V_T, 15 ml/kg; RR, 80/min; $n = 7$); dashed line = *inj-20* subgroup (V_T, 20 ml/kg; RR, 52/min; $n = 5$); dashed-dotted line = *inj-15* and *inj-20* subgroups pooled.

Seven mice served as sham control animals.

In Vivo Measurements

Respiratory input impedance was measured every hour using the broadband forced oscillation technique with the flexiVent ventilator. Tissue elastance was calculated by fitting the constant phase model to the respiratory impedance (26). Inspiratory capacity was determined from the recruitment maneuver, which is expected to reach total lung capacity (27). Pressure–volume curves to 30 cm H₂O were generated every hour to compute

hysteresis. An upper pressure limit of 30 cm H₂O was chosen, both because it is considered to correspond to total lung capacity (28) and because it corresponded to the pressure of the recruitment maneuver, which the curve replaced at hourly intervals (see flowchart in Figure E1 in the data supplement). Elastance, inspiratory capacity, and hysteresis were normalized by the first (baseline) value of each subject. Lung strain and weighted lung strain (i.e., the average of the strain applied to the lung during inspiration and expiration, weighted by the inspiratory-to-expiratory time ratio) were computed as previously described (29). Arterial blood gas samples were collected from the carotid cannula when possible ($n = 13$) (Table 1).

Ex Vivo Measurements

Protein concentrations of IL-1 β and IL-6 in lung homogenate were measured by ELISA (protective group, $n = 10$; injurious group, $n = 7$). The mRNA concentrations of IL-1 β , IL-6, and MIP-2 (macrophage inflammatory protein 2) were measured by real-time qRT-PCR. The right upper lobe was harvested to measure wet-to-dry ratio.

Human Data

We compared plasma concentrations of IL-6 on Day 3 between the lower- and higher-V_T groups for the patients who did or did not survive to hospital discharge in the ARMA study (Lower Tidal Volume Trial) (1).

Statistical Analysis

Changes in elastance, inspiratory capacity, and hysteresis relative to baseline, and their differences between the protective and injurious groups, were analyzed with

two-way repeated measures analysis of variance and the Holm-Sidak method for pairwise comparisons. Hourly values were fit to a mixed-random and fixed-effects longitudinal model (30) to assess the temporal behavior of these variables regardless of differences in timing of the final value (defined as the last hourly value before the animal died or at 16 h). Cytokine concentrations were logarithmically transformed for normality and compared by one-way analysis of variance and the Holm-Sidak method. Lung strain and blood gases, wet-to-dry ratio, and human IL-6 were analyzed with Student’s *t*, Kruskal-Wallis, and Mann-Whitney-Wilcoxon rank-sum tests, respectively. $P < 0.05$ was considered significant. Data are reported as mean \pm SEM.

Results

Blood pressure was not significantly different between the two groups (Figure E2). Survival rate at 16 hours was higher in the protective (60%) group than in the injurious (25%) group ($P < 0.05$) (Figure 1).

Respiratory Mechanics

Elastance increased during the study in all groups (protective, +19.3%; *inj-15*, +25.7%; *inj-20*, +29.1%; $P < 0.001$ for all) (Figure 2A), but at a significantly faster rate in the injurious group, particularly the *inj-20* subgroup (Figure 2B). Inspiratory capacity decreased in all groups (protective, -3.4% ; $P < 0.05$; *inj-15*, -4.9% ; $P < 0.05$; *inj-20*, -15.1% ; $P < 0.001$), and the relative magnitude of this decrease was significantly higher in the *inj-20* subgroup ($P < 0.001$ vs. both *inj-15* and protective groups) (Figure 2C). In fact, the model revealed that

Table 1. Arterial Blood Gases

	pH	PaCO ₂ (mm Hg)	PaO ₂ (mm Hg)	Bicarbonate (mM)	Lactate (mM)
Reference*	7.39 \pm 0.02	30 \pm 2		18.4 \pm 0.8	4.6 \pm 0.7
Protective ($n = 7$)	7.11 \pm 0.05	43 \pm 3	99 \pm 27	13.9 \pm 1.4	2.8 \pm 0.7
Injurious ($n = 6$)	7.17 \pm 0.09	33 \pm 4	102 \pm 24	13.5 \pm 2.9	5.0 \pm 2.1
<i>P</i> value	0.541	0.062	0.929	0.894	0.534
Survivors ($n = 6$)	7.23 \pm 0.04	38 \pm 2	123 \pm 24	16 \pm 1.2	1.6 \pm 0.4
Nonsurvivors ($n = 7$)	7.06 \pm 0.07	39 \pm 5	81 \pm 23	11.7 \pm 2.4	5.7 \pm 1.6
<i>P</i> value	0.035	0.945	0.241	0.149	0.008

Definition of abbreviations: PaCO₂ = arterial carbon dioxide tension; PaO₂ = arterial oxygen tension; pH = arterial pH.

Data are mean \pm SEM.

*Reference values from Reference 73.

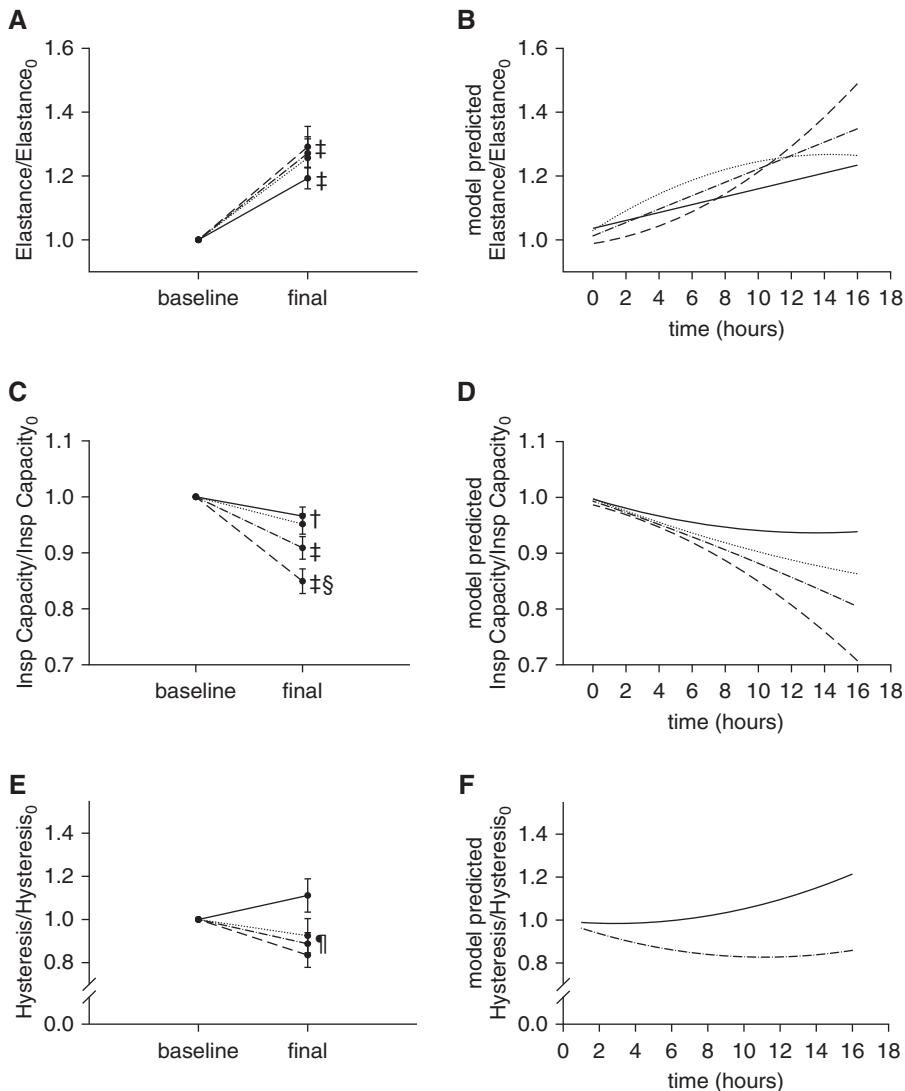


Figure 2. (A) Elongation increased significantly in all groups from the beginning (E_{0}) to the end of the study ($^{\dagger}P < 0.001$ vs. baseline). (B) The longitudinal model indicated a faster rise of elongation in the injurious subgroups ($P < 0.001$ for interaction between time and group), with the *inj-20* subgroup showing a marked increase after 8 hours of mechanical ventilation. (C) Inspiratory (Insp) capacity decreased in all groups ($^{\dagger}P < 0.001$ and $^{\ddagger}P < 0.05$ vs. baseline), and the relative magnitude of this decrease was significantly larger in the *inj-20* subgroup ($^{\S}P < 0.001$ vs. protective and *inj-15*). † Refers to both protective and *inj-15* subgroups. (D) The longitudinal model indicated a progressively faster rate of decline of inspiratory capacity with increasing V_T ($P < 0.001$). (E) Final hysteresis normalized by its initial value (hysteresis $_0$) was significantly lower in the injurious than in the protective group ($^{\parallel}P < 0.01$). (F) Whereas hysteresis increased progressively in the protective group, it decreased until approximately 11 hours of ventilation in the injurious group and then started to increase ($P < 0.05$ for interaction between time and group). The longitudinal model did not detect significant differences in the time course of hysteresis between the two injurious subgroups. Solid line = protective group (V_T , 6 ml/kg; RR, 180/min; $n = 10$); dotted line = *inj-15* subgroup (V_T , 15 ml/kg; RR, 80/min; $n = 7$); dashed line = *inj-20* subgroup (V_T , 20 ml/kg; RR, 52/min; $n = 5$); dashed-dotted line = *inj-15* and *inj-20* subgroups pooled. Data are mean \pm SEM.

the rate of decrease became progressively faster with increasing V_T , such that 16 hours were necessary for inspiratory capacity of the protective group to decrease by the same fraction as in the *inj-20* subgroup

at 5 hours (Figure 2D). Taken together, these results indicate that several hours are necessary for respiratory mechanics to deteriorate in models of VILI using V_T values closer to physiologic values.

The relative change of hysteresis was significantly higher in the protective group than in the injurious group ($P < 0.01$) (Figure 2E). In contrast to elongation, hysteresis rose at a faster rate in the protective group than in the injurious group, in which hysteresis declined initially, reached a minimum at approximately 11 hours, and then started to increase (Figure 2F).

Relationship between Inflammation, Mechanics, and Survival

IL-6 protein concentration correlated with the relative increase in elongation by the end of the study among all ventilated animals ($r = 0.53$; $P < 0.05$). However, inspection of the individual data points (Figure 3) revealed two distinct clusters: In one cluster, IL-6 was invariant, whereas in the other cluster, IL-6 showed a strong, direct relationship with the change in elongation. Individual data points could not be segregated into these two clusters on the basis of V_T (i.e., injurious vs. protective). Instead, what clearly clustered them was survival status. For animals that survived the 16 hours, there was no relationship between IL-6 and the change in elongation. In contrast, a strong correlation was observed in nonsurvivors ($r = 0.93$; $P < 0.001$; dotted line in Figure 3). Within this nonsurviving cluster, protectively ventilated animals corresponded to data points with lower IL-6 and smaller increases of elongation than injuriously ventilated animals. In fact, IL-6 was higher in the injurious group nonsurvivors than in the protective group nonsurvivors ($1,651 \pm 432$ pg/ml vs. 397 ± 88 pg/ml; $P < 0.01$) and higher in both these groups than in the survivors and sham control group ($P < 0.001$). IL-6 was not increased in the survivors of either group compared with the sham group (Figure 4A).

Similarly, IL-1 β was lower in the survivors than in the nonsurvivors (protective, 761 ± 190 pg/ml vs. $2,287 \pm 312$ pg/ml, respectively; injurious, 836 ± 189 pg/ml vs. $4,004 \pm 1,033$ pg/ml, respectively; $P < 0.01$ for both). In contrast to IL-6, IL-1 β was mildly elevated also in the survivors of both groups compared with the sham group (264 ± 55 pg/ml; $P < 0.01$), similar to previous findings (31), but again it did not differ between the two survivor groups (Figure 4B). Moreover, IL-1 β was negatively correlated with the relative change in hysteresis by the end of the study ($r = -0.84$; $P < 0.001$) (Figure 5). In

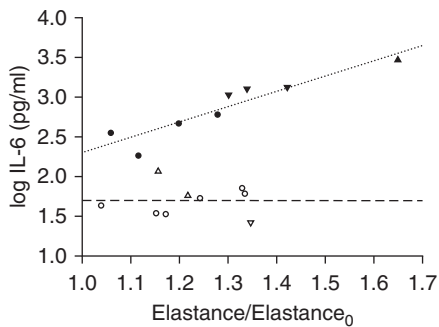


Figure 3. Lung IL-6 protein concentration as a function of the relative increase in elastance from the beginning to the end of the study in mice that survived (dashed line) or did not survive (dotted line) ($r = 0.93$; $P < 0.001$) 16 hours of mechanical ventilation. Open symbols = survivors; solid symbols = nonsurvivors; circles = protective group (V_T , 6 ml/kg; RR, 180/min; $n = 10$); triangles = *inj-15* subgroup (V_T , 15 ml/kg; RR, 80/min; $n = 7$); inverted triangles = *inj-20* subgroup (V_T , 20 ml/kg; RR, 52/min; $n = 5$).

contrast to the relationship shown in Figure 3, all data points followed a single line. However, the survivors clustered on the portion of this relationship corresponding to an increase in hysteresis and lower IL-1 β , whereas the nonsurvivors showed a decrease in hysteresis and higher IL-1 β , particularly in the injurious group. A similar inverse relationship with the relative change in hysteresis was found for IL-6 ($r = -0.79$; $P < 0.001$). Both these relationships remained statistically significant after normalizing the cytokine measurement by the wet-to-dry ratio of the individual animal (for IL-1 β , $r = -0.60$; $P < 0.05$; for IL-6, $r = -0.77$; $P < 0.001$).

For both IL-1 β and IL-6, their protein concentrations correlated with those of mRNA ($r = 0.94$; $P < 0.001$; and $r = 0.59$; $P < 0.05$, respectively) (Figure E3), indicating transcriptional control of these cytokines. MIP-2 mRNA was highest in the nonsurvivors and tended to be higher in the survivors than in sham control animals (Figure E4), similar to IL-1 β . In fact, MIP-2 mRNA was strongly correlated with IL-1 β mRNA ($r = 0.94$; $P < 0.001$), as expected on the basis of previous studies that showed induction of MIP-2 mRNA by IL-1 β (32). Hysteresis changes showed the same inverse relationship with mRNA concentrations of IL-1 β ($r = -0.76$; $P < 0.001$), IL-6 ($r = -0.56$; $P < 0.05$), and

MIP-2 ($r = -0.67$; $P < 0.01$) as with IL-1 β and IL-6 protein concentrations. Taken together, these results show concordant responses of various pulmonary cytokines and consistent relationships with changes in hysteresis.

There was a small difference in lung strain at the onset of mechanical ventilation between the protective group survivors and nonsurvivors (0.796 ± 0.024 vs. 0.860 ± 0.006 , respectively; $P < 0.05$), but not between the injurious group survivors and nonsurvivors (1.790 ± 0.154 and 1.812 ± 0.075 , respectively). When the applied lung strain was weighted by the inspiratory-to-expiratory time ratio, there was no significant difference between survivors and nonsurvivors of either the protective (0.486 ± 0.015 and 0.521 ± 0.010 , respectively) or injurious (0.936 ± 0.054 and 0.911 ± 0.036) group. No threshold could discriminate survivors from nonsurvivors, regardless of how strain was calculated (Figure 6).

Prolonged mechanical ventilation caused a mild increase in lung wet-to-dry weight ratio (sham, 4.4 ± 0.1 ; protective, 5.4 ± 0.1 ; injurious, 5.7 ± 0.3 ; $P < 0.05$) (Figure 7). There was no significant difference between the protective and injurious groups or between survivors and nonsurvivors (5.5 ± 0.2 and 5.5 ± 0.3 , respectively).

Arterial carbon dioxide tension tended to be lower in the injurious group. When analyzed according to survival status, the nonsurvivors had lower pH ($P < 0.05$) and higher lactate concentration ($P < 0.01$) than the survivors (Table 1).

Plasma IL-6 in the ARMA Trial

Plasma IL-6 on Day 3 was similarly low in the 6 and 12 ml/kg V_T groups for patients who survived to hospital discharge in the ARMA trial, whereas the 12 ml/kg nonsurvivors had significantly higher plasma IL-6 than the 6 ml/kg nonsurvivors ($P < 0.05$) (Figure 8).

Discussion

Thanks to its extended duration, this study separated the survival animals, who tolerated mechanical ventilation well regardless of the V_T , from the nonsurvivors. In the survivors, pulmonary expression of cytokines was not substantially increased and was unaffected by V_T .

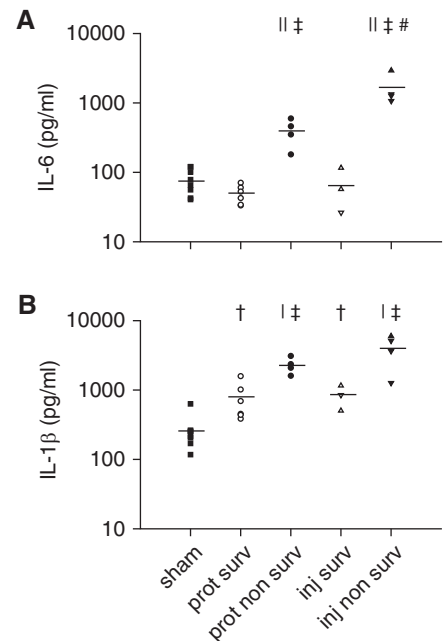


Figure 4. (A) Lung IL-6 protein concentration at the end of the study. (B) Lung IL-1 β protein concentration at the end of the study. $^{\dagger}P < 0.001$ and $^{\ddagger}P < 0.01$ versus sham; $^{\parallel}P < 0.001$ and $^{\perp}P < 0.01$ versus corresponding survivor group; $^{\#}P < 0.01$ versus protective nonsurvivors. Squares = sham; open symbols = survivors (surv); solid symbols = nonsurvivors; circles = protective (prot) group (V_T , 6 ml/kg; RR, 180/min; $n = 10$); triangles = *inj-15* subgroup (V_T , 15 ml/kg; RR, 80/min; $n = 7$); inverted triangles = *inj-20* subgroup (V_T , 20 ml/kg; RR, 52/min; $n = 5$); lines = mean.

In contrast, nonsurvivors showed marked elevation of cytokines even in the low- V_T group, in which lung strain was as low as about 0.8. In the nonsurvivors, but not in the survivors, the increase of elastance reflected the concentration of IL-6 (Figure 3). The concentration of all measured cytokines was instead negatively correlated with the relative change of hysteresis during the study.

Time Course of Respiratory Mechanics: Why Longer-Duration Models of VILI Are Needed

All groups showed an increase of elastance. This differs from shorter studies that did not report any deterioration in respiratory mechanics when V_T and PEEP similar to the protective group of our study were applied for 3 to 6 hours (23, 33). Furthermore, within the range of V_T s

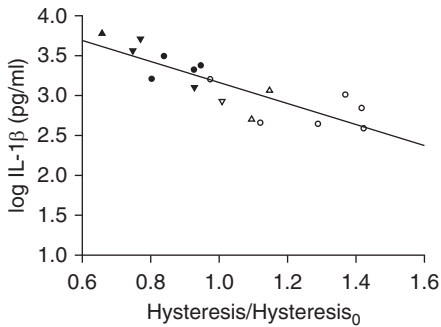


Figure 5. Lung IL-1 β as a function of the relative change in hysteresis by the end of the study ($r = -0.84$; $P < 0.001$). Open symbols = survivors; solid symbols = nonsurvivors; circles = protective group (V_T , 6 ml/kg; RR, 180/min; $n = 10$); triangles = *inj-15* subgroup (V_T , 15 ml/kg; RR, 80/min; $n = 7$); inverted triangles = *inj-20* subgroup (V_T , 20 ml/kg; RR, 52/min; $n = 5$).

employed (6–20 ml/kg), the detrimental effect of higher V_T became apparent only after 6–8 hours of ventilation, when elastance started to deteriorate rapidly in the *inj-20* subgroup (Figures 2B and 2D). This time interval is much longer than the 1.5 hours required to develop VILI with V_T of 45 ml/kg (34). Consequently, the first message of this study is that several hours are necessary to model VILI with V_T values

closer to those encountered clinically. In fact, PEEP and V_T combinations used in this study were deemed noninjurious on the basis of lung injury cost function models validated on data from shorter studies (35, 36).

The measurement of hysteresis allowed insights into the cause of the increase of elastance. Lung hysteresis increased in the protective group, suggesting that progressive alveolar derecruitment leading to less open lung was the cause of the increase in elastance (13, 37).

Derecruitment occurred despite a recruitment maneuver performed every 5 minutes, a frequency that maintained respiratory mechanics stable in shorter studies (23, 24, 37). In contrast, hysteresis decreased in the injurious group, at least up to 11 hours (Figure 2F), suggesting that 1) up to this time, the larger V_T maintained the lung in a recruited state, possibly by stimulating surfactant function, which can also independently decrease hysteresis, and by generating higher mean airway pressures that prevented derecruitment; and consequently, 2) the increase of elastance was predominantly due to the prolonged effect of moderate overstretching, which indeed resulted in mild pulmonary edema, rather than to derecruitment. In fact,

V_T of the injurious group was two to three times that of spontaneously breathing mice (38). These results thus temper conclusions of shorter studies that V_T values up to 20 ml/kg lead only to “atelectrauma” in mice (25) and instead suggest that mechanisms of both high- and low-volume injury can be modeled over the same V_T spectrum for which we would expect them in humans (i.e., 6 to 15–20 ml/kg), provided that ventilation is extended for a sufficiently long period (39–41).

Relationship between Respiratory Mechanics, Cytokines, and Survival

The protective group had better survival than the injurious group, resembling what was reported previously with a larger V_T difference (42). Although it has recently been shown that 20 ml/kg V_T for 4 hours leads to activation of the integrated stress response pathway and release of cytokines (43), there are conflicting data on whether low- V_T ventilation of initially uninjured lungs leads to increased pulmonary expression of cytokines *in vivo* (44–47). In our study, there was no effect of V_T magnitude on cytokine concentrations in the survivors. In contrast, cytokines were markedly elevated in the nonsurvivors, particularly of the injurious group. Because an increase of elastance is expected to result from both volutrauma (14, 16, 48) and derecruitment (24), we expected that such increase would correlate with the concentration of cytokines as it would be causally related to the pathogenetic mechanisms. Our results revealed that such correlation was present only in nonsurvivors, despite the fact that elastance and wet-to-dry ratio increased to a comparable extent also in survivors (Figures 3 and 7). The second message of this study is thus that the increase of elastance does not necessarily reflect the degree of underlying pulmonary inflammation due to VILI.

To identify the reason why some mice developed pulmonary inflammation and died whereas others showed only minimal inflammation and tolerated mechanical ventilation well, we computed lung strain. Although strain was slightly higher in the nonsurvivors than in the survivors of the protective group, there was no significant difference in the injurious group. Furthermore, strain less lower than 2 in most animals of both groups (Figure 6), and no threshold could be identified, in contrast

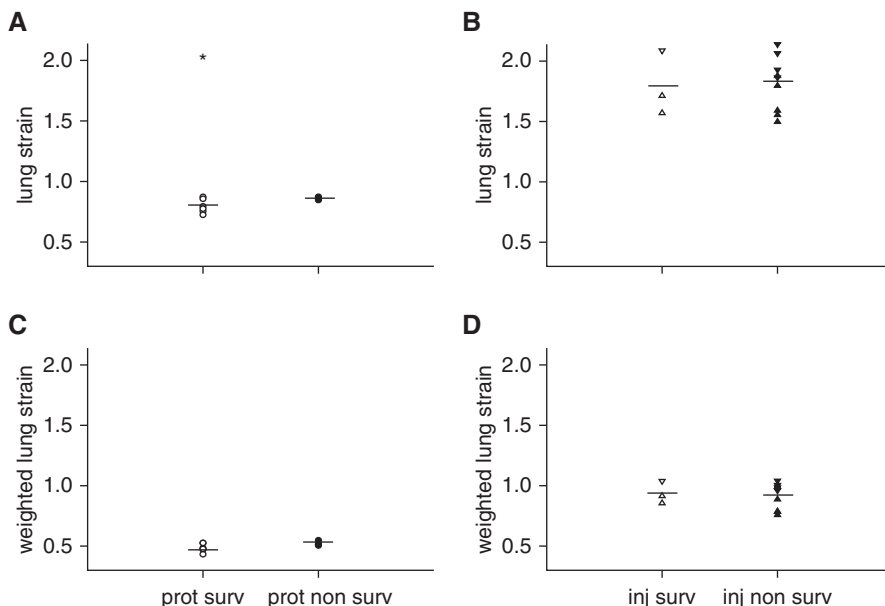


Figure 6. Lung strain in (A) protective and (B) injurious groups at the beginning of the study. Lung strain weighted by the inspiratory-to-expiratory time ratio in (C) protective and (D) injurious groups. * $P < 0.05$ survivors versus nonsurvivors. Open symbols = survivors (surv); solid symbols = nonsurvivors; circles = prot group (V_T , 6 ml/kg; RR, 180/min; $n = 10$); triangles = *inj-15* subgroup (V_T , 15 ml/kg; RR, 80/min; $n = 7$); inverted triangles = *inj-20* subgroup (V_T , 20 ml/kg; RR, 52/min; $n = 5$); lines = mean.

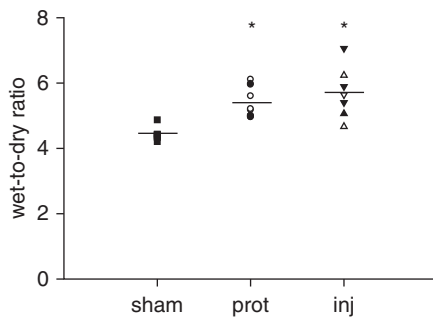


Figure 7. Lung wet-to-dry weight ratio at the end of the study. * $P < 0.05$ versus sham. Squares = sham; open symbols = survivors; solid symbols = nonsurvivors; circles = prot group (V_T , 6 ml/kg; RR, 180/min; $n = 10$); triangles = *inj-15* subgroup (V_T , 15 ml/kg; RR, 80/min; $n = 7$); inverted triangles = *inj-20* subgroup (V_T , 20 ml/kg; RR, 52/min; $n = 5$); lines = mean.

to previous studies (49, 50). Studies that demonstrated that VILI would develop and be lethal above a strain threshold of 1.5–2 in various species were based on experiments employing high V_T (29, 50). In contrast, values of weighted lung strain such as those of this study, particularly in the protective group (Figure 6C), have been speculated to be associated with VILI due to alveolar derecruitment and intratidal cyclic alveolar opening and closing, rather than to overdistension (29). The finding that protective nonsurvivors presented consistent and significant elevation of pulmonary cytokines (Figures 4 and E4) proves that, even with strain below 1.5–2, VILI developed and was the likely cause of death (51, 52). Metabolic acidosis and cardiovascular collapse are indeed consistent with the systemic inflammatory response that characterizes the terminal stage of VILI (16, 48, 51). In fact, IL-6, for

example, has been associated with mortality attributable to VILI in patients with ARDS. Interestingly, IL-6 plasma concentrations in the ARMA trial revealed a pattern similar to that of pulmonary IL-6 in mice (Figures 4A and 8). Consequently, we propose that VILI results from an interaction between the magnitude of the mechanical insult and an intrinsic susceptibility to pulmonary inflammation induced by mechanical ventilation. If *each* subject had an *individual* strain threshold above which VILI ensued, then tidal strain below this threshold would not cause significant inflammation, which would instead develop in a V_T -dependent fashion above this threshold. Accordingly, ventilator parameters that are protective in one subject might be injurious in another. Although caution must be exercised in extrapolating to humans, these results offer a rationale for personalized mechanical ventilation. Interindividual differences in the ratio of elastin to collagen, cross-linking of collagen, and magnitude of the cytokine response could represent potential determinants of this threshold, similarly to what has been hypothesized to explain age-related variation in susceptibility to VILI (53, 54).

Possible explanations for the observation that VILI occurred for V_T and strain values lower than in prior experimental studies are: 1) the same V_T per kilogram results in greater strain in smaller than in larger species because functional residual capacity is proportional to body weight to a power of approximately 1.2 (29, 55). In addition, the murine chest wall is more compliant than the human (56, 57), and hence a given PEEP is expected to cause greater distension in the former. We accounted for these factors by limiting

PEEP to 2 cm H_2O and V_T of the protective group to slightly less than that of spontaneously breathing mice (38). 2) Subtle bloodstream infection may have ensued and increased susceptibility to VILI (58). If so, however, we would have expected the mortality rate to increase over the course of the study because the cumulative probability of contamination should increase with time. Furthermore, this risk should have been present also in other long-term studies that identified a strain threshold (49, 50).

Role of Derecruitment in VILI

Because several *ex vivo* studies demonstrated that low- V_T ventilation of a derecruited lung can induce injury (10, 11, 59), we hypothesized that the increase of hysteresis in the protective group would predict pulmonary cytokine concentrations. In fact, a direct relationship between hysteresis and BAL fluid protein content, which is an index of inflammation, was reported in other models of murine lung injury characterized by alveolar derecruitment (60). Instead, we found that the higher the increase of hysteresis, the lower the expression of cytokines. One interpretation of this result is that derecruited airspaces are protected from injury (61, 62). An alternative interpretation is that, in this study, derecruitment was simply an epiphenomenon of resilience to VILI: Animals that did not mount a substantial pulmonary inflammatory response to mechanical ventilation survived longer and, as a result of being ventilated for longer times, developed greater derecruitment, particularly in the protective group. Regardless of the interpretation, the third message of this study, supported also by others (63, 64), is that derecruitment *per se* does not appear to be a potent inflammatory trigger *in vivo*, even during low- V_T ventilation. However, recent evidence suggests that derecruitment may act predominantly indirectly in the intact respiratory system: Because of alveolar interdependence, derecruitment may induce overdistension of the airspaces that remain aerated (65, 66). This could constitute an additional mechanism of low-volume injury. Furthermore, atelectasis can be injurious by mechanisms other than VILI, such as right ventricular dysfunction and increased pulmonary microvascular permeability (67).

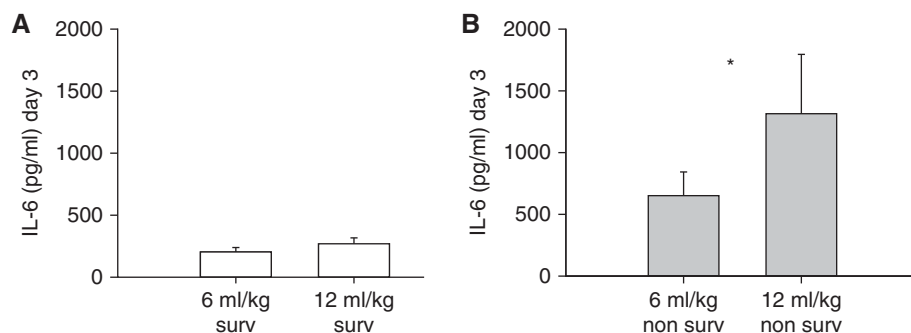


Figure 8. Plasma IL-6 on Day 3 in (A) survivors and (B) nonsurvivors of the 6 and 12 ml/kg V_T groups in the ARMA trial (Lower Tidal Volume Trial) (1). * $P < 0.05$ for 12 ml/kg versus 6 ml/kg. Data are mean \pm SEM.

Limitations and Critique of the Study

Although a recruitment maneuver every 5 minutes may sound excessive for larger mammals, it is important to properly scale the murine variables. Because the spontaneous respiratory rate of C57/BL6 mice is approximately 350 breaths per minute (68), one maneuver every 5 minutes would correspond to one every 2.5 hours at a respiratory rate of 12 per minute. This 30× scaling is consistent with that reported in the literature (69). According to this scaling, the 16 hours of ventilation in mice should correspond to approximately 20 days for a human.

We measured a limited number of cytokines, selected on the basis of the consistency with which they were reported to be elevated in VILI studies in mice (24, 45–47, 70) and humans (1, 71, 72), as well

as on the basis of their kinetic profile. Other cytokines, such as TNF- α , also play a role in VILI, but because TNF- α expression is transient and peaks in the early stage of VILI (33), it did not seem the best mediator to assess prolonged VILI.

Conclusions

In a 16-hour model of murine mechanical ventilation, mortality was 1) associated with development of pulmonary inflammation despite lung strain as low as 0.8, and 2) lower with V_T 6 ml/kg than with V_T 15–20 ml/kg. Survivors developed only modest pulmonary inflammation, regardless of V_T and despite an increase of hysteresis whose magnitude was, in fact, inversely related to the concentration of pulmonary cytokines. These results suggest that there is no single

strain threshold for developing VILI and that alveolar derecruitment during low- V_T ventilation does not necessarily lead to pulmonary inflammation. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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